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Arsenic exposure determinants on toddlers in Arica, Chile

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An abstract of
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Abstract

Arsenic exposure determinants on toddlers in Arica, Chile

By Maureen Hitschfeld Bushell

Inorganic arsenic and arsenic compounds are considered class 1 chronic non-threshold carcinogenic to humans. Long-term oral exposure to arsenic is associated with several multi-systemic health conditions. Furthermore, in utero and early-life exposures to arsenic have been linked to increased risks for several types of cancer and other diseases during adulthood. Understanding sources of early exposure is crucial to prevent detrimental health effects. Our goal was to determine whether breast milk protects toddlers from arsenic exposure and whether formula, rice consumption, drinking tap water or other dietary and environmental factors contribute to arsenic exposure in toddlers in Arica, Chile. For this secondary analysis study, we used data from a subgroup of 192 subjects from a recently completed cohort study to examine the relationship between determinants in the last 48 hours followed by urinary arsenic speciated concentration. Multivariate models were developed for each arsenic specie: arsenobetaine (AsB), arsenite (AsIII), arsenate (AsV), dimethylarsinic (DMA), monomethylarsonic (MMA) and total arsenic (excluding AsB). Our study suggests that drinking 6 or more breast milk portions protects toddlers from arsenic exposure in this population. Further, our study confirms formula, rice consumption and drinking tap water as potential source of arsenic exposure in this population and hints to other dietary and environmental modulators.

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Table of contents

Introduction

Arsenic (As) presence in the environment is due to natural and anthropogenic causes. It is widely and variably distributed throughout the environment in the air, water, and land in both inorganic, reduced arsenite (trivalent, As III) and oxidized arsenate (pentavalent, AsV), and organic forms (e.g. monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), and arsenocholine (AsC)). In the body, AsV is reduced to AsIII, which is then oxidatively methylated to MMA and DMA, with subsequent excretion primarily in the urine. Inorganic arsenic (iAs) metabolites have elimination half-lives of approximately 2-4 days (NRC, 2001). Inorganic forms of As and its metabolites are considered toxic in the following general order DMA(III)>MMA(III)> AsIII > AsV > MMA(V) > DMA(V), while AsB and AsC are considered nontoxic (Hughes, 2002; Mass et al., 2001; NRC, 2001; Yehiayan et al., 2009). The distribution of arsenic in human urine is generally 10 to 30 percent iAs, 10 to 20 percent MMA and 60 to 70 percent DMA. (Vahter, 2000)

According to World Health Organization's (WHO) soluble As is one of ten chemicals of major public health concern (WHO, 2012). Arsenic and arsenic compounds are considered class 1 chronic non-threshold carcinogenic to humans (IARC, 2004). Long-term oral exposure to iAs is associated with several multi-systemic health conditions. The first symptoms of long-term exposure to high levels of iAs are usually observed in the skin, and include pigmentation changes, skin lesions and hyperkeratosis (Argos et al., 2011). In addition, long-term exposure to arsenic may cause cancers of the skin (Yu et al., 2006), bladder (Ferreccio, Yuan, et al., 2013; Marshall et al., 2007), lung (Smith et al., 2009; Steinmaus et al., 2014), kidney (Ferreccio, Smith, et al., 2013; Yuan et al.,

2010) and liver (Liaw et al., 2008) on a linear dose response fashion. Based on epidemiological studies a benchmark dose between 0.3 and 8 μg/kg per body weight per day was estimated to result in a 1 percent increased risk of lung, skin and bladder tumors in humans (EFSA, 2010). Other health effects of long-term ingestion of inorganic arsenic levels include type 2 diabetes (Islam et al., 2012; Wang et al., 2014) high blood pressure (Abir et al., 2012) and acute myocardial infarction, among other. Importantly, As can pass through the placenta (Concha, 1998a; Punshon et al., 2015). Pregnant women chronically exposed to arsenic-contaminated drinking water are at increased risk for adverse birth outcomes such as late fetal and toddler mortality (Ahmad et al., 2001; Hopenhayn-Rich et al., 2000), reduced birth weight (Hopenhayn et al., 2003; Rahman et al., 2009) and preterm birth (Laine et al., 2015). Furthermore, in utero and early-life exposures to arsenic have been linked to increased risks for several types of cancer and other diseases during adulthood (Naujokas et al., 2013; Smith et al., 2006). In Chile, a study showed that early life (i.e. in utero or ≤ 18 years of age) high arsenic exposure (870 μg/l) resulted in increased mortality from bladder cancer (SMR = 18.1; 95% CI: 11.3, 27.4), laryngeal cancer (SMR = 8.1; 95%CI: 3.5, 16.0), liver cancer (SMR = 2.5; 95% CI: 1.6, 3.7), and chronic renal disease (SMR = 2.0; 95%: 1.5, 2.8) (Smith et al., 2012). Like with other environmental exposures growing evidence supports that even at low exposure levels (10-100 μg/l) arsenic may have important health consequences (Garcia-Esquinas et al., 2013; George et al., 2015; James et al., 2013; Moon et al., 2013; Schmidt, 2014; Steinmaus et al., 2014).

