Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Medicine and Health Sciences
Department of Public Health and Nursing

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Associations Between Maternal Lead Exposure and Birth Outcomes in Argentina. The EMASAR Study

Master's thesis in Global Health

Supervisors: Jon Øyvind Odland, Kam Sripada and Solrunn Hansen

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ABSTRACT

Background: The EMASAR assessed concentrations of environmental toxins in the blood of delivering women to investigate maternal and fetal health risks related to food security and exposure to persistent toxic substances in two regions of Argentina (Ushuaia and Salta).
Objectives: To determine the blood lead levels and identify related risk factors among delivering women in Argentina and to evaluate the relationships between maternal lead exposure and birth outcomes.
Methods: Blood samples derived from EMASAR study were analyzed. A total of 696 maternal serum samples collected from Ushuaia (n = 198) and Salta (n = 498) singleton women at 36±12 hours after delivery. Data of health and sociodemographic characteristics of the mother and their child were obtained from the medical records and questionnaires. Multiple linear regression models were applied to describe the relationships between lead exposure and related maternal risk factors, as well as the associations between levels of maternal blood lead and birth outcomes while adjusting for the possible confounders and covariates. Adjusted logistic regression analyses were used to examine the relationships between quartile of maternal blood lead levels and preterm birth, and low birth weight.
Results: Blood lead levels were higher in women from Salta than those in Ushuaia ($p < 0.001$), with geometric mean blood lead levels of 15.8 µg/L and 10.1 µg/L, respectively. The geographic differences in blood lead levels can be explained by the differences in socioeconomic conditions, prior or current industry emissions and potential contributors of regional pollution. Age and smoking were positively associated with the blood lead levels. Women with primary education had significant higher levels of lead in serum than those who attended tertiary education. Blood lead levels were higher in women in urban areas than in those that live in rural areas. Adjusted models of multiple linear regression analyses suggested that increasing blood lead levels were related to increase in the gestational age in the overall sample. Significant negative associations were found between blood lead levels and birth weight, and birth length in the Salta sample. No significant associations between lead exposure and birth outcomes were observed in the Ushuaia sample. There were no clear relationships between quartile of blood lead levels and preterm birth or low birth weight were evident in the adjusted logistic regression models.
Conclusions: Maternal lead exposure was mainly related with residence, age, smoking and education. Blood lead levels showed a positive association with gestational age in the overall sample. Inversely, even maternal low-level lead exposure may adversely affect birth weight and birth length in the Salta sample. There was no evidence to suggest dose-response relationships for the effect of blood lead levels on birth outcomes. Blood lead levels should be kept as low as possible especially during pregnancy to minimize undesirable effects.
Keywords: EMASAR; Lead; Maternal serum; Risk factors; Birth outcomes; Argentina

ACKNOWLEDGEMENTS

I would like to extend sincere appreciation and gratitude to all those who helped me during the completion of this Master's thesis.

My deepest gratitude goes first and foremost to my main supervisor Dr. Jon Øyvind Odland, Professor in Global Health, Department of Public Health and Nursing, Faculty of Medicine and Health Sciences, Norwegian University of Science and Technology, NTNU, Trondheim, Norway, who with extraordinary patience and constant encouragement, gave me great help by providing me with dataset for this Master's thesis, advice of great value and inspiration of new ideas. He has walked me through all the stages of the writing this thesis. Without his consistent and illuminating supervision, this thesis could not have reached its present form.

I am deeply indebted to my co-supervisor, Kam Sripada, a post-doctoral fellow in the Center for Global Health Inequalities Research (CHAIN), Department of Sociology and Political Science, Faculty of Social and Educational Sciences, NTNU, who was always there to help me. I really appreciate your precious suggestions, thorough input and English editing of my manuscripts.

My sincere thanks also go to my co-supervisor Dr. Solrunn Hansen, Department of Health and Care Sciences, The Arctic University of Norway, Tromsø, Norway, for helping to obtain dataset of my Master's thesis, and the revision of my manuscripts.

I would like to thank NTNU for providing me excellent opportunity to continue my higher studies in a reputed institution. I am grateful to Global Health Master program offered by NTNU that planted the importance of global-scale issue of public health to me. I would like to thank all the people working at Global Health Master program for their participation and contribution for this Master's program.

I am grateful to those people who made efforts in the EMASAR study, for providing me with good data source for this Master's thesis.

I would also like to thank Professor Stian Lydersen, Department of Mental Health, Faculty of Medicine and Health Sciences, NTNU, for his help of statistical problem.

I also owe my sincere gratitude to my friend and my fellow classmate, Maria Jensen, for her advice and assistance in searching of Master's thesis project and translation of Spanish literature.

Last my thanks would go to my beloved parents who have always been assisting, supporting and caring for me all of my life.

Shanshan Xu

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LIST OF ABBREVIATIONS

AMAP	Arctic Monitoring and Assessment Program
BBB	Blood brain barrier
BMI	Body Mass Index
CDC	United States Centers for Disease Control and Prevention
CI	Confidence interval
DALYs	Disability-adjusted life years
EMASAR	Estudio del Medio Ambiente y la Salud Reproductiva (study on the Environment and Reproductive Health)
EDCs	Endocrine disrupting chemicals
LogMBLLs	log10 transformed maternal blood lead levels
IQR	Interquartile range
ORs	Odds ratios
OCs	organochlorine compounds
PTS	Persistent toxic substance
SD	Standard deviation
SDGs	Sustainable Development Goals
UN	United Nations
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1 Background

1.1.1 Sources and pathways of human lead exposure

The growth of population and the speeding up of industrialization resulting the demands for greater mineral resources, metal process and smelting, the development of industrial chemicals, fertilizers, pesticides and pharmaceuticals have increased rapidly in modern society. Although many of these chemicals are utilized or destroyed, a high prevalence of these chemicals in air, water and soil poses a potential hazard to the environment and creating public health concerns [1, 2].

Lead (Pb) is one of the most ubiquitous blush gray metals that occurs naturally in the earth crust, with trace amounts of found in soil, plants and water [3]. Lead has been used by mankind for over 6,000 years, and lead's toxicity has occurred for at least 2,500 years in record history [4]. Despite early recognition of lead hazards, exposure to lead from variety of sources persists to nowadays [2]. Lead is practically immobile but becomes highly toxic after anthropogenic activities such as mining, burning, industrial processing and manufacturing [2, 5]. Lead has many different industrial, agricultural and domestic applications [2]. Environmental lead level has increased more than 1,000 times over the past three centuries as a result of human activities, the largest increase occurred between 1950 and 2000, reflecting the increasing use of leaded gasoline worldwide [6]. Before the global phase out, leaded gasoline is accounting for more than 90% of airborne lead pollution [7]. The applications of lead compounds in paints, dyes, ceramic glazes and caulk and the lead alloys in pipes, storage batteries, ammunition, cables, solders and shielding equipment and so forth are also regarded as other important sources of lead pollution. In recent years, the consumption of lead in these products has been reduced to minimize the harmful effects of lead on human beings and animals [6]. One of the most notable impacts is removal of lead from gasoline in 1990, it has

been presented worldwide that the drop in mean blood lead level along with the restrictions in leaded gasoline [3, 8, 9]. Although all these precautionary measures were effective, human exposure to lead still remains the one of the serious environmental health problems because it does not degrade and strongly adheres to the soil and sediment [10].

People are exposed to lead through occupational and environmental sources. Lead is mainly absorbed through the respiratory and gastrointestinal systems, which the principal exposure route in the occupational population is through respiratory system. While food is the primary source of non-occupational exposure in the general population [11, 12]. In Argentina, the most important sources of lead exposure derived from contaminated soil, air or food by industry waste or due to mining activities and presence of foundries [13]. Lead can be bioaccumulated and biomagnified through the food chain and finally consume by human resulting in health hazards [14]. Food may be polluted as a result from lead-contaminated soil, water and air, consuming meat harvested by lead projectiles or metal equipment applying in the food production and packing materials of food. In an epidemiological study in Nigeria, Tirima et al. found that most dietary lead exposure was associated with contamination of staple cereal grains and legumes during post-harvest processing and contaminated home preparation [15]. Similarly, other investigators from Guangzhou, China obtained the results that rice and rice products, leafy vegetables, and wheat flour and its products were the largest food sources of lead intake, followed by pork, fruiting vegetables, algae, and fruit [12]. Maternal dietary intake may be the potential confounding factor in the associations between lead levels in maternal serum and fetal growth outcomes. Meanwhile, fetal growth may influence by various factors, among which nutritional supply to the development of fetus is crucial. Consumption of nutritious food during pregnancy is known to be benefit for birth outcomes. Maternal intake of some nutrients and minerals have been observed to help to reduce the adverse effects of lead during the pregnancy. Dietary intake of iron and calcium during pregnancy was negatively related to neonatal blood lead levels [16]. West et al. found vitamin-mineral supplement users had significantly higher serum levels of vitamin E and ascorbic which could protect the fetus against lead toxicity and or free radical damage through antioxidant actions [17].

The Institute for Health Metrics and Evaluation estimates that in 2017, lead exposure caused 1.06 million deaths and 24.4 million years of healthy life lost worldwide (disability-adjusted life years (DALYs)) as a result of long-term health effects, accounting for 63.2% of the global burden of idiopathic developmental intellectual disabilities, 10.3% of the global burden of hypertensive heart disease, 6.2% of the global burden of stroke and 5.6% of the global burden of ischemic heart disease [19].

1.1.2 Health effects in pregnant women and children

It is acknowledge that children and pregnant women (as surrogates for fetuses) are most sensitive populations to lead exposure [18, 19]. Early pregnancy is a critical stage for fetal development, during which exposure to environmental pollutants can have a negative impact on pregnancy as well as on neonatal, early childhood and later life outcomes [20]. Blood brain barrier (BBB) has long been known to be a target for lead toxicity [21]. Because the BBB is not fully developed during fetal development of the nervous system, the fetus is more sensitive to all neurotoxins [22]. Lead can readily access to central nervous system through BBB even when BBB is fully developed, the ability of lead to pass through the BBB is due to large part of its ability to substitute for calcium ions [21, 23]. Previous studies have been reported that maternal blood lead levels were positively associated with spontaneous abortion [24], preterm, small-for-gestational-age birth [25], gestational hypertension and pre-eclampsia [26]. Lead in the body is distributed to the brain, liver, kidney and bones. Over 95% of maternal lead is stored in bone [27]. Bone is also the storage source of calcium in human body. Many of lead's toxic properties are due to its ability to mimic or compete with calcium [28]. The demand for calcium is higher during pregnancy than the non-pregnant state, in order to meet the increasing physiological needs, the body absorb the calcium from the bone [27]. Lead stored in the bones will be released into the maternal blood, causing the elevated of maternal blood lead level. Lead can freely cross the placental membranes, thus maternal lead level can significantly affect the growth of fetus [26]. A considerable body of scientific evidence shows that the associations between maternal blood lead levels and a variety of birth outcomes, including the low level lead exposure was risk for decreasing of birth weight [24, 29-31], a negative association was also

found between maternal blood lead levels and head circumference as well as crown-heel length of neonates [29, 32, 33]. While some of other epidemiological studies were not find such associations [34, 35].

It has been well studied that lead mainly affects the central nervous system, especially the development of brain. Young children are particularly vulnerable to the toxic effects of lead and lead exposure can have serious consequences for the health of children even at a low level. In children, lead exposure results in deficiencies in such global indicators as intelligence quotient and attention deficit disorder [36]. A number of epidemiological studies have also demonstrated that lead exposure in children is associated with cognitive impairment, mental retardation, development delays, behavioral deficits, which including hyperactivity, deficits in fine motor function, hand-eye coordination and reaction time [23, 37, 38]. In addition, the studies conducted by Needleman et al. [38] and Nevin et al. [39] found that lead exposure affects the risk of juvenile delinquency and criminal behavior later in life.