Major human environmental health concern has been associated with iAs bioavailability in drinking water. In Chile, the drinking water regulation NCh 409/1 of the Ministry of

Health reduced the arsenic concentration limit from 50 µg/l to 10 µg/l in 2005 (INN, 2005), in line with WHO's latest recommendation from 1993. However, it has been estimated that adhering that standard still causes 30 deaths per 10,000 people (NRC, 2001). For perspective, based on a daily drinking water consumption of 2 l by a person weighing 65 kg, a drinking water concentration of 10 µg/l equals 0.31 µg/kg per body weight per day, which is already in the range of the aforementioned 1 percent benchmark dose of carcinogenesis. In northern Chile, populations exposed to mean arsenic concentrations as high as 900 µg/l in drinking water have been reported (Fernandez et al., 2012; Marshall et al., 2007; Rivara et al., 1997) Nonetheless, historically since 1930 drinking water in Arica – the site of the present study - has consistently exhibited a lower As concentration of around 10 µg/l (Dauphine et al., 2011; Ferreccio et al., 2000; Ferreccio, Smith, et al., 2013; Sancha & Frenz, 1997; Smith et al., 2012; Steinmaus et al., 2014)

More recently, health concerns have been raised regarding the extent of iAs exposure via food (Gundert-Remy et al., 2015). Due to absorption from the soil and water, and particularly the use of arsenic-based fertilizers, herbicides, pesticides and animal feed, iAs is often present in many foods, including but not limited to grains, vegetables, fruits, meat, poultry and dairy products. Due to low concentrations of iAs, seafood is not a primary contributor to dietary iAs intake (Schoof et al., 1999). However, as suggested by some authors, seafood intake is a major determinant of not only increased urine concentrations of AsB but also of DMA, potentially through metabolism of other organoarsenicals (Aylward et al., 2014; Molin et al., 2012; Navas-Acien et al., 2011). Concern has been raised with regard to iAs bioavailability from food, because despite

reduced drinking water concentrations, elevated exposures to iAs have been reported (Kippler et al., 2016; Yager et al., 2015). It has been estimated that food may contribute significantly (up to 54-85 percent) to iAs intake where iAs in water concentration is not high among non-seafood eaters (Kurzius-Spencer et al., 2014). Nevertheless, one study in Arica found that water contributed to around 57 percent and food to around 42 percent of the iAs daily intake (Sancha & Frenz, 1997). At the time of the study the average As concentration in water in Arica was 9 μg/l. In the U.S., since the 2003–2004 the National Health and Nutrition Examination Survey (NHANES) begun to measure speciated As as well as total As in the diet (Caldwell et al., 2009). NHANES allows estimates of the food groups that make the greatest contribution to dietary iAs intake, which in turn provides a guide to potential most important foods in assessing iAs bioavailability. Using NHANES the following contributions to iAs by food group in the overall population were found: vegetables (24%); fruit juices and fruits (18%); rice (17%); beer and wine (12%); and flour, corn, and wheat (11%) (Xue et al., 2010). Further, according to a study published by the European Food Safety Authority (EFSA) on dietary iAs exposure, the estimated exposure of toddlers (≥ 12 months to < 36 months old) to iAs was 3 to 4 times the exposure of adults to iAs. This group exhibited the highest exposure with 95th percentiles dietary exposure to iAs ranging from 0.62 (lower bound min) to 2.09 μg/kg (upper bound max) per body weight per day. That is similar to the results from Xue et al. where the 1 to 2 years old group 95th percentiles dietary exposure was also the highest (2.06 μg/kg/day from food). In the European toddler population, the main contributors to iAs exposure were: 'Milk and dairy products' (13-24%, median 17%), 'Grain-based processed products (non rice-based) (9-17%, median 11%) and 'Rice' (median 7%). 'Drinking water' (3-

16%, median 10%) was also an important source of dietary iAs in this population class (EFSA, 2014).

Globally, special attention has been given to rice because it contains significant amounts inorganic arsenic with concentrations often between 100 to 400 µg arsenic/kg dry mass and sometimes considerably higher (EFSA, 2010; Sun et al., 2008) and because it may introduce significant concentration of iAs particularly into toddlers' and young children's diets due its use in toddler's formulas and first foods (CR, 2012; Davis et al., 2012; Hojsak et al., 2015; Jackson et al., 2012; Signes-Pastor et al., 2016). The JECFA proposed a maximum level of 200 µg/kg of iAs in polished rice (JECFA, 2014). Other food groups are less well characterized with regard to the relative proportions of iAs and organic As. While in Europe, US or WHO there are no limits for either total or inorganic arsenic in food, including rice, the Chilean MoH's food regulation Nº 977/96 set a total arsenic content limit for food ranging from 50 µg/kg (bottled mineral water) to 2000 µg/kg (sugar). Cereals' (including rice) limit is set to 500 µg/kg (MINSAL, 2014). China regulates the level of iAs exclusively in rice, where the maximum contaminant level permitted is 150 µg/kg.

In contrast to the aforementioned free As transfer through the placenta during pregnancy, when exposure levels are similar in the mother and the fetus, there is limited passage over the mammary gland. It has been reported that in spite of high concentrations of iAs in maternal blood and urine, the concentration in maternal milk has been reported as low, on average 3.4 µg/l, even in high exposed mothers (drinking water iAs content about 200 µg/l) and, for instance, arsenic concentrations from two-day old newborns dropped from 80 µg/l to less than 30 µg/l after four months of exclusive breastfeeding (Concha, 1998a).

Toddlers who were not exclusively breastfed, and were given formula in addition to breast milk showed higher urine arsenic methylated metabolites (Concha, 1998b). Urinary As concentrations in breast milk have been reported as low (0.17-2.9 μg/l) but 7.5 times higher for 6-week toddlers exclusively fed with formula than for toddlers exclusively fed with breast milk in the U.S. (Carignan, Cottingham, et al., 2015). A study conducted in an Arica's mine waste polluted area found that toddlers with higher breastfeeding level were more protected than not exclusively breastfed toddlers and that As concentration in breast milk were not significantly different to those taken from a control area, and both were low between 0.04 and 2.82 μg/l (Castro et al., 2014).

This study explores potential dietary and environmental determinants for acute arsenic exposure, and examines, first, whether breastfeeding is a protective factor for arsenic exposure in this population. Second, this study looks to verify whether dietary exposures, particularly rice, cereals, tap water, formula and meats, reported in the literature are applicable to this specific population. Given the differential distribution and toxicity of arsenic metabolites this study aims to describe determinants for each metabolite and is pioneer in doing so. Also, compared to previous studies, the current study is novel in specifically exploring dietary determinants for low-level arsenic exposure in early life in this population, and also exploring breastfeeding effect in children beyond one year of age. This study should be viewed as exploratory and hypothesis-generating.

Methods

Study sample

The participants of this secondary data analysis study were a subgroup of subjects from a recently completed cohort study, which explored pre- and postnatal exposure to arsenic and its relationship with cognitive development in a representative sample of mother-toddler pairs. The study was conducted in the port city of Arica, which is Chile's northernmost city, located just 18 km south of the border with Peru. According to the 2012 Population and Housing Census of Chile Arica's population was 223,486 (INE, 2012).

In 1983, a Swedish mining company disposed of 20,000 tons of lead, cadmium and arsenic contaminated smelter waste in Arica. The polymetal waste deposit was removed in 1998 and resident relocation process started in 2013 (EDLC, 2016).

A month before recruitment and during the month the last postnatal interview was performed (May 2013-April 2015), the As levels in drinking water reported by the Superintendent of Sanitary Services (Superintendencia de Servicios Sanitarios) were normal (i.e. below or equal to 10 μg/l) in the area of study (SISS, 2016).

Originally, 257 pregnant women in the second trimester, aged between 16 and 44 years old attending primary healthcare centers in Arica from May to November 2013 were recruited into the study, a total of 210 mothers (81.7%) completed their postnatal follow-up interview between September 2014 and April 2015. Reasons for loss to follow-up of the other 47 mothers were: lost contact (5.8%), refusal (6.2%), moving out of town (3.9%), failure to attend the appointment (1.2%) and stillbirths (1.2%). For this specific study we considered a subset of 192 mother-child pairs, which besides their assessment at

postnatal follow-up interview had their toddler speciated urine arsenic concentration information available. This represents approximately a sample of a 5 percent of total natality in Arica, considering a natality rate of 19 (INE, 2009).

Study procedures and data collection

- Prenatal initial interview: during pregnancy second trimester. Informed consent, recruitment and collection of maternal demographic (i.e. age in years, civil status, ethnicity, education, household income and nationality) and exposure information (i.e. home street traffic level and whether it was asphalted, drinking water source (bottled or tap) and portions of tap water ingested in the last 48 hours). Maternal self-collected spot urine samples were retrieved the day after recruitment.
- Postnatal follow-up interview: between 11 and 19 months of toddler age. Collection of toddler demographic (age in months, gender) and exposure information (time of sampling, detailed food and portions in the last 48 hours: including breast milk, cereal, chicken or turkey, baby formula, fruit, vegetables, meat, rice or pasta, seafood, bottled; drinking water source (bottled, tap or both), daycare attendance, second hand smoking exposure). Toddler urine samples were taken during the appointment. When not possible to obtain the urine sample during the evaluation, a pediatric collector was provided to the mother and was retrieved the next day (14.7% of the urine samples were obtained by this method)

Analytical methods

Prenatal and postnatal acute arsenic urine concentration analysis was performed at Trace Metals Facility Core, Columbia University Mailman School of Public Health. Speciation of arsenic metabolites, AsIII, AsV, MMA and DMA, in maternal and toddler urine

samples used high pressure liquid chromatography on line with hydride generation and inductively coupled mass spectrometry (HPLC-ICPMS). In addition, the organic arsenic forms, AsB and AsC were reported for toddler urine samples.