There is no known safe level of exposure to lead [40, 41]. Blood lead level is considered the primary biomarker for lead exposure [42]. In 2006, the Scientific Committee on Neurotoxicology and Psychophysiology and the Scientific Committee on the Toxicology of Metals of the International Commission on Occupational Health (ICOH) issued the Declaration of Brescia, which supported the revision of action level for children's blood lead concentration to 50 μg/L immediately [43]. In 2012, the United States Centers for Disease Control and Prevention (CDC) changed the terminology from 'level of concern' to 'reference value' and reduced this reference value of blood lead level from 100 μg/L to 50 μg/L in U.S. children aged 1-5 years based on the 97.5 percentile [44]. Argentina Ministry of Health took 50 μg/L as intervention level of blood lead for children in the guideline in 2013 [13]. CDC guidelines also suggested that blood lead level ≥ 50 μg/L in pregnant women requires following-up and interventions [45]. Figure 1 depicts the gradual decline in children's acceptable blood lead levels over time [46], which indicates that as the understanding of lead toxicity has deepened, the concept of lead poisoning has fundamentally changed, and people's focus has shifted from the early high-dose clinical effects of lead poisoning to the consequences of exposure at lower doses

that cause chronic cumulative toxicant [28]. Blood lead concentrations that were previously considered safe have repeatedly been shown to be harmful to health.

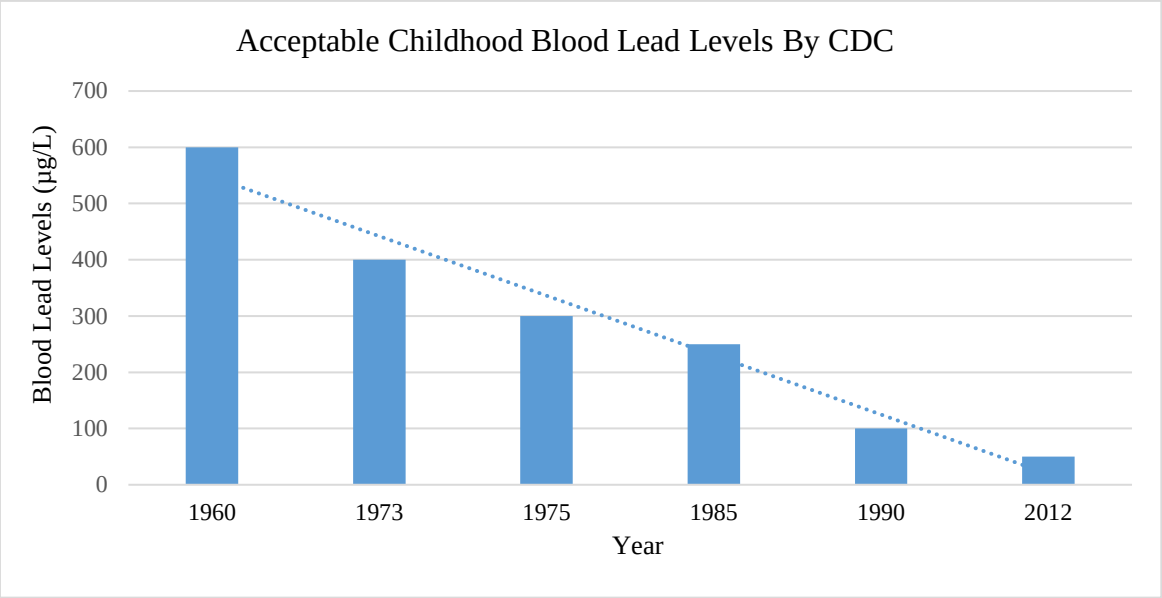

Figure 1 Acceptable childhood blood lead levels by CDC
The bars present the acceptable value of blood lead levels in children at different time. Dotted line shows the acceptable value have been decreasing gradually from 1960 to 2012 by CDC [46].

1.1.3 World Health Organization (WHO) response to lead exposure

WHO has identified lead as one of the 10 chemicals of major public health concern and encourages all the countries to take action to protect the health of workers, children and women of childbearing age [40]. WHO is currently developing evidence-based guidelines on prevention and management of lead toxicity and a set of related information to lead are available through its website. As lead paint is a continuous source of exposure in many countries, WHO has joined the United Nations (UN) Environment Program to form the Global Alliance to Eliminate Lead Paint. The phasing out of lead paint by 2020 is one of the priority actions for governments included in the WHO *Road map to enhance health sector engagement in the Strategic Approach to International Chemicals Management towards the 2020 goal and beyond*. But a new WHO report, only 73 governments confirmed that they have legally binding control measures on lead paint, thus there is still a significant gap to achieve the goal that by 2020 all the countries should ban the leaded paint [47]. Eliminating lead paint will help achieve the UN Sustainable Development Goals (SDGs) by 2030.

1.1.4 Argentina’s context

1.1.4.1 Country profile

Argentina is the eighth largest country by area in the world with multicultural, diverse geography and abundant natural resources. It is located mostly in the southern half of South America, bordering the South Atlantic Ocean on the east, facing Antarctica across the sea in the south, sharing the Andes Mountains with Chile on the west, neighboring Bolivia and Paraguay to the north, adjoining Uruguay and Brazil on the northeast and with a mainland area of 2,780,400 km^2.

The economy of Argentina is an upper middle-income economy for fiscal year 2019 according to the World Bank. Argentina’s mining industry has undergone massive growth since the 2000s, which is closely related to the growth of its economic activities [48]. Argentina offers gold, silver, copper, zinc, lead, molybdenum, iron, lithium, potassium, nuclear minerals, rare earth metal and a diversified resource. The level of urbanization in Argentina is high, with 92% of its population living in cities [49].

1.1.4.2 Health system

Argentina’s health care system is composed of public sector, social security sector and private sector. The country’s federal structure and the health system is highly decentralized, which means that public health is administered at the municipal level and primary health care is usually managed independently by each city. The Ministry of Health oversees the three sectors of the health care system and is responsible for the development of regulations, evaluation and collection of statistical data. Although all the people have access to services provided by the public sector. The differences of health care expenditure and human as well as material conditions among various territories and different population groups cause the inequity of the medical service [50].

Figure 2 Map of South America with study areas

Yellow Stars in the map show the study sites Salta and Ushuaia, Argentina [51].

1.1.4.3 Related epidemiological studies of childhood and maternal lead exposure in Argentina

Although not many, some studies regarding children and maternal environmental lead exposure have been conducted in Argentina. In a study in Córdoba, obtained results that the average blood lead level of children was observed to drop from 77.0 µg/L in 1995-1996 to 25.8 µg/L in 2009 and 2010, following the ban of leaded gasoline. Moreover, the study showed the percentage of children with blood lead levels above 100 µg/L was reduced to 3.72% in 2009-2010 compared to 26.7% in 1995-1996. The study also pointed out the children living in suburbs have higher

blood lead levels than the children in the city [8]. Disalvo et al. analyzed blood level levels from 93 children age between 6 months and 5 years of La Plata during the months of July to October 2006, geometric mean blood lead level was 42.6 µg/L and with 10.8% of the total children presenting blood lead levels values above 100 µg/L. They also reported higher blood lead levels were found in children living in households with lead-handling contaminating activities and in very low-income households [52]. One cross-sectional study of La Plata city and outskirts between 2009 and 2012 found that 12.6% of children (n = 319) between 1 to 6 years old had a blood lead levels above 50 µg/L and the mean blood lead level was 29 µg/L and median value was 22 µg/L. The main risk factors for lead exposure were age equal 3 years old or below and pica behavior. Other factors included anemia, maternal education < 7 years, overcrowding, and dirt floors showed less relevant risks [10]. Another cross-sectional study performed in the public maternity of the Hospital in La Plata between 2010 and 2011 revealed that geometric mean for cord blood lead level was 21 µg/L and 5.5% of cord blood lead level was above 50 µg/L and none was greater than 100 µg/L, the study also found that 25% of newborns might have some degree of risk for lead poisoning [53].

These epidemiological studies revealed a notable reduction in blood lead level in the pediatric population of Córdoba and La Plata and its vicinity, as well as a relatively low umbilical cord blood lead level in the city of La Plata. Probably, this parallel reduction in blood lead level along with the prohibition of lead applications in gasoline and regulation of lead paints in 1996 and 2004 respectively in Argentina [8, 10].

1.2 Statement of purpose

It is known that poor birth outcomes are associated with health and development problem during infancy and throughout childhood, as well as with long-term implications for adult health. Thus, it is important to characterized the effects of blood lead concentrations to lead-exposed pregnant women and neonates. In Argentina, the majority of lead related health research focus on children near industrial and mining areas, a few studies reported umbilical cord lead levels [8, 10, 52, 53]. Though these studies showed a reduction of children's blood lead level and neonatal cord blood lead level under the suggested 50 µg/L. The information available on the maternal

blood lead levels and its effects on the newborns is still very limited till now [53].

The present study is devoted to contribute to fill this gap by analysis of maternal blood lead levels from EMASAR (Estudio del Medio Ambiente y la Salud Reproductiva; study on the Environment and Reproductive Health). The previous publications from EMASAR study revealed that organochlorine compounds (OCs) concentrations in the Argentina were mostly in the lower range compared with other countries [54, 55]. Age was found positively associated with OCs concentrations while parity showed an inverse relationship [54]. Body Mass Index (BMI, kg/m^2) and region of residence also indicated dependencies of the OCs concentrations [55]. Higher weight accumulation during pregnancy involved dilution of these OCs [55].The differences of OCs concentrations between Ushuaia and Salta were explained by contrasting domestic sources, historical and current use, industrial emissions, dietary patterns, lifestyle factors and long-range transport [54].

Previous EMASAR papers provided good evidence of blood OCs levels and determinants of OCs exposure in Argentinean women after delivery. The regional difference of OCs concentrations in Ushuaia and Salta also explained the environmental, social economic and lifestyle factors can be considered determinants of undesirable toxic exposure. People experience the living environment as a combination of physical, chemical, biological, social, cultural, and economic conditions that vary depending on geographical location, infrastructure, time and activities performed. These living environmental factors play an important role in toxic exposure. The situation for human exposure and human biological levels of contaminants in South America has not been studied adequately [51].

Therefore, the present study, a location-specific research based on Argentina, has expanded the EMASAR data analyses by investigating maternal blood lead levels related risk factors and examining the effects of lead in maternal blood on measures of birth outcomes could provide an important complement to traditional toxicological studies that have already demonstrated the toxicity of lead.

1.3 Objectives of the study

To determine the blood lead levels and identify related risk factors among delivering women in two regions of Argentina (Ushuaia and Salta) and to evaluate the relationships between maternal lead exposure and birth outcomes. The specific objectives of the study are as follows:

1. To obtain maternal blood lead levels in Ushuaia and Salta.
2. To compare differences of the maternal blood lead levels between Ushuaia and Salta.
3. To investigate the risk factors of the maternal blood lead levels.
4. To investigate the associations between maternal blood lead levels and birth outcomes.
5. To compare the associations between maternal blood lead levels and birth outcomes in Ushuaia and Salta.
6. To investigate the relationships between quartile of maternal blood lead levels and two binary outcomes including preterm birth (gestational age < 37 weeks) and low birth weight (birth weight < 2500 grams).

1.4 Research questions

1. What are the risk factors for maternal lead exposure?
2. Is maternal lead exposure associated with gestational age and neonatal anthropometric measures (including birth weight, birth length, head circumference and ponderal index*) after adjusting for potential covariates and confounders?

   *Ponderal index was used to estimate the nutritional status for the neonates in this study. Ponderal index = Weight (kg) / Height $(m)^3$.
3. Is magnitude of maternal blood lead levels have different effects on the birth outcomes?

CHAPTER 2

METHODOLOGY

2.1 Study area

The EMASAR study was conducted at Hospital Público Materno Infantil de Salta and its Obstetrics and Neonatology units and at Clínica San Jorge in Ushuaia. The hospital in the City of Salta is a public hospital and is responsible for all in-hospital deliveries in the city and is a referral hospital in Salta Province. While the Clínica San Jorge is one of the big private health care centers in Ushuaia, jointly responsible with another public hospital for the in-hospital deliveries in the city and surrounding areas.