Ethical considerations

This study is a research thesis in partial fulfillment of the requirements for the degree of Master of Public Health in Applied Epidemiology of the Rollins School of Public Health of Emory University. In that context, it was submitted to the Emory University Institutional Review Board, which determined on December 22nd, 2015 that this study did not require IRB review because it would analyze data collected in a previous study without access to identifiers (secondary analysis) not meeting the definitions of research with "human subjects" or "clinical investigation" as set forth in Emory policies and procedures and U.S. federal rules, if applicable (Appendix A).

Statistical analysis

Four coded data sets without personal identifiers were merged. Data was inspected for appropriateness and cleaned. Missing data were assumed to be missing at random and left in the dataset. Overall, study data was complete. It was arbitrarily decided not to use variables with 22% or higher missing data points. Toddler and mother urine speciated arsenic concentrations were log transformed. Values below the limit of detection (LOD) which is 0.1 µg/l were censored and substituted with 0.1 µg/l divided by the squared root of 2. Variables were categorized and reference groups were selected. Variables maternal age and toddler age were kept as continuous. Free text sections of the postnatal follow-up interview questionnaire were extracted using the INDEX function and categorized for analysis. Descriptive analyses were conducted for demographic characteristics and

covariates. Frequency tables were generated for categorical variables, including missing data. Differences between tested and non-tested as well as with or without urine sample groups were tested using chi square or Fisher's exact test (when cell count less or equal to 5) for categorical variables. Continuous variables were tested using the Mann–Whitney–Wilcoxon test, when normality assumption was violated. Normal distribution of the variables and residuals was assessed using the Shapiro-Wilk test. Correlation among variables was evaluated using Spearman or Pearson test of correlation. Mean toddler urine arsenic metabolites concentrations were determined for the categories of each variable, and the differences between categories were tested by using the Mann–Whitney–Wilcoxon test, when there were two categories and the Kruskal-Wallis test when there were three or more categories. Multilevel linear models (PROC GLM) were used to identify determinants associated with differences in levels of each log-transformed mother and toddler urine arsenic concentrations. To identify the determinants of toddler and maternal urine arsenic speciated metabolites concentrations, a regression model was developed for each one. All maternal variables, toddler variables and exposure information were examined as potential determinants of urine arsenic metabolites concentrations. First, we specified a crude model without any adjustment to estimate the association between each determinant of interest and arsenic concentrations, only one explanatory variable at a time (univariate models). Second, for maternal and toddler models we included in the final models all explanatory independent variables with $p < 0.10$, and then eliminated variables below that same level of significance variables one by one using backward stepwise regression. If two variables were significant in the univariate models, but highly correlated, only the most significant

variable was included in the final model. Correlations between variables were computed using Spearman's correlation coefficient. We adjusted multivariate models for potential confounding. Maternal models included maternal age (continuous), ethnicity, income, educational level, civil status and nationality. Toddler models were further adjusted by toddler age (continuous) and sex. All analyses were performed using SAS® Software version 9.4 or later (SAS Institute, Cary, NC, USA).

Results

Study participants' characteristics

Non-participants (n=47) were no different than participants (n=210) regarding all variables considered (Tables 1a and 1b). The group who participated but did not have urine samples (n=18) was also no different from those who participated but had toddler urine samples (n=192), regarding variables of interest, with the exception of maternal urinary DMA (Tables 2a and 2b). Toddler outcome variables were complete (n=192) and maternal outcome variables were almost complete (n=188). Maternal information obtained at the initial interview (demographic (i.e. age in years, civil status, ethnicity, education, household income, nationality) and exposure information (i.e. home street traffic level and whether it was asphalted, drinking water source (bottled or tap) and portions of tap water ingested in the last 48 hours) were complete (n=192). Toddler missing data from the follow-up interview ranged from 0 to 56%. Variables with up to 22% missing data were included in the analyses. Relevant toddler exposure information such as portions of tap water, portions of bottled water, daycare program (part- or fulltime), number of smokers in the household and certain kind of foods ingested in the last 48 hours were not included in the analyses. The toddler and maternal descriptive characteristics the urine samples are summarized in Table 3. Given the large number of data below the limit of detection (80.7%), AsC was excluded from further analyses. Mean maternal age was 26±6.2 years at the moment of the prenatal initial interview. Most of the mothers graduated from high school (79.7%), earned CL$400,000 (approximately US$600) or less (82.3%), were single (58.3%), Caucasian (59.6%), Chilean (91.15%) and were not current smokers (61.5%). Mean toddler age was 13.1±1.4

months at postnatal follow-up interview. Most of the children (84.4%) lived in a house at an asphalted street. Less than 50% of the toddlers ate meat, cereal, legumes or seafood 48 hours before the postnatal follow-up interview. Over 50% of the toddlers ate pasta, rice, dairy products and chicken, and over 80% of them had fruits and vegetables in that timeframe. 55.21% of the infants were breastfed and 71.9% had formula 48 hours before the postnatal follow-up interview