The geographical location of the study sites are shown in the Figure 2. The City of Salta (24.78° S, 65.42° W) is the capital of Salta Province and is located in the Lerma Valley, northwest part of Argentina. The metropolitan area has a population about 647,796 inhabitants in 2019. Salta's present-day economy is based on diversified farming, lumbering, stock raising and mining, but relatively under-developed; poverty is a general feature and there are large socioeconomic inequalities [51].

Ushuaia (54.80° S, 68.30° W) has long been described as the southernmost city in the world and the capital of Tierra del Fuego, Antártida e Islas del Atlántico Sur Province. The city has a population around 75,658 people in 2019. Lumbering, sheep raising, fishing, trapping, and tourism are the city's principal economic activities.

2.2 Study design and study population

The EMASAR study is an observational study, with a cross-sectional design that was conducted to investigate maternal and fetal health risks related to food security and exposure to persistent toxic substances (PTS) in two regions of Argentina, Salta in the north and Ushuaia in the south. A total of 698 pregnant women (200 from Ushuaia and 498 from Salta) were consecutively enrolled in this study before or at admission to the delivery unit, between April 2011 and March

2012. The current study component is limited to 696 singleton subjects (198 from Ushuaia and 498 from Salta). Two cases from Ushuaia were excluded due to the twin pregnancies.

2.3 Data collection

Present study sample was derived from the EMASAR study. The data from EMASAR study includes questionnaires, clinical information and maternal blood samples.

Participants were examined and interviewed by the midwife and obstetrician. The data collected included: maternal age, previous children, socioeconomic factors (education, employment), environmental factors, lifestyle (use of tobacco) and dietary habit before and during pregnancy. The questions about diet pertained to the frequency of consumption (never or seldom, at least once a week and almost every day) of various food categories. Which include meat (red meat, poultry, processed or tinned meat); fish (fresh water fish, seafood, saltwater fish, tinned fish, smoked or processed fish); eggs; dairy products (milk, butter and cheese); fruits and vegetables (root, leafy or ground and others); carbohydrates (bread, cereals); fats and sugar; and fluids (fresh fruit juices, soft drinks and bottled water) [51].

Clinical obstetric data was based on hospital records. Information on the history of early pregnancy, as well as obstetric and neonatal data of current delivery were sought. The latter included the date of delivery, gestational age, birth weight, birth length and head circumference, gender of the newborns and any significant malformations.

Non-fasting maternal blood samples were obtained at 36±12 hours after the delivery. The usage of personal items before blood collection and the time of last meal and last cup of coffee, as well as the blood sampling and freezing time were recorded. Measuring the maternal height (cm) and weight (kg) after delivery, and try to be consistent with the time of blood sampling.

Whole blood was drawn from the maternal antecubital vein with standard equipment into a BD Vacutainer® for trace elements (HemogardTM/Royal Blue, Ref# 368381; plastic, 6-ml,

with 10.8 mg K_2 EDTA; Becton Dickinson, Plymouth, UK). The whole blood was transferred to 4.5 ml cryovials and were stored at minus 20 °C at the local hospital pending shipping to Norway. In Norway, the biological samples were stored in the EMASAR Biobank at the UiT, The Arctic University of Norway at minus 35 °C until analysis.

2.4 Chemical analysis and quality control

The chemical analyses for lead in the whole blood samples were performed using an Octapole Reaction System (ORS) Inductively Coupled Plasma Mass Spectrometer (7500ce, Agilent) equipped with an ASX-510 Autosampler (Cetac). Briefly, an aliquot of 0.3 mL of blood sample was diluted ten times with an alkaline solution containing Triton X-100 and ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) in a contamination free tube [56]. An aliquot of an internal standard solution containing Sc, Ga, Y and Gd was added. For calibration, the standard addition procedure was performed. Instrumental conditions were: Babington nebulizer, Scott-type spray chamber, reaction cell gas helium, isotopes monitored ^{206}Pb, ^{207}Pb, ^{208}Pb. Tuning of the instrument was made daily using a solution containing Li, Mg, Y, Ce, Tl and Co. Quantification on all isotopes was performed using one central point of the spectral peaks and three repetitions.

Analytical precision was 5% for Pb. Limits of detection for lead, calculated as three times the standard deviations of the blank sample. Lead was entirely detected in this study. A reference material Seronorm Trace Elements Whole Blood L-1 (SERO AS, Norway) was used to check the accuracy of the results every 12 samples.

2.5 Statistical analysis

Data were given as arithmetic means, standard deviations (SDs), median, minimum and maximum or proportion (%) for describing clinical and sociodemographic characteristics. Due to the non-normal distributions, Mann-Whitney U test was applied to compare the clinical data (quantitative variables) differences between Ushuaia and Salta. Chi-square test was used to compare the sociodemographic characteristics (categorical variables) differences between Ushuaia and Salta. Arithmetic means, geometric means with 95% confidence intervals (CI),

minimum, maximum and percentiles were used for the maternal blood lead levels descriptive analyses. Statistical difference of lead between Ushuaia and Salta was test for significance using Mann-Whitney U test. After log10 transformation, the blood lead concentrations maintained approximate normality according to the Q-Q plot. One extreme outlier (Q3+3*IQR, third quartile plus three times of interquartile range) of log10 transformed lead was detected in the Salta group. But sensitivity analyses allowed them to be included in further statistical analyses. Multivariate association was evaluated with multiple linear regression model to describe the relationships between lead exposure and related risk factors.

The participants' dietary intake frequency items were selected with multiple linear regression analyses using a stepwise approach [57]. Specifically, all these dietary items were introduced in the multiple linear regression model and applied an automatic procedure to selected the dietary intake variables by stepwise method in the regression. In each step, stepwise regression will exclude the weakest correlated variable, and criteria for inclusion and exclusion were based on the probability of F, namely p value < 0.05 and p value > 0.1, respectively. Finally, dietary items related to both maternal blood lead levels and any of the birth outcomes at $p < 0.05$ to be potential confounders and those only related to birth outcomes to be covariates [57]. Multiple linear regression models were also used to examine the effects of maternal blood lead levels on birth outcomes, i.e., gestational age, birth weight, birth length, head circumference and ponderal index. The potential covariates and confounders that were adjusted for in the regression models were selected based on the previous literature [29, 31, 32] or on their associations with maternal blood lead concentrations and or birth outcomes ($p < 0.05$) in this study. Generally, there were two kinds of models in multiple linear regression analyses, regression model I for each birth outcome was adjusted for residence area (Ushuaia and Salta), maternal age (continuous), parity (parity 1, multiparity), pre-pregnancy BMI (continuous), educational levels (primary, secondary, tertiary, university), smoking status(no, yes), gender of neonates (boy, girl). In addition, birth weight and birth length were added into gestational age regression model, and gestational age was introduced into birth weight, birth length, head circumference and ponderal index regression models [25]. Regression model II was additionally adjusted maternal dietary intake

frequency of items based on regression model I. To evaluate whether the associations between maternal lead exposure and neonatal birth outcomes differed by geographical location, stratified multiple regression analyses by area of residence were also conducted.

In order to evaluate the effects of maternal low level lead exposure on birth outcomes, several sensitivity analyses were conducted by excluding the 11(1.6%) participants with blood lead levels over 50 μg/L (intervention level of blood lead for pregnant women by CDC [45]), meanwhile, elimination of outliers (value that fall outside 3 standard deviations in multiple linear regression analysis) on the robustness of the associations between blood lead concentrations and birth outcomes.

Furthermore, logistic regression analysis was use to examine the associations between the quartile of maternal blood lead levels (≤ 10.03 μg/L; 10.04 μg/L to 13.40 μg/L; 13.41 μg/L to 18.51 μg/L; 18.52 to 152.31 μg/L) and binary outcomes including preterm birth (gestational age < 37 weeks) and low birth weight (birth weight < 2500 grams). The criteria for selecting and retaining confounders and covariates in the logistic regression was similar to those for linear regression. Preterm birth was introduced into low birth weight model and low birth weight was included in the preterm birth model [25]. Adjusted odds ratios and 95% confidence interval were used to report the relationships between quartile of maternal blood lead levels and preterm birth as well as low birth weight.

Complete case analysis was used for handling missing data, which means that participants with any missing data were excluded in the statistical analyses. Before inclusion in analysis, maternal blood lead levels were log10 transformed. A significance level of $p < 0.05$ (two tailed) was used for all analyses. Statistical analyses were carried out using the IBM SPSS Statistics for Windows (version 26; SPSS Inc., Chicago, IL, USA).

2.6 Ethical considerations

The study (#2010/7317) was approved by the Ethics Committee of the Salta Medical Association and the Ministries of Health in both provinces. The Norwegian Regional

Committee for Medical and Health Research Ethics (REC North) approved the study (#2011/706), and it was conducted in accordance with the Helsinki declaration. Written informed consent was obtained from all participants for any use of data in the study.

CHAPTER 3

RESULTS

3.1 General characteristics of the study population

The selected maternal and neonatal clinical and sociodemographic characteristics of the n = 696 individuals are presented in the Table 1 (quantitative variables) and Table 2 (categorical variables). The participants ranged in age from 14 to 45 years, with a mean (SD) age of 25.9 (6.6) years. The women in Ushuaia were 4 years older than the women from Salta on average (28.8 vs. 24.7 years). Women in Ushuaia were nearly average 5cm higher and 3kg heavier (pre-pregnancy) than the Salta mothers ($p < 0.001$), while participants from both sites share a similar pre-pregnancy BMI, namely 23.5 kg/m^2. During the pregnancy, women in Ushuaia gained 5.3 kg more body weight than those in Salta. The median gestational age was 39 weeks in both sites. The average number of children of these participants were around 2 but 43.5% women just experience one parity. Most of women living in urban area, with 91.4% and 86.7% women living in Ushuaia and Salta urban area respectively. Nevertheless, there was no statistically significant difference of smoking ratio among participants in both sites, women from Salta (41.2%) were exposed significantly higher ratio of passive smoking at home than the women in Ushuaia (28.3%), $p = 0.001$. In Ushuaia, 48.3% women had tertiary or university education background while this group only accounted for 10% in Salta. Similarly, 66% women in Ushuaia had a permanent job while just 17% women had permanent job in Salta. The proportion of women reporting using lead-containing materials in Salta was significantly higher than the those in Ushuaia (46.4% vs. 26.3%, $p < 0.05$).

The information about the gender for 677 neonates, 318 boys (47%) and 359 girls (53%), Table 2. The average birth weight, length and head circumference were 3319 grams, 48.8 cm and 34.5 cm, respectively in the overall sample. Detailed information about neonates in different sites can be found in Table 1. Although neonates from Ushuaia average weighed 90 grams more and 1cm longer than those from Salta, mean ponderal index in Ushuaia neonates was slightly lower than those in Salta.