Log-transformed arsenic species concentrations correlation

Because not all log-transformed arsenic concentrations were normally distributed, correlation was assessed with nonparametric Spearman correlation unless specified otherwise (Table 4). Most toddler metabolites and all maternal metabolites were correlated among themselves. With respect to correlation of arsenic concentrations between toddlers and mothers, the only meaningful correlations were toddler urine AsB log-concentrations weakly and negatively related to maternal AsV and MMA log-concentrations.

Toddler urinary arsenic metabolites concentration according to significant characteristics.

As shown in Tables 5a and 5b , the distribution of mean arsenic metabolites in toddler urine was 15.4% (AsIII + AsV), 10.6% MMA and 74.0% DMA. Across overall participants mean AsB accounted for 9.2% when included along with inorganic and methylated arsenic species. Mean differences were determined for each arsenic urinary metabolite:

Arsenobetaine (AsB): The mean AsB concentrations among corn- and seafood-eaters were 4.97 and 2.65 times higher comparing to non-eaters. Proportion of AsB in urine was

incremented accordingly. Formula consumers exhibited a AsB mean concentration and proportion reduction by approximately a half.

Arsenite (AsIII): AsIII mean concentrations were significantly augmented among daycare attendees (61% higher) and formula consumers (2.61 times higher). Those reporting tap water portions in the last 48 hours (n=85, not included in further analyses) showed increased AsIII urine levels as the ingested portions increased 2.41 times higher among those drinking 1200 ml or more and those drinking a maximum of 400 ml). AsIII urinary levels were significantly lower among those living at an asphalted street (37% reduction) and those breastfed (15% reduction), that reduction was of 33% and 51% in toddlers fed with 6 or more breast milk portions and ad-libitum, respectively versus those fed with 5 or less breast milk portions in the last 48 hours.

Arsenate (AsV): AsV mean concentrations were increased among daycare attendees (45% more), among those sampled in the afternoon (62% more), and among cereal- (30% more), melon- (by 2.1 times, data not shown) and meat-eaters (36% more). Eating broccoli diminished AsV mean concentration by 3 times (data not shown), and compared to drinking tap water, drinking bottled water diminished AsV mean concentration by a quarter.

Dimethylarsinic acid (DMA): Those drinking tap water, those eating 2 portions of chicken or turkey, daycare attendees and melon-eaters presented a 29%, a 32%, a 55% and a 67% increase of DMA in urine, respectively. While those living at an asphalted street (27%) and eating cauliflower (3.4 times) presented the mentioned significant reduction of DMA levels in the urine.

Monomethylarsonic acid (MMA): MMA levels were increased by 16% among those

eating 2 portions of chicken or turkey, by 67% among daycare attendees, by 68% among watermelon eaters and by double among melon eaters. Living at an asphalted street, eating tangerine and eating cauliflower, reduced MMA urine levels by 27%, by 3.1 times and by 3.3 times, respectively.

Total AsIII + AsV + DMA + MMA: As per total inorganic and methylated species concentration in urine, daycare increased their sum concentration by 55%, eating melon and watermelons increased them by 73% (data not shown) and 59%, respectively, and eating 2 portions of chicken or turkey by 30%. Tap water drinkers presented 23% more of these species in their urine. Eating cauliflower diminished these species presence in urine by 3.4 times and living at an asphalted street by 55%.

Toddlers urinary arsenic metabolites concentration modelling

Adjusted multivariate model effects are summarized in Table 6. Several other variables had a significant effect on arsenic species concentration in the univariate models but were dropped from multivariate models. All models were adjusted by toddler sex and age, and maternal age, education, income, civil status and ethnicity. All linear regression assumptions were verified for the models.

Arsenobetaine (AsB): In the adjusted multiple regression model corn and seafood consumption were the strongest positive independent predictors of AsB log-concentration in the urine. Formula also was a statistically significant predictor decreasing total urinary AsB log-concentration. The variables dairy product's portions, vegetables portions (continuous) and civil status (separated) were significant in the univariate model but no longer significant after adjusting for variables in the multivariate model. The variable 'eating yogurt' was also significant but had 40% missing data.

Arsenite (AsIII): The model included formula portions in the last 48 hours as a positive significant predictor of urinary AsIII and living at a house at an asphalted street as a negative significant predictor of the metabolite. Test for trend for formula portions was significant. Variables that were significant in the univariate models but were no longer significant after adjusting for variables in the multivariate models include categorical breast milk portions and drinking cow's milk, both decreasing AsIII levels. The variable 'portions of tap water' was also significant in the univariate model but had over 50% missing data.

Arsenate (AsV): The significant independent predictors of AsV log-concentration increase were sampling time, drinking water source, eating rice, eating mea and drinking tap water in the last 48 hours. Having 1 to 3 cereal portions and living in a house at a street with heavy traffic were predictors of AsV log-concentration decrease. Eating melon, attending daycare and. eating broccoli were also significant in the univariate analysis but were not retained in the multivariate analysis.

Dimethylarsinic acid (DMA): In the adjusted multiple regression model daycare attendance, eating watermelon and having two portions of chicken or turkey were the significant positive independent predictors of DMA log-concentration in the urine. On the other hand, drinking 6 portions of breast milk or more and eating cauliflower decreased DMA urinary concentration. Source of drinking tap water, living in a house at an asphalted street, eating rice, and eating melon were significant in the univariate models but after adjusting for the other variables were no longer significant nor lower equal then 0.10.

Monomethylarsonic acid (MMA): Similarly, the independent predictors of MMA log-concentration increase were: attending daycare and eating watermelon in the last 48 hours. Having 6 or more portions of breast milk in the last 48 hours, eating cauliflower and eating tangerine were significant predictors reducing MMA log-concentration in urine. Drinking tap water, living in a house at an asphalted street, eating rice, eating melon although significant in the univariate model, did not remain in the multivariate model.

Total AsIII + AsV + DMA + MMA: Lastly, the following independent were predictors of total inorganic and methylated arsenic (AsIII + AsV + DMA + MMA) log-concentration: attending daycare, having 6 or more portions of breast milk, eating watermelon, eating cauliflower and having 2 portions of chicken or turkey. The variables drinking tap water, living in a house at an asphalted street, eating rice and eating melon were significant in the univariate model but no longer significant after adjusting for variables in the multivariate model.

Mothers urinary arsenic metabolites concentration modelling

Multivariate model effects are summarized in Table 7. Besides maternal demographic characteristics (age, education, income, ethnicity, nationality and civil status), water source for drinking, living at an asphalted street, traffic level of residence street were analyzed as predictors. Water source for drinking reported for mother at the initial interview and for toddlers at the follow-up interview were correlated between them.

Arsenite (AsIII): In the adjusted multiple regression tap water source for drinking was the strongest positive independent predictor of AsIII log-concentration in the urine, with 54% more AsIII than those drinking bottled water. Being single was associated with a 41%

reduction of urinary AsIII log-concentration.

Arsenate (AsV): The significant independent predictors of AsV log-concentration were living at a street with light or moderate traffic (34% less urinary AsV than those living at a private street), tap water as drinking source (47% more urinary AsV than those drinking bottled water) and mother's age (each one-year increase in age was associated with 3% decrease in AsV urinary log-concentration)

Dimethylarsinic acid (DMA): In the adjusted multiple regression model, being single (40% less DMA than married subjects), drinking tap water (47% more DMA than those drinking bottled water), having superior education (48% increase in comparison with no high school) were the significant independent predictors of DMA log-concentration in the urine.

Monomethylarsonic acid (MMA): the independent predictors of MMA log-concentration in urine were being single (33% less MMA than married subjects), being separated (64% less MMA than married subjects) and living with a partner (38% less then married subjects), living in a house at a street with light or moderate traffic (38% less MMA than those living in a house at a private street), and living in a house at a street with heavy traffic (35% less MMA than those living in a house at a private street), and drinking tap water (32% more MMA than those drinking bottled water)

Total AsIII + AsV + DMA + MMA: Civil status was the only predictor of total arsenic: living with a partner (34% less than married subjects), being single (34% less than married subjects), being separated (64% less than married subjects). Drinking tap water was associated with a 24% rise in total arsenic levels.

Discussion

Toddlers' and mothers' analyses key results

Arsenobetaine (AsB): Positive correlation of arsenobetaine and seafood has been well described (Aylward et al., 2014; Molin et al., 2012; Navas-Acien et al., 2011). However, the significant increase in urine AsB after 48 hours of corn consumption and the significant decrease in AsB urinary excretion after formula consumption reported by this study is novel. No effect was found from water sources, so diminished AsB concentration related to formula consumption may involve other mechanisms. Several studies have reported elevated concentrations of urinary AsB in populations that consumed little or no fish or seafood (Brima et al., 2006; Lai et al., 2004; Rivera-Nunez et al., 2012). Our results may point to an additional non-seafood sources of AsB. Corn origin and whether it was canned, frozen or otherwise processed is unknown. Further, contrary to recent studies that reported higher concentrations of DMA in individuals that have consumed fish or seafood during the days before urine sample collection (Francesconi et al., 2002), seafood did not have a significant impact on DMA urinary concentrations. Toddler urinary AsB and DMA were weakly correlated. Since AsB is deemed non-toxic and strongly related to seafood ingestion there are no many studies analyzing other determinants. However, if AsB does indeed impact DMA concentrations it would be relevant to learn more about its sources.