Table 1 Health characteristics of the study population

Quantitative variables	Total (n = 696)			Ushuaia (n = 198)			Salta (n = 498)			*P* value*
	N (Missing)	Mean (SD)	Median (Min-Max)	N (Missing)	Mean (SD)	Median (Min–Max)	N (Missing)	Mean (SD)	Median (Min–Max)	
Maternal Age (years)	696 (0)	25.9 (6.6)	25.0 (14.4–44.5)	198 (0)	28.8 (6.6)	28.6 (15.9–44.5)	498 (0)	24.7 (6.2)	23.4 (14.4–44.2)	<0.001
Maternal Height (cm)	694 (2)	159.0 (6.2)	159.0 (140.0–181.0)	198 (0)	162.3 (6.0)	162.0 (147.0–181.0)	496 (2)	157.7 (5.8)	158.0 (140.0–176.0)	<0.001
Pre-pregnancy weight (kg)	639 (57)	59.5 (11.1)	58.0 (35.0–111.0)	190 (8)	61.9 (11.0)	60.0 (40.0–111.0)	449 (49)	58.5 (11.0)	57.0 (35.0–109.0)	<0.001
Postpartum weight (kg)	688 (8)	67.5 (11.8)	66.0 (40.0–120.0)	198 (0)	73.7 (11.0)	72.0 (50.0–120.0)	490 (8)	65.0 (11.2)	64.0 (40.0–111.0)	<0.001
Pre-pregnancy BMI (kg/m^2)	637 (59)	23.5 (4.1)	22.7 (14.8–40.8)	190 (8)	23.5 (4.1)	22.6 (16.1–40.8)	447 (51)	23.5 (4.2)	22.8 (14.8–39.6)	0.884
Postpartum BMI (kg/m^2)	687 (9)	26.7 (4.2)	26.2 (16.4–44.1)	198 (0)	28.0 (3.8)	27.5 (18.6–44.1)	489 (9)	26.1 (4.2)	25.5 (16.4–43.4)	<0.001
Total children born	696 (0)	2.1 (1.3)	2 (1–8)	198 (0)	1.9 (1.0)	2 (1–7)	498 (0)	2.2 (1.4)	2 (1–8)	0.244
Gestational age (weeks)	658 (38)	39.1 (1.0)	39.1 (32–42)	197 (1)	38.8 (1.3)	39.0 (32.0–41.0)	461 (37)	38.8 (1.3)	39.0 (33.0–42.0)	0.579
Birth weight (gram)	687 (9)	3318.6 (467.3)	3330.0 (1650.0–5200.0)	196 (2)	3383.2 (438.9)	3375.0 (2120.0–4500.0)	491 (7)	3292.8 (476.1)	3300.0 (1650.0–5200.0)	0.016
Birth length (cm)	688 (8)	48.8 (2.2)	49.0 (41.0–55.0)	196 (2)	49.7 (2.1)	50.0 (42.0–54.0)	492 (6)	48.5 (2.2)	49.0 (41.0–55.0)	<0.001
Head circumference (cm)	686 (10)	34.5 (1.4)	34.0 (28.0–40.0)	195 (3)	34.9 (1.5)	35.0 (31.0–40.0)	491 (7)	34.3 (1.4)	34.0 (28.0–38.0)	<0.001
Ponderal index (kg/m^3)	686 (10)	28.5 (2.8)	28.2 (12.6–42.1)	196 (2)	27.6 (2.7)	27.3 (20.3–42.1)	490 (8)	28.8 (2.8)	28.6 (12.6–38.5)	<0.001

* Mann-Whitney U test.

Table 2 Sociodemographic characteristics of the study population

Categorical variables	Category	Total (n = 696)		Ushuaia (n = 198)		Salta (n = 498)		*P* value*
		Count	Percentage %	Count	Percentage	Count	Percentage	
Parity	Para 1 / multiparity	303 / 393	43.5 / 56.5	82 / 116	41.4 / 58.6	221 / 277	44.4 / 55.6	0.477
Newborns gender	Boy / Girl / Missing data	318 / 359 / 19	45.7 / 51.6 / 2.7	96 / 102 / 0	48.5 / 51.5 / 0	222 / 257 / 19	44.6 / 51.6 / 3.8	0.612
Population density of residence	Urban / Semi – urban / Rural	613/50/33	88.1 / 7.2 / 4.7	181 / 15 / 2	91.4 / 7.6 / 1.0	432 / 35 / 31	86.7 / 7.0 / 6.2	0.014
Smoking	No / Yes	512 / 184	73.6 / 26.4	141 / 57	71.2 / 28.8	371 / 127	74.5 / 25.5	0.375
Home indoor smoking	No / Yes / Missing data	431 / 261 / 4	61.9 / 37.5 / 0.6	142 / 56/ 0	71.7 / 28.3 / 0	289 / 205/ 4	58.0 / 41.2 / 0.8	0.001
Education	Primary / Secondary / Tertiary / University / Missing data	168 / 379 / 96 / 52 / 1	24.1 / 54.5 / 13.8 / 7.5/ 0.1	7 / 95 / 56 / 40 / 0	3.5 / 48.0 / 28.3 / 20.2 / 0	161 / 284 / 40 / 12 / 1	32.3 / 57.0 / 8.0 / 2.4 / 0.2	<0.001
Permanent job	No / Yes / Missing data	476 / 216 / 4	68.4 / 31.0 / 0.6	68 / 130 / 0	34.3 / 65.7 / 0	408 / 86 / 4	81.9 / 17.3 / 0.8	<0.001
Marital status	Married / Divorced / Single / Living together	127 / 4 / 177 / 388	18.2 / 0.6 / 25.4 / 55.7	71 / 1 / 21 / 105	35.9 / 0.5 / 10.6 / 53.0	56 / 3/ 156 / 283	11.2 / 0.6 / 31.3 / 56.8	<0.001
Use of lead-containing materials	No / Yes / Do not know / Missing data	328 / 283 / 84 / 1	47.1 / 40.7 / 12.1 / 0.1	90 / 52 / 56 / 0	45.4 / 26.3 / 28.3 / 0	238 / 231 / 28 / 1	47.8 / 46.4 / 5.6 / 0.2	<0.001

* Chi-squared test.

3.2 Distribution of maternal blood lead levels

Maternal blood lead levels by area of regions are shown in Figure 3 and Figure 4. The geometric mean of lead level in maternal blood for entire samples was 13.9 μg/L (95% CI:13.4-14.5), with a range of 2.9-152.3 μg/L. The 25th, 50th and 75th percentiles of blood lead levels in the total sample were 10.03 μg/L, 13.40 μg/L, 18.51 μg/L respectively. Mann-Whitney U test indicated that mother living in Salta had significantly higher levels of blood lead than those living in Ushuaia, $p < 0.001$, with geometric mean blood lead levels of 15.8 μg/L and 10.1 μg/L, respectively. A total of 11 mother (1.6%) had blood lead levels higher than 50 μg/L (CDC recommended intervention level [45]) and all of them came from Salta (Data not shown). Supplementary Table S1 presents the specific concentrations of the blood lead in each group.

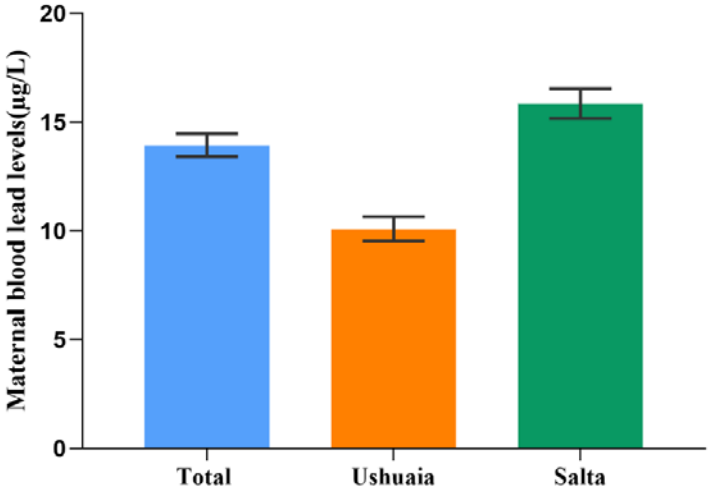

Figure 3 Geometric means of the maternal blood lead concentrations

The bars present the geometric means of blood lead levels (μg/L) with 95% confidence intervals in mothers from the entire sample and stratified by residence location (Ushuaia and Salta).

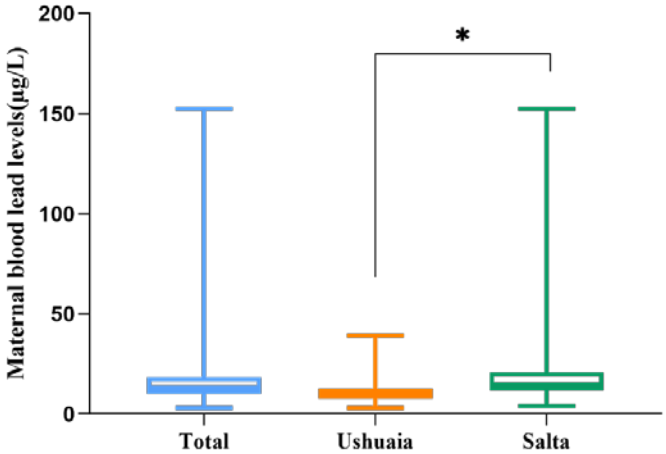

Figure 4 Distribution of maternal blood lead levels in Ushuaia and Salta

In each box plot, the median value is indicated by a horizontal line in the box. The vertical whiskers stop at minimum or maximum. $^{*}p < 0.001$, there was significant difference of maternal blood lead levels between Ushuaia and Salta in Mann-Whitney U test.

3.3 Associations between lead in blood serum and maternal characteristics

Multiple linear regression analysis of maternal sociodemographic and lifestyle characteristics and blood lead concentrations provided a relatively comprehensive description of the main maternal risk factors related with the blood lead levels (Table 3). As it mentioned above, blood lead levels in Salta women were significantly higher than those in Ushuaia. Area of residence was the main determinant for the lead exposure. The regression model showed the highest β coefficient for residence area (β = 0.41; 95% CI: 0.15-0.24; $p < 0.001$) among the determinant variables considered. Aside from residence area, maternal age was the main risk factor for lead levels (β = 0.11; 95% CI: 0.000-0.007; p = 0.031). Smoking was also influencing the lead concentrations. Lead levels in maternal blood were significant lower in women with tertiary education than those who only attended primary education (β = -0.10; 95% CI: -0.12 to -0.01; p = 0.037). Participants who live in rural areas had significant lower blood lead levels than those that live in urban areas (β = -0.09; 95% CI: -0.19 to -0.02; p = 0.016). There was no difference of blood lead levels between the women live in urban areas and semi-urban areas. Parity, pre-pregnancy BMI and permanent job were not show any significances associated with blood lead concentration in this model.

Table 3 Results of multiple linear regression analysis measuring effects of various determinants in maternal blood lead levels

Variable	Maternal blood lead levels[a]		
	n	β[b] (95%CI)	*p* value
Maternal age (years)	635	0.11 (0.000-0.007)	0.031
Parity (Para 1 / Multiparity)[c]	635	0.02 (-0.03 to 0.05)	0.630
Pre-pregnancy BMI (kg/m^2)	635	0.04 (-0.002 to 0.01)	0.363
Smoking (No / Yes)[d]	635	0.09 (0.01-0.08)	0.014
Permanent job (No / Yes)[e]	635	0.02 (-0.03 to 0.05)	0.661
Residence area (Ushuaia / Salta)[f]	635	0.41 (0.15-0.24)	<0.001
Education			
Primary education		Reference category	
Secondary education	635	-0.08 (-0.08 to -0.00)	0.097
Tertiary education	635	-0.10 (-0.12 to -0.01)	0.037
University education	635	-0.07 (-0.14 to 0.01)	0.103
Population density of residence			
Urban		Reference category	
Semi-urban	635	-0.00 (-0.07 to 0.06)	0.921
Rural	635	-0.09 (-0.19 to -0.02)	0.016

[a] Maternal blood lead levels were log10 transformed.
[b] β coefficients of the multiple linear regression model after standardizing all the variables.
[c] One parity as reference category.
[d] Women who do not smoke as reference category.
[e] Women who do not have permanent job as reference category.
[f] Ushuaia as reference category for residence area.

3.4 Maternal blood lead concentrations and birth outcomes

Table 4 shows the maternal dietary intake frequency of 24 items associated with maternal lead levels, gestational age and neonatal anthropometric characteristics. The dietary items that were significantly associated with birth outcomes were included as covariates in the further multiple linear regression analyses. In the present study, the blood lead levels were negatively associated with mother's frequency of dietary intake of smoked fish, tinned fish and processed fish during pregnancy. After stratified by the area of residence, these relationships were not observed in the Ushuaia sample, none of the dietary intake frequency items were significantly associated with blood lead concentrations in Ushuaia sample. Tinned fish still kept the negative significant association with lead levels in Salta sample, aside tinned fish, meat and vegetable leafy and ground also showed inverse associations with levels of blood lead in Salta delivering women. Supplementary Table S2 presents the details of dietary intake frequency of items during pregnancy in women from Ushuaia and Salta.