Arsenite (AsIII): For toddlers consuming formula (and its portions in a linear trend fashion) positively affected AsIII urinary concentration and distribution. Drinking formula did not only increase the AsIII mean in urine, but also increased by 2.3 times AsIII presence in the urine out of the sum of inorganic and methylated species. Although

it did not remain in the multivariate model 'drinking water source' was predictor with p= 0.0624 in the univariate model. Also, 'tap water portions (200 ml)', that was not included in the modelling because it had over 50% missing data, showed to significantly increase AsIII levels in both the Kruskal Wallis test and the univariate linear model. Drinking tap water was a significant predictor of AsIII increase in the maternal model. Thus, it could be hypothesized that some AsIII formula contribution might come from the water used. In addition, analysis of local baby formulas would also help understand the arsenic source since some studies have reported presence of inorganic arsenic in baby formula in the U.S and Europe (Jackson et al., 2012; Ljung et al., 2011)

Arsenic in dust can be be present from several sources. House dusts in an area around a smelter were among the highest reported in the literature. Considering the contaminated smelter waste disposal in Arica, arsenic can be re-incorporated into the atmosphere from traffic travelling on unpaved roads (Fowler, 1983). Huang et al. (Huang et al., 2014) collected road dust samples and inorganic As species were the predominant species in dust and airborne particles. Living at an asphalted street would hence be a protective factor, in line with our findings. Atmospheric particles are important exposure sources of inorganic As. Collection of air, soil and water samples for arsenic tests would help understanding this environment.

Arsenate (AsV): Interestingly, AsV toddler log-concentration besides being incremented by samples taken in the afternoon, rice, meat and cereal consumption, also shared two significant predictors with the maternal model: water source for drinking and home street type (living at a street with heavy traffic), both with the same direction of the effect. In addition, water source for drinking reported for mother at the initial interview and for

toddlers at the follow-up interview are correlated between them.

Time of sampling was a significant predictor for AsV in the toddler model. Apparently, the proportion of the arsenical species in urine depends on the time of sampling or in vitro transformations could have occurred if sample logistic differed. Standardization of time of sampling and logistics would be recommended for next studies.

Since it has been reported the rice contains significant amounts inorganic arsenic (AsIII and AsV) (Davis et al., 2012; EFSA, 2010; Sun et al., 2008), it was expected that rice would be a significant predictor of inorganic arsenical species. In Chile, a study analyzing arsenic content in local rice brands showed an average of 400 μg arsenic/kg dry mass (Schencke, 2010), which is beyond the codex maximum level for arsenic in rice of 200 μg/kg..Meat though has been reported earlier to contain mainly DMA and negligible content of inorganic arsenic (EFSA, 2010; Xue et al., 2010). Regarding cereals, it has been reported to contain inorganic arsenic (Llorente-Mirandes et al., 2014), in particular if cereal is rice based (Carbonell-Barrachina et al., 2012). Further analysis of cereals reported to be consumed by the toddlers in the last 48 hours would help understanding the source of arsenic in cereal (Carignan, Karagas, et al., 2015). Regarding maternal AsV determinants, as mentioned, water source for drinking and home street type (living at a street with light or moderate traffic), were significant in both models. Protection by drinking bottled water has been already described, presumably because of lower content of arsenic in bottled water, which is something to confirm because the Chilean MoH's food regulation Nº 977/96 sets a total arsenic content limit 50 μg/kg for bottled mineral water. As per home street type, local soils, paved road dusts, automobile exhaust emissions along with construction activities have also been identified as potential

airborne arsenic sources. Considering that the the soil in Arica may be highly contaminated with arsenic, the protection could be attributed to light or moderate traffic and heavy traffic roads to be more likely asphalted then smaller streets. Unpaved streets, particularly in a dry environment as Arica, may increase the risk of exposure due to existence of inorganic arsenic in dust. In addition, it has been shown that children are particularly susceptible to inhalation exposure due to their increased likelihood of coming into contact with dust and children inhale a greater volume of air than adults relative to their size, may therefore be exposed to correspondingly higher levels of airborne contaminants than adults. Children also have an increased likelihood of coming into contact with soil and dust through playing in dirt contaminated with arsenic residues (Martin et al., 2014)

DMA, MMA and total arsenic (iAs + DMA + MMA): DMA, MMA and total arsenic log-concentration in urine shared several predictors in their toddler models. The overall mean inorganic and methylated arsenic metabolites distributions in toddler urine were in line with the literature (Vahter, 2000), that means that an average of over an 80% of the total arsenic in urine corresponds to MMA and DMA species, which could explain why total arsenic concentration may be explained by their predictors.