The possible associations between exposure to lead and gestational age or neonatal birth weight, birth length, head circumference and ponderal index have been examined by multiple regression analysis adjusting for potential covariates and confounders (Table 5). As shown in Table 5, -Model I, among the entire sample, there was significant positive relationship between log10-transformed maternal blood lead levels (logMBLLs) and gestational age (β = 0.08; 95% CI: 0.003-0.882; p = 0.049). However, the association between logMBLLs and gestational age was no longer significant after additionally adjusting for dietary intake frequency of items, as shown in Table5, Model II.

After stratified the residence, no significant associations between blood lead exposure and birth outcomes were observed in the Ushuaia sample (Table 5). However, in the Salta group of Model -I, logMBLLs were found significantly inversely associated with birth weight (β = -0.09; 95% CI: -409.24 to -13.26; p = 0.037) and birth length (β = -0.11; 95% CI: -1.95 to -0.21; p = 0.015). The statistically significant relationships between logMBLLs and gestational age, head circumference and ponderal index were not observed. After adding dietary intake frequency items in Salta group (Model II), the negative relationships still remained significant in birth weight and birth length models and the regression coefficient β for lead levels had slightly decline in the birth length model.

Supplementary Table S3 presents the results of several sensitivity analyses were conducted to evaluate the effects of low-level lead exposure on birth outcomes by excluding 11(1.6%) participants with blood lead levels over 50 μg/L, and elimination of outliers on the robustness of associations between blood lead levels and birth outcomes in the multiple linear regression analyses. These sensitivity analyses helped to confirm and support the previous stratified analyses results from Ushuaia and Salta, meanwhile, the associations were stronger in the sensitivity analysis models. In addition to previous analyses results, these sensitivity analyses also observed that logMBLLs were significantly negatively associated with birth weight and birth length in the overall sample, and positively associated with gestational age in Model I in Salta sample (Table S3).

Table 4 Dietary intake frequency related to blood lead levels and birth outcomes in multiple linear regression analyses

Total				Ushuaia				Salta			
Items	**n**	**β[a] (95%CI)**	**P value**	**Items**	**n**	**β[a] (95%CI)**	**p value**	**Items**	**n**	**β[a] (95%CI)**	**p value**
lead levels (μg/L)[b]	690								492		
Smoked fish		-0.21 (-0.14 to -0.03)	0.001	-				Tinned fish		-0.11 (-0.04 to -0.004)	0.018
Tinned fish		-0.10 (-0.04 to -0.01)	0.005					Meat		-0.11 (-0.07 to -0.01)	0.013
Processed fish		-0.16 (-0.12 to -0.02)	0.012					Vegetable leafy/ground		-0.10 (-0.09 to -0.01)	0.024
Gestational age (weeks)	653				197				456		
Dairy products		0.10 (0.04-0.28)	0.009	Fresh fruit juice		0.16 (0.04-0.50)	0.024	Dairy products		0.13 (0.05-0.31)	0.005
Tinned fish		-0.08 (-0.23 to -0.01)	0.038	Processed meat		-0.14(-0.53 to -0.00)	0.049	Tinned fish		-0.12 (-0.27, -0.03)	0.013
Birth weight (gram)	681								485		
Dairy products		0.10 (12.47-98.06)	0.011	-				Dairy products		0.11 (8.87-102.91)	0.020
Vegetables leafy/ ground		0.08(2.25-166.25)	0.044					Cereals		0.10 (17.45-235.46)	0.023
Birth length (cm)	683				196				487		
Processed fish		0.21 (0.57-1.28)	<0.001	Dairy products		0.15 (0.04-1.04)	0.036	Cereals		0.79 (0.28-1.29)	0.002
Cereals		0.11 (0.16-0.89)	0.005					Fats		-0.41 (-0.69 to -0.12)	0.005
Dairy products		0.11 (0.09-0.49)	0.006					Dairy products		0.26 (0.03-0.48)	0.025
Fats		-0.88 (-0.43 to -0.02)	0.032								
Head circumference (cm)	681				195						
Saltwater fish		0.18 (0.23-0.52)	<0.001	Saltwater fish		0.16 (0.04-0.80)	0.031	-			
Ponderal index (kg/m3)	681				196				485		
Processed fish		-0.22 (-1.68 to -0.81)	<0.001	Smoked fish		0.16 (0.19-2.75)	0.025	Tinned meat		0.12 (0.11-0.71)	0.007
Tinned meat		0.10 (0.08-0.65)	0.013	Butter/cheese		-0.15 (-1.10 to -0.051)	0.031				

[a] β coefficients of the multiple linear regression models after standardizing all the variables.

[b] Maternal blood lead levels were log10 transformed.

24 variables were introduced into every model including dietary intake frequency of meat, poultry, processed meat, tinned meat, eggs, fresh water fish, tinned fish, smoked fish, sea food, salt water fish, processed fish, vegetables root, vegetables leafy/ground, vegetables other, fruit, dairy products, butter and cheese, Fat (oil, margarine), cereals, bread, sugar, fresh fruit juice, soft drinks, bottled water. A stepwise approach was used, and the criteria for inclusion and exclusion were $p < 0.05$ and $p > 0.1$, respectively.

Dietary intake frequency was categorized as never or seldom, at least once a week and almost every day.

Table 5 Results of multiple linear regression models showing effects of blood lead levels in neonatal birth outcomes

	Outcomes	Lead levels (μg/L)[a] Total			Lead levels (μg/L)[a] Ushuaia			Lead levels (μg/L)[a] Salta		
		n	β[b] (95% CI)	*P* value	n	β[b] (95% CI)	*P* value	n	β[b] (95% CI)	*P* value
Model I	Gestational age (weeks)	585	0.08 (0.003-0.882)[c]	0.049	187	0.004 (-0.90 to 0.96)[e]	0.953	398	0.08 (-0.02 to 1.00)[e]	0.057
	Birth weight (gram)	586	-0.05 (-263.60 to 67.13)[d]	0.244	187	0.11 (-38.64 to 588.50)[f]	0.085	399	-0.09 (-409.24 to -13.26)[f]	0.037
	Birth length (cm)	587	-0.07 (-1.41 to 0.05)[d]	0.069	187	0.04 (-0.97 to 1.88)[f]	0.531	400	-0.11(-1.95 to -0.21)[f]	0.015
	Head circumference (cm)	585	-0.05 (-0.88 to 0.22)[d]	0.235	186	-0.06 (-1.72 to 0.66)[f]	0.38	399	-0.04 (-0.88 to 0.38)[f]	0.443
	Ponderal index (kg/m^3)	585	0.03 (-0.80 to 1.47)[d]	0.056	187	0.12 (-0.52 to 4.13) [f]	0.127	398	0.00 (-1.33 to 1.32)[f]	0.990
Model II	Gestational age (weeks)	581	0.07 (-0.06 to 0.83)[g]	0.090	187	-0.004 (-0.96 to 0.90)[l]	0.953	394	0.07 (-0.12 to 0.92)[p]	0.133
	Birth weight (gram)	581	-0.04(-258.58 to 73.91)[h]	0.276		-		394	-0.09 (-402.17 to -5.89)[q]	0.044
	Birth length (cm)	583	-0.07 (-1.38 to -0.07)[i]	0.078	187	0.03 (-1.02 to 1.79)[m]	0.587	396	-0.10 (-1.86 to -0.14)[r]	0.023
	Head circumference (cm)	581	-0.05 (-0.88 to 0.22)[j]	0.241	186	-0.06 (-1.69 to 0.66)[n]	0.386		-	
	Ponderal index (kg/m^3)	581	0.03 (-0.74 to 1.52)[k]	0.499	187	0.09 (-0.95 to 3.66)[o]	0.249	394	0.007 (-1.23 to 1.41)[s]	0.892

[a] Maternal blood lead levels were log10 transformed.
[b] β coefficients of the multiple linear regression models after standardizing all the variables.
[c] Adjusted for residence (Ushuaia and Salta), maternal age, parity, pre-pregnancy BMI, educational, smoking status, gender of neonates and newborn's birth weight and length.
[d] Adjusted for residence (Ushuaia and Salta), maternal age, parity, pre-pregnancy BMI, educational, smoking status, gender of neonates and gestational age.
[e] Adjusted for maternal age, parity, pre-pregnancy BMI, education, smoking status, gender of neonates and newborn's birth weight and length.
[f] Adjusted for maternal age, parity, pre-pregnancy BMI, education, smoking status, gender of neonates and gestational age.
[g] Adjusted for covariates list in *c* and for dairy products and tinned fish.
[h] Adjusted for covariates list in *d* and for dairy products and vegetables leafy / ground.
[i] Adjusted for covariates list in *d* and for processed fish, cereals, dairy products and fats.
[j] Adjusted for covariates list in *d* and for saltwater fish.
[k] Adjusted for covariates list in *d* and for processed fish and tinned meat.
[l] Adjusted for covariates list in *e* and for fresh fruit juice and processed meat.
[m] Adjusted for covariates list in *f* and for dairy products.
[n] Adjusted for covariates list in *f* and for saltwater fish.
[o] Adjusted for covariates list in *f* and for smoked fish, butter and cheese.
[p] Adjusted for covariates list in *e* and for dairy products and tinned fish.
[q] Adjusted for covariates list in *f* and for dairy products and cereals.
[r] Adjusted for covariates list in *f* and for cereals, fats and dairy products.
[s] Adjusted for covariates list in *f* and for tinned meat.

3.5 Relationships between quartile of blood lead levels and birth outcomes

Figure 5 presents the associations between the quartile of maternal blood lead levels and dichotomous outcomes: preterm birth (gestational age < 37weeks) and low birth weight (birth weight < 2500grams). There were in total 32 babies experienced preterm birth, among them 15 babies were delivered in Ushuaia and 17 babies were in Salta. 5 babies in Ushuaia had a low birth weight while 18 babies in Salta had low birth weight. No clear dose-response relationships between quartile of blood lead levels and preterm birth or low birth weight were evident in the adjusted logistic regression analyses.

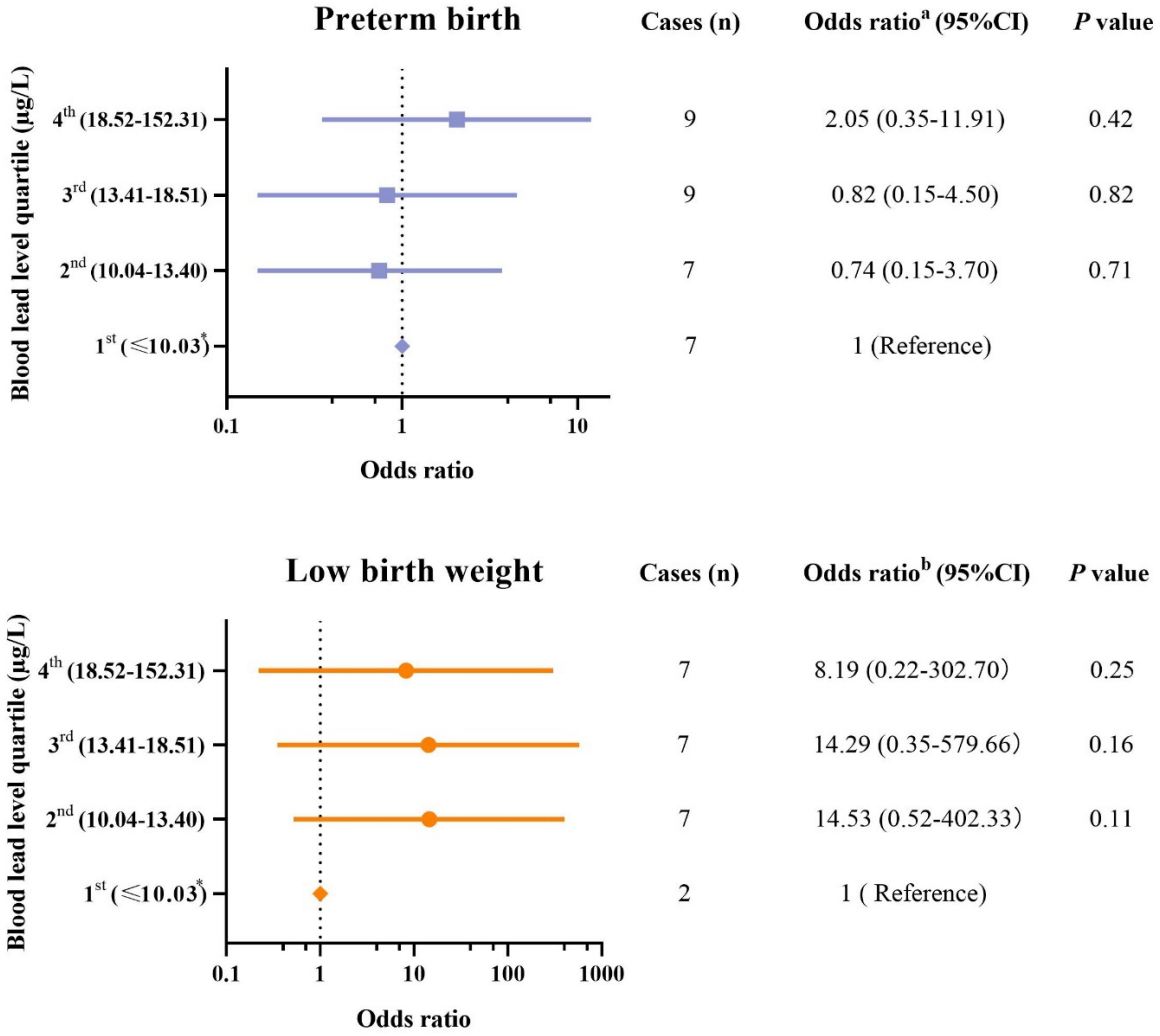

Figure 5 Results of logistic regression analyses showing the relationships between quartile of blood lead levels and preterm birth as well as low birth weight

Adjusted odds ratios plot for preterm birth and low birth weight. The horizontal bars plot the 95% confidence intervals. The quartiles of blood lead levels were untransformed. * The First quartile of maternal blood lead concentration (≤ 10.03 μg/L) as the reference category.

[a] Odds ratios (ORs) are estimated from logistic regression after adjustment for residence, maternal age, parity, pre-pregnancy BMI, smoking, education, gender of neonates and low birth weight.

[b] Odds ratios (ORs) are estimated from logistic regression after adjustment for residence, maternal age, parity, pre-pregnancy BMI, smoking, education, gender of neonates and preterm birth.

CHAPTER 4
DISCUSSION

This study, which stems from the ongoing EMASAR study in Argentina, showed a positive significant relationship between log10 transformed maternal blood lead levels and gestational age in the overall sample, besides, log10 transformed blood lead levels were inversely associated with birth weight and birth length in Salta sample, based on multiple linear regression analyses adjusting for possible confounding variables and covariates. The EMASAR was the first study to describe the body burden of persistent toxic substance (PTS) in Argentinian women after delivery [54], the present thesis expands upon EMASAR study in two regions of Argentina (Ushuaia and Salta city).

Descriptive analyses found distinct socioeconomic and anthropometrical differences between participants from Ushuaia and Salta as well as their neonates. Participants from Salta had higher blood lead levels than those mother from Ushuaia, correspondingly, neonates from Salta had lower average birth weight, birth length and head circumference than those from Ushuaia. Residence area, maternal age, smoking and education were the main risk factors related to blood lead levels. Logistic regression analysis revealed that there was no evidence to suggest a dose-response relationship between maternal blood lead levels and preterm birth or low birth weight.

4.1 Differences between Ushuaia and Salta dwellers

Blood lead levels were higher in women from Salta than in Ushuaia. This result was similar with those of previous EMASAR study [54] in which the concentrations of most of OCs were significantly higher in Salta than those in Ushuaia.

The social and economic gap between Salta and Ushuaia could be one of the explains for the elevated maternal blood lead level in Salta women. Salta is relatively underdeveloped while the socioeconomic conditions in Ushuaia are among the one of the most prosperous in Argentina [51]. Educational levels served as proxies for socioeconomic status of the samples in the present study. Lower maternal educational status is an indirect measure relatively poorer socioeconomic status. In the present study, maternal blood lead levels were statistically

significantly higher in mother with primary education than the mother attended tertiary education, meanwhile, 90% women in Salta only have primary or secondary education, this number was account for around 50% in Ushuaia. Lower maternal education level has been widely associated with higher risk for lead exposure. For example, the mean blood lead level of children in the low maternal education group was greater than that in the high maternal education group was found in a Korean children study as low socioeconomic status was a major risk factor for elevated blood lead levels through environmental factor [58].

Lead has been depicted to be a multimedia environmental pollutant owing to multitudinous and a variety of sources and pathways of potential exposure [58]. Lead released into the environment makes its way into air, soil, water and even inside our home. Prior or current industrial emissions might be another reason account for the difference of blood lead levels between Ushuaia and Salta. Because there are different industries development in Ushuaia and Salta. Lumbering, sheep raising, fishing, tapping, and tourism constitute the main economic activities in Ushuaia. Salta is one of the most promising mining provinces in Argentina. Uranium, lithium and silver are the main resources in Salta [59]. Lead is an element of the natural uranium radioactive decay series. Besides, currently lead is usually found in ore with zinc, silver and copper and it is extracted together with these metals. Lead contamination generally is higher in mining area than other areas where there is no specific of identified polluted source. The lead concentrations among pregnant women in present study revealed a lower blood lead level than those in the neighboring Bolivia [60]. This may be because the study in Bolivia was conducted in a specific mining district. There are many different mining operations that could responsible for releasing lead into the environment. Mining and ore processing are not always stored or disposed properly that lead toxins can seep into the soil and penetrate the water systems or release into the air. Although it was not clear that whether participants from Salta living in a mining area, there are the more mining activities in Salta than those in Ushuaia. Geographically, it seems likely that the mother in Salta were have a higher chance to expose to environmental contamination by industrial emission compare to those mothers live in Ushuaia.

It is acknowledged that nutritional benefits of fish consumption related to the high-quality protein, various vitamins (A, B_3, B_6, B_{12}, E, and D) and minerals (calcium, iron, selenium, zinc, etc.) that fish provide. Tinned fish, such as salmon and sardines that include bones, provide a significant amount of absorbable calcium. High demand for additional calcium during

pregnancy is recognized and the main storage for calcium are bones. Ettinger et al. found that calcium supplementation was associated with modest reduction in blood lead and it may constitute an important secondary prevention effort to reduce circulating maternal lead and fetal exposure [61]. Although the consumption of tinned fish, smoked fish and processed fish during pregnancy were negatively associated with maternal blood lead levels in the total sample in this study. Consuming fish seems to be a protective factor for elevating blood lead levels. However, there were only a small group of women ate tinned fish, smoked fish and processed fish in this study. Thus, these inverse relationships observed might by chance. Generally speaking, fish intake was low in Ushuaia and Salta in this study.

Notably, although fish is a good source of calcium and other important nutrients, the study from Arctic Monitoring and Assessment Program (AMAP) found that many of the marine mammals, some fish species can be highly contaminated with persistent bioaccumulate chemicals due to the wind, ocean currents and rivers transportation, resulting toxic metals like lead could enter the food chain [62]. Fish may be contaminated lead during fish growth, transportation, and storage. Tinned fish may contain extra lead compare to other fresh fish due to the production handling and canning process. Olmedo et al. assessed a variety of fish and shellfish species in fresh, canned and frozen products in Spain found that a significant number of shellfish and fish species were below the limit of detection and the highest lead levels corresponded to canned bivalve mollusks [63]. Fish contributed around 10-15% of Argentina diet [54]. One study conducted in Argentina revealed that high concentration of lead was found in some water bodies and in the muscle of silverside (a native fish species of Argentina) [64]. Some other studies conducted in Argentina also suggested that freshwater species contain metal contaminants and toxic PTS due to pollution of rivers [65, 66]. Such sources would probably affect the inland residence of Salta more.

4.2 Risk factors for lead exposure among pregnant women

Maternal age. The observed significant positive association between age and maternal blood lead concentrations in this study was in agreement with previous studies [67-69]. This may be related to the older people may have been exposure to more lead on average in their life compare to those young people. Besides, lead mobilization from bone to blood could also account for age related elevated blood lead levels [68, 69]. Furthermore, it has been reported that lead can long term store in bone, with half-life ≥ 10 years [70], accumulative body burden of lead

increase over time.

***Smoking and alcohol consumption*.** Smoking was found elevated blood lead levels in this study. Active and passive smoking both with increased blood lead concentrations during pregnancy, as has been found previously [71-73]. It is known that tobacco has a high lead content as the plants absorb lead from the soil, which is reflected in the smoke produced by cigarettes [74]. This represents an important factor in prenatal lead exposure for fetuses and neonates. Earlier epidemiological studies suggested that alcohol consumption, especially of wines, have been reported as a source of lead intake and as a confounding factor in the apparent relationship between smoking and blood lead levels [31, 75]. A survey study conducted by Smart et al. in 1990s found that wine may contain high levels of lead possibly because the contamination from fittings used in manufacturing and processing at that time. However, a recent study examined lead concentrations of wine in Argentina found that lead levels from wine samples were lower than the upper established by the Office International de la Vingne et du Vin [76]. Towle et al. also found that lead content from 18 country's wine sample does not pose a health risk to adult wine consumers [77]. Furthermore, smoking as well as alcohol consumption have long been recognized as the risk factors for low birth weight or preterm birth [78-80]. However, present study lack of information about alcohol consumption, this might weaken the results somewhat.

BMI. BMI prior to pregnancy is an important indicator of maternal nutritional status and also closely related with birth outcomes and maternal health. No association was detected between pre-pregnancy BMI and blood lead levels in present study. This was consistent with a study in neighboring Chile that no correlation between BMI and blood lead levels in women was found [81]. However, SPECT-China study suggested that blood lead levels were positively associated with BMI in women but this association was not found in man samples [82]. From the data of National Health and Nutrition Examination Survey in 1999-2006 among children, adolescents, and adults in the U.S. population, blood lead levels were associated with a decreased BMI in adults [83]. Lead is considered to be one of endocrine disrupting chemicals (EDCs), which may be associated with hypothalamic-pituitary-adrenal axis function, thyroid hormones and bone metabolism [82, 84]. Some epidemiological studies found that EDCs were associated with obesity [85, 86]. Further investigation of its relationship between blood lead levels and BMI should be conducted in larger studies in larger group.

***Parity*.** Parity was not significantly associated with blood lead levels in this study. A recent

report by Ugwuja et al. suggested that elevated blood lead levels were found in parity above 3 times [68]. A possible explanation for this may be found in the speculation by Rothenburg et al., which the changes in the placental structure with each pregnancy would alter the blood flow and enable a greater transfer of lead with increasing parity [74, 87]. However, some studies suggested that increased parity seems to protect against increased lead level in pregnancy [69, 74, 88]. This was because if bone lead is mobilized during pregnancy [26, 28], it is expected that the multiparous women would have lower blood lead levels since previous pregnancy would have depleted the bone lead stores [68].

Urban vs. rural. Present study suggests that maternal blood lead levels in urban setting were significantly higher than those live in rural area and no significant relationship was observed between urban and semi-urban setting. Conversely, a study conducted in Iowa, the USA by Carrel et al. found that there were no differences in blood lead levels of newborns between urban and rural maternal residence but the highest newborn blood lead level was found in the urban site [89]. However, Martínez et al. observed that blood lead levels of children were higher in suburban areas than in those live in urban areas in Córdoba, Argentina [8]. This inconsistency results of blood lead levels in different residence settings from various studies implied that the same risk factors for lead exposure apply regardless of population density [89]. 92% population live in urban areas in Argentina [49] and there was 88% of participants live in urban in this study. Despite a low population density in semi-urban or rural areas, we should not overlook the potential hazard exposure among those populations.

4.3 Effects of maternal lead exposure on birth outcomes

Despite the fact that blood lead levels in pregnant women have been decreasing over the previous decades, there is no safe level of lead, adverse health effects of even small amounts of lead exposure in pregnancy remain concerning [41]. Present study provided evidence for associations between maternal blood lead levels and birth outcomes. The log10 transformed maternal blood lead levels were positively associated with gestational age in the overall sample in present study. Significantly higher blood lead levels were reported in women in their third trimester than those in first trimester of pregnancy [90]. Hansen et al. reported in the MISA study that the sort ascending by blood lead levels during pregnancy and subsequently was second trimester < 3 days postpartum < 6 weeks postpartum [70]. Maternal physiological and biochemical changes occur during pregnancy for the development of the uterus and fetus and

the preparation for labor and lactation. Pregnant women will undergo significant changes during and after pregnancy, which include the expansion of blood volume, plasma and erythrocytes; enhanced metabolism of glucose, lipid, protein and calcium; as well as changes in skeletal, bone density and endocrine and so forth [91]. It is plausible that the blood lead levels occur changes during gestational and postpartum periods. Many studies have showed that the association between blood lead levels and calcium needs, as lead can mimic or compete with calcium in biological processes [28, 72]. The peak demand for calcium is in the third trimester, maternal response to meet this demand may allow calcium resorption bone, especially when dietary calcium intake is inadequate [72, 91]. Thus, pregnant women who were exposed to lead prior to pregnancy may mobilize lead from bone to blood, increasing maternal blood lead levels in late pregnancy. However, West et al. found inverse correlation between maternal levels of lead and gestational age in African American pregnant women [17]. Jelliffe-Pawlowski et al. reported that among women with blood lead levels above 100 μg/L, each unit increase in lead level was associated with an average 0.3 day decrease in gestational age [25]. In a cross sectional study in Nigeria, no statistically significant association between maternal blood lead levels and gestational age was observed [92]. A review by Andrews et al. suggested that prenatal lead exposure is unlikely to increase the risk of premature rupture of fetal membranes but does appear to increase the risk of preterm delivery and it is also not clear that prenatal lead exposure will reduce the gestational age of the infants [93]. There have been contradictory reports on the effects of maternal blood lead levels on gestational age during pregnancy, further studies are necessary to clarify this effect.

The log10 transformed blood lead levels were inversely associated with birth weight and birth length only in Salta sample. Numerous previous studies have reported similar relationships [29-33, 94-96]. Potential mechanisms of maternal lead exposure could have adverse effects on birth outcomes have been revealed. One possible explanation is lead could impair normal growth of fetal bone due to the competition with calcium and deposited into fetal bone, this is because lead and calcium share a similar intracellular metabolic pathway, and calcium is the indispensable element for fetal growth [28]. Lead readily cross the placenta and to accumulate in fetal tissues which would compete with calcium resulting fetal growth impairment. Hernandez-Avila et al. suggested that lead may reduce size at birth by reducing circulating levels of thyroid hormones, serum thyroxin is an important predictor of birth weight and fetal growth [97]. In addition, accumulation of lead in placenta may cause dysfunction placenta activities, resulting in impair the transportation of nutrients for the fetal growth [95].

Therefore, negative effects of lead exposure on neonatal anthropometric parameters are biologically plausible. However, other studies found no associations between prenatal exposure and birth outcomes [34, 35].Thus, findings in present study need to be confirmed by further studies based on larger sample groups.

When dietary intake status was further adjusted in multiple linear regression model in the overall sample, the relationship between maternal lead levels and gestational age did not hold significance. This change may due to the effects of the dietary intake of the mother on the gestational age. This indicated that inclusion of confounders or covariates in regression models may influence the effects of lead exposure. Dietary intake frequency of items may be sensitive to the association between blood lead levels and gestational age.

4.4 Effects of magnitude of blood lead levels on birth outcomes

The evidence that relationships between quartile of maternal blood lead levels associated with preterm birth or low birth weight were not observed in this study. These findings were similar with the study reported by Taylor et al. that no superlinear dose-response relationship between maternal blood levels and birth outcomes in the ALSPAC study [32]. In a study conducted in New York, Zhu et al. reported no clear dose-response was observed between quartile of lead levels and preterm birth while low level of lead was associated with small risk of decreased birth weight with a superalinear dose-response relationship [30]. However, Zhang et al. reported that that higher blood lead exposure was associated with an elevated risk of preterm low birth weight in China [96]. In a study conducted in Iran suggested that 1 unit increase in blood lead levels led to an increased risk of preterm birth (OR = 1.41, 95% CI: 1.08-1.84) [98]. Although no clear dose-response relationship was observed in this present study, elevated maternal blood lead levels would be expected to have adverse effects on fetal growth have been witness by other scientific studies, as there is no safe threshold for blood lead level, blood lead concentration should be kept as low as possible, especially during pregnancy to minimize adverse outcomes.

4.5 Maternal blood lead levels compared with other relevant studies

Table 6 shows a comparison of maternal blood lead levels from the two study sites in present study and previously published maternal blood or umbilical cord blood lead concentrations from non-occupationally exposed populations worldwide. The observed concentrations in

present study had a similar levels of blood lead compared to other studies in some countries varying degrees of industrial and socioeconomic development, such as Japan [94], the USA [99], Colombia [100] and South Africa [101]. The lead levels in maternal serum in Ushuaia and Salta were higher than those reported in Australia [102] and lower than in Mexico [103], China [29] and Bolivia [60]. Besides, compared with a study conducted in La Plata, Argentina [53], geometric means of maternal blood lead concentrations in present study were lower than the geometric mean of neonatal umbilical cord blood level reported in that study. Although only cord blood levels were reported in that study, as it indicated by other studies that there was a strong correlation between maternal blood lead and umbilical cord lead [31, 92]. Generally, the observed maternal blood concentrations for Ushuaia and Salta study participants were in the middle to lower range compared with other regions of the world.

Table 6 Comparison of blood lead levels from the present study and previous studies

References	Place	Sampling years	N	Samples source	Lead levels (μg/L)*	Range (μg/L)
Present study	Ushuaia	2011-2012	198	Maternal blood samples were collected at 36±12 hours after delivery	10.1[a]	2.9-39.1
Present study	Salta	2011-2012	498	Maternal blood samples were collected at 36±12 hours after delivery	15.8[a]	3.9-152.3
La-Llave-Leon et al. (2017) [103]	Mexico	2014-2016	633	Maternal blood samples were collected during prenatal health care	20.9[b]	4.8-268.5
Nishioka et al. (2014) [94]	Japan	2010-2013	506	Maternal blood samples were collected at 36 weeks of pregnancy	9.9[b]	< 0.9-39.6
Martins et al. (2013) [53]	Argentina	2010-2011	159	Umbilical cord blood samples were collected at delivery	21[a]	< 16.0-71.0
Xie et al. (2013) [29]	China	2010-2011	252	Maternal blood samples were obtained during admission to the hospital in preparation for delivery (within 3 days before delivery)	35.3[b]	10.0-119.1
Sanders et al. (2012) [99]	USA	2009-2011	211	Maternal blood samples were collected in the third trimester of pregnancy	8.9[a]	1.9-77.2
Suarez-Ortegon et al. (2013) [100]	Colombia	2009-2010	357	Maternal blood samples were collected in the third trimester of pregnancy	9.5[b]	0.01-21.7
Rollin et al. (2017) [101]	South Africa	2008-2013	640	Maternal blood samples were collected in pre-partum	13.2[a]	0.4-317.0
Hinwood et al. (2013) [102]	Australia	2008-2011	172	Maternal blood samples were collected approximately 2 weeks prior to delivery	5.02[b]	< 0.5-25.5
Barbieri et al.(2016) [60]	Bolivia	2007-2008	419	Maternal blood samples collected during pregnancy	26.5[a]	4.38-801.6

* Blood lead levels reported by each author: [a] geometric mean; [b] arithmetic mean.

4.6 Strengths and limitations

***Strengths*:** There are few limited data in relation to effects of lead exposure on birth outcomes in Argentina. The findings in the present study address the knowledge gap of maternal lead exposure in Argentina and provide theoretical basis for relevant further studies of others. The study conducted in two different geographical locations, the findings could provide evidence to compare lead exposure of the representative two groups of people in Argentina - Ushuaia features the most southern towns and settlements in the world while Salta situated at the foothills of Andes mountains in the north. Besides, demographic, socioeconomic, and lifestyle characteristics that differ between coastal inhabitants and high-altitude populations in Argentina offer novel insights in this area of maternal environmental health. Furthermore, method validation and analytical approaches employed in AMAP studies and the laboratory participated successfully in the most recent AMAP Analytical Ring Test [54].

***Limitations*:** Despite the strengths, present study also has some limitations. (1) Complete case analyses were applied to deal with missing data but it reduced sample size and were perceived to lead to reduced statistical efficiency of estimations while increasing the potential for bias [104]. (2) Maternal blood samples were only measure once after the delivery that may not accurately reflected lead exposure burden during the pregnancy and after postpartum. Previous studies have reported U-shaped curve in maternal blood lead concentrations during the pregnancy, for example, Nishioka et al. found that blood lead levels were decreased at 25 weeks and then increased at 36 weeks of gestation [94]. MISA study found that blood lead concentrations increased during the pregnancy and from birth to 6 weeks postpartum [70]. Therefore, it might require to analyze the associations between blood lead levels in different trimester during pregnancy as well as after postpartum. (3) Umbilical cord blood samples were not collected in original EMASAR study that the lead exposure on neonates was not assess in present study. Although a significant association of maternal blood lead levels and umbilical cord blood lead concentrations was proved in substantial studies [31, 92], measuring umbilical cord blood could provide a more accurate picture of the redistribution of lead between mother and neonates. (4) Even though the multiple regressions were performed, there were still

potential confounders or covariates were not adjusted due to the lack of availability from original study. For instance, socioeconomic characteristics such as income and occupational status; calcium and iron supplements intake and alcohol consumption were not assessed in present study. (5) Previous papers of EMASAR study found the body burden of OCs and associations between OCs and maternal BMI, parity and gestational age [54, 105]. Thus, the possibility of the findings might be confounded by other toxins or risk factors which were not include into this study. (6) Genetic information could be associated with birth outcomes was not include in the present study [106]. (7) The data of maternal dietary intake frequency of various food were somewhat rough and not adequately reflected the nutrients or minerals that participants absorbed during pregnancy. Recall bias was inevitable. Also, response bias was unavoidable as the ability of participants to answer the questionnaire might depending on their expectations of the correctness.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

In summary, the results of the present study provide useful information concerning the blood lead levels among the delivering women, for which few reference values are available in Argentina. The residence was one of the main determinants for the blood lead levels, involving higher blood lead levels in those living in Salta than those living in Ushuaia. This difference can be inferred to reflect differences in socioeconomic conditions, prior or current industry emissions and potential contributors of regional pollution. Other maternal characteristics such as age, smoking and education are also main determinants of the concentrations of blood lead. Maternal blood lead exposure showed a positive association with gestational age in the overall sample. Inversely, even maternal low-level lead exposure may adversely affect birth weight and birth length in Salta sample. No significant associations between blood lead levels and birth outcomes were observed in Ushuaia sample. There is no evidence to suggest clear dose-response relationships between blood lead levels and preterm birth, and low birth weight. As there is no safe threshold for lead exposure, blood lead level should be kept as low as possible especially during pregnancy to minimize undesirable outcomes. Birth outcomes are multifactorial problems, further studies may be required to confirm the findings of the present study.

5.2 Recommendations

- It is recommended that health authorities and policy makers identified the risk factors as a screening tool for lead exposure in pregnant women.
- It is recommended health authorities and policy makers to reassess the reference value of blood lead in pregnant women. In accordance with CDC's recommended schedules, pregnant women with blood lead levels of 50 µg/L or higher source of lead exposure should be identified and women should receive follow-up activities and interventions regard to avoid further exposure and receive specific nutritional recommendations to decrease blood

lead levels [45]. However, in present study suggests that even a very low level of lead exposure (< 50 µg/L) is risk factor for reduced birth weight and birth length.

- It is recommended women quit smoking and avoid passive smoking during the pregnancy to reduce the risk of restricted fetal growth.
- It is recommended that ban the lead containing materials for repairing home.
- Women should encourage to eat iron, calcium and vitamin rich food during pregnancy.

These recommendations could help to achieve one of the targets in United Nation SDGs, that is substantially reduce the number of deaths and illnesses from hazardous chemicals and air, water and soil pollution and contamination by 2030 to help people obtain good health and well-being.

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Appendices: Supplementary Materials

Appendix 1: Table S1 Details of blood lead levels in the population of study (µg/L)

Statistics	Total (n = 696)	Ushuaia (n = 198)	Salta (n = 498)	P value[c]
AM (SD)[a]	16.1 (11.2)	10.9 (4.7)	18.2 (12.4)	< 0.001
GM (95%CI)[b]	13.9 (13.4-14.5)	10.1 (9.5-10.7)	15.8 (15.2-16.5)	
Minimum	2.9	2.9	3.9	
Maximum	152.3	39.1	152.3	
Percentile				
25th	10.0	7.6	11.8	
50th	13.4	9.8	15.0	
75th	18.5	13.0	20.9	
95th	34.2	20.0	37.4	

[a] Arithmetic mean

[b] Geometric mean with 95% confidence interval.

[c] Mann-Whitney U test.

Appendix 2: Table S2 Maternal intake frequency of dietary items during pregnancy

Dietary items	Total (n = 690)			Ushuaia (n = 198)			Salta (n = 492)		
	Never / Seldom (%)	At least once a week (%)	Almost every day (%)	Never / Seldom (%)	At least once a week (%)	Almost every day (%)	Never / Seldom (%)	At least once a week (%)	Almost every day (%)
Meat	6.6	19.6	73.8	8.0	25.3	66.7	6.1	17.3	76.6
Poultry	5.8	31.2	63.0	5.6	38.9	55.6	5.9	28.0	66.1
Processed meat	49.4	30.3	20.3	62.6	25.8	11.6	44.1	32.1	23.8
Tinned meat	87.1	9.4	3.5	91.9	6.1	2.0	85.1	10.8	4.1
Fresh water fish	78.3	13.9	7.8	82.3	12.1	5.6	76.6	14.6	8.7
Sea food	94.1	4.6	1.3	81.8	14.1	4.0	99.0	0.8	0.2
Saltwater fish	89.3	9.4	1.3	69.7	26.8	3.5	97.2	2.4	0.4
Tinned fish	67.5	25.1	7.4	67.2	27.8	5.1	67.7	24.0	8.3
Smoked fish	97.7	1.9	0.4	93.9	5.1	1.0	99.2	0.6	0.2
Processed fish	99.2	0.4	0.3	97.5	1.5	1.0	100.0	-	-
Eggs	17.8	27.1	55.1	34.3	30.8	34.8	11.2	25.6	63.2
Dairy products	13.5	17.8	68.7	6.1	18.7	75.3	16.5	17.5	66.1
Butter and cheese	13.6	25.9	60.4	14.1	27.3	58.6	13.4	25.4	61.2
Vegetables root	1.3	4.2	94.5	1.5	7.6	90.9	1.2	2.8	95.9
Vegetables leafy / ground	2.4	9.3	88.3	1.5	14.6	83.8	2.8	7.1	90.0
Other vegetables	4.1	13.3	82.6	5.6	19.7	74.7	3.4	10.8	85.8
Fruits	8.1	6.2	85.7	8.1	12.1	79.8	8.1	3.9	88.0
Bread	10.7	8.4	80.9	29.8	24.2	46.0	3.0	2.0	94.9
Cereals	2.9	8.0	89.1	5.6	19.7	74.7	1.8	3.3	94.9
Fats	22.0	18.6	59.4	50.5	11.6	37.9	10.6	21.3	68.1
Sugar	16.2	6.8	77.0	37.9	19.7	42.4	7.5	1.6	90.9
Fresh fruit juices	32.9	20.1	47.0	43.9	31.8	24.2	28.4	15.4	56.1
Soft drinks	37.4	24.6	38.0	50.0	26.8	23.2	32.3	23.8	43.9
Bottled water	48.5	13.5	38.0	19.7	26.3	54.0	60.2	8.3	31.5

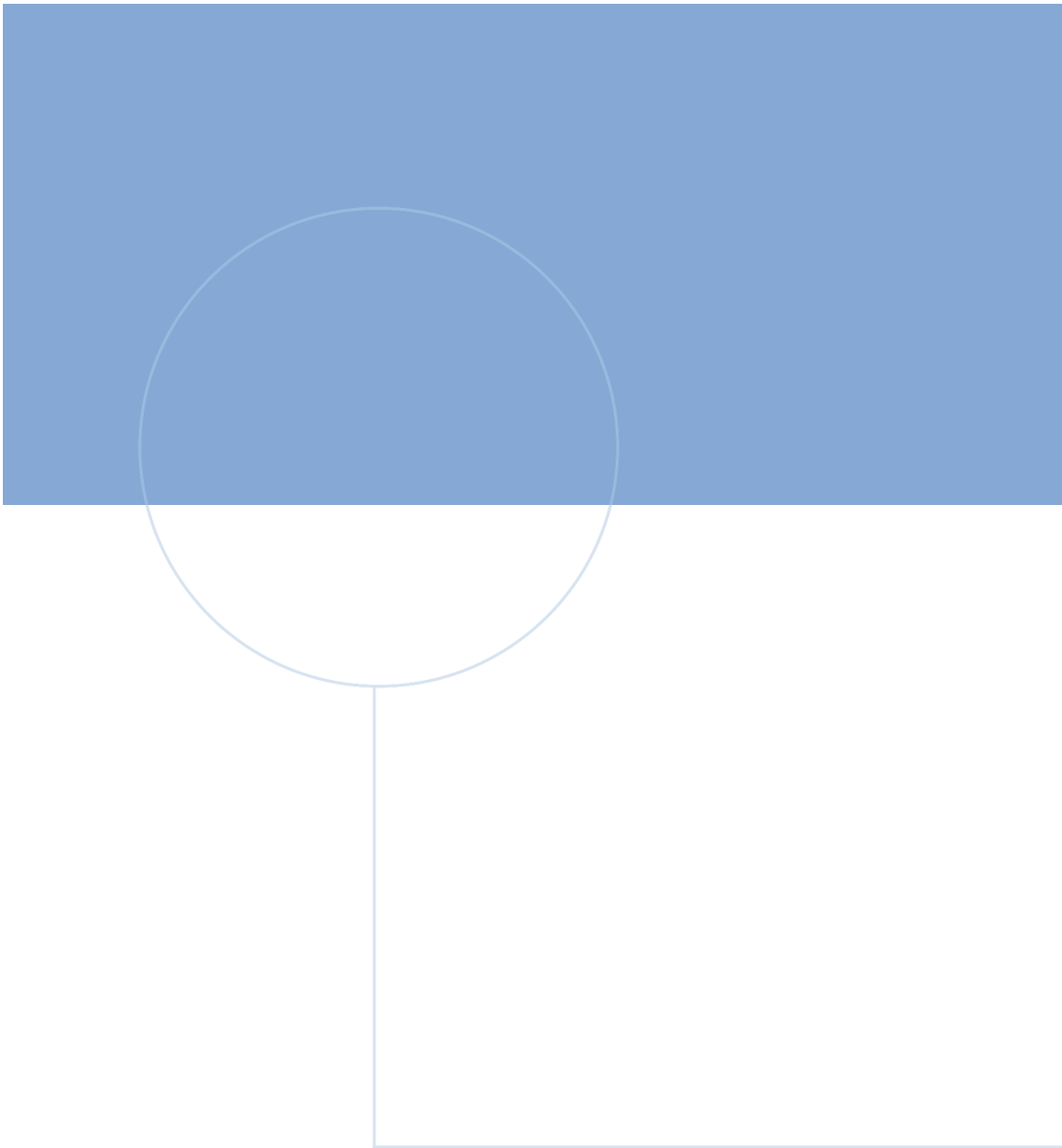
* There were 6 participants with dietary intake frequency data missing and all of them came from Salta group.

Appendix 3: Table S3 Results of sensitivity analyses

Several sensitivity analyses showing effects of maternal low-level lead exposure in birth outcomes after exclusion of blood lead levels above 50 µg/L and elimination of outliers on the robustness of association between blood lead levels and birth outcomes in the multiple linear regression analyses.

	Outcomes	Lead levels (µg/L)[a] Total			Lead levels (µg/L)[a] Ushuaia			Lead levels (µg/L)[a] Salta		
		n	β[b] (95% CI)	*P* value	n	β[b] (95% CI)	*P* value	n	β[b] (95% CI)	*P* value
Model I	Gestational age (weeks)	572	0.10 (0.12-1.04)[c]	0.014	185	0.01 (-0.79 to 0.97)[e]	0.842	386	0.09 (0.01-1.13)[e]	0.047
	Birth weight (gram)	571	-0.09 (-369.97 to -36.80)[d]	0.017	185	0.09 (-75.55 to 537.35)[f]	0.139	387	-0.13 (-526.85 to -120.55)[f]	0.002
	Birth length (cm)	573	-0.08 (-1.58 to -0.08)[d]	0.030	185	0.06 (-0.65 to 1.97)[f]	0.321	389	-0.13 (-2.33 to -0.46)[f]	0.004
	Head circumference (cm)	574	-0.06 (-1.01 to 0.15)[d]	0.143	184	-0.04 (-1.50 to 0.79)[f]	0.544	389	-0.06 (-1.01 to 0.27)[f]	0.236
	Ponderal index (kg/m^3)	567	-0.40 (-1.59 to 0.60)[d]	0.376	182	-0.04 (-2.26 to 1.32)[f]	0.603	386	0.02 (-1.73 to 1.06)[f]	0.636
Model II	Gestational age (weeks)	568	0.09 (0.05-0.99)[g]	0.031	185	0.002 (-0.87 to 0.90)[l]	0.975	383	0.07 (-0.14 to 1.01)[p]	0.134
	Birth weight (gram)	566	-0.09 (-362.61 to -27.47)[h]	0.023		-		382	-0.13 (-511.87 to -105.31)[q]	0.003
	Birth length (cm)	570	-0.08 (-1.55 to -0.04)[i]	0.039	185	0.06 (-0.70 to 1.91)[m]	0.360	386	-0.12 (-2.34 to -0.45)[r]	0.004
	Head circumference (cm)	570	-0.06 (-1.00 to 0.15)[j]	0.147	185	-0.05 (-1.57 to 0.73)[n]	0.473		-	
	Ponderal index (kg/m^3)	564	0.03 (-1.54 to 0.68)[k]	0.448	182	-0.05 (-2.40 to 1.20)[o]	0.508	384	-0.02 (-1.69 to 1.14)[s]	0.703

[a] Maternal blood lead levels were log10 transformed.
[b] β coefficients of the multiple regression models after standardizing all the variables.
[c] Adjusted for residence area (Ushuaia and Salta), maternal age, parity, pre-pregnancy BMI, educational, smoking status, gender of neonates and newborn's birth weight and length.
[d] Adjusted for residence area (Ushuaia and Salta), maternal age, parity, pre-pregnancy BMI, educational, smoking status, gender of neonates and gestational age.
[e] Adjusted for maternal age, parity, pre-pregnancy BMI, educational levels, smoking status, gender of neonates and newborn's birth weight and length.
[f] Adjusted for maternal age, parity, pre-pregnancy BMI, educational levels, smoking status, gender of neonates and gestational age.
[g] Adjusted for covariates list in *c* and for dairy products and tinned fish.
[h] Adjusted for covariates list in *d* and for dairy products and vegetables leafy / ground.
[i] Adjusted for covariates list in *d* and for processed fish, cereals, dairy products and fats.
[j] Adjusted for covariates list in *d* and for saltwater fish.
[k] Adjusted for covariates list in *d* and for processed fish and tinned meat.
[l] Adjusted for covariates list in *e* and for fresh fruit juice and processed meat.
[m] Adjusted for covariates list in *f* and for dairy products.
[n] Adjusted for covariates list in *f* and for saltwater fish.
[o] Adjusted for covariates list in *f* and for smoked fish, butter and cheese.
[p] Adjusted for covariates list in *e* and for dairy products and tinned fish.
[q] Adjusted for covariates list in *f* and for dairy products and cereals.
[r] Adjusted for covariates list in *f* and for cereals, fats and dairy products.
[s] Adjusted for covariates list in *f* and for tinned meat.



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