Epidemiologic studies have shown that a lower capacity to methylate iAs to DMA, based on relative amounts of As metabolites in urine, is positively associated with risk for several diseases (Hall et al., 2013). High urinary proportions of MMAs and high ratios of MMAs/DMAs, which are thought to be indicators of an inefficient methylation of iAs, have been associated with the development of several adverse outcomes in humans. Normally children present a more active 2nd methylation step than adults and hence a

higher arsenic excretion (Chowdhury et al., 2003) and is known that pregnancy improves metabolism efficiency of iAs (Laine et al., 2015). This study confirms the aforementioned, as in average toddler presented a DMA/MMA ratio of 6.9 and mothers of 9.9. Further, decrease in arsenic methylation efficiency during weaning has been reported (Fangstrom et al., 2009). However, some groups think that it is essential to re-evaluate the hypothesis that methylation is the detoxification pathway for inorganic arsenic, provided that the trivalent forms of DMA and MMA are the most toxic metabolites. (Mandal et al., 2001)

In univariate models, attending daycare is a determinant for increased arsenic for all species (excluding AsB) in urine. Further information would be required about how daycare attendance might impact arsenic concentrations, it would be necessary to know the daycare location and taking soil and water samples. Food samples would also add value to a following study. Moreover, presence of contaminated wooden facilities and equipment (e.g. playground sets), which could contain potentially hazardous levels of arsenic due to the use of Chromated Copper Arsenate (CCA) as a wood preservative and insecticide would need to be examined (Kwon et al., 2004; Zagury & Pouschat, 2005). That chemical has been banned in the U.S. and Europe, however it has not been regulated in Chile yet and remains being widely used. Furthermore, children attending daycare may be potentially more exposed to airborne arsenic in the environment than children staying at home. No significant interactions were found between daycare attendance and formula or breast milk consumption in the models, however this would need to be further explored.

In line with our hypothesis, reporting 6 or more portions of breast milk in the last 48 hours was a protective factor for DMA, MMA and total arsenic (Carignan, Cottingham, et al., 2015; Carignan, Karagas, et al., 2015; Fangstrom et al., 2009).

Watermelons are over 90% water, so it would be interesting to learn where they were grown and if they took up arsenic from the soil or water. Similar effect could be hypothesized for eating melon, which was significant in the univariate model but did not remain significant in the multivariate model.

The chemoprotective effect of cruciferous vegetables has been hypothesized to be due to their high glucosinolate content and the capacity of glucosinolate metabolites, this could have to do with arsenic metabolism (Lampe & Peterson, 2002).

DMA content has been reported in poultry fed with fish meals (Silbergeld & Nachman, 2008). In addition, the use of arsenic-based drugs, such as Nitarsone and Roxarsone could be also the reason for the significant increase in DMA and total arsenic levels after reporting eating 2 portions of chicken in the last 48 hours. The FDA has formally withdrawn its approvals for those drugs, while in Chile both are still approved for animal use, particularly in poultry.

Concerning maternal models, DMA was increased significantly by drinking tap water, which was a variable that was significant in the univariate toddler model but was dropped. Home street type (light or moderate traffic and heavy traffic) and drinking tap water were significant predictors of maternal logDMA. Drinking tap water was the main predictor of total arsenic increase in the mothers.

Tap water contribution to arsenic exposure is a relevant aspect to consider even when levels are at or below the standard. Water portions reported by toddler and mothers 48

hours before their urine samples were taken imply an ingestion of 2 to 24 µg/l and 0 to 62.5 µg/l arsenic when tap water arsenic level is below or equal to 10 µg/l, respectively. According to WHO's weight-for-age one-year-old boys weight in average 10.2 kg and one-year-old girls weight 9.5 kg in average. That means that in average toddlers may be ingesting 2.35 µg/l (boys) and 2.53 µg/l (girls) exclusively from tap water consumption, which which is already in the range of the aforementioned 1 percent benchmark dose of carcinogenesis.

Strenghts and limitations

This is one of the few studies analyzing breast feeding and other dietary determinants of urinary arsenic in this specific age-group with such a high breastfeeding rate. One of the greatest strengths of this study is the analysis of determinants for individual arsenic species, highlighting their differential toxicity profile and natural distribution. However, we identified at least two aspects that were missing. First, because of the high number of results below the LOD, arsenocholine was not considered in the analysis and would have been interesting to analyze it, since AsC is converted to AsB and DMA(Christakopoulos et al., 1988). Second, since the methylated trivalent metabolites, MMA(III) and DMA(III), are significantly more toxic than their pentavalent counterparts and either AsIII or AsV, further characterization of the DMA and MMA species including their oxidative state would help understanding their potential toxicity (Mass et al., 2001; Yehiayan et al., 2009)

There were some other various limitations that can be identified in this study. First, there were no measures to account for urine dilution in spot urine samples such as including urine creatinine as a control covariate. Further, urinary creatinine can be a strong

predictor of arsenic methylation efficiency (Barr et al., 2005; Gamble et al., 2006). Although stringent training and consistent standardization of the measurements used in this study seem to leave little chance for measurement bias, there is likely some variation in measurements between examiners, leading to measurement bias. Also, samples were not taken always at the same time and that might have impacted the study results. The use of questionnaires and interviews that require participants to provide information on events that have already happened, makes the study prone to threats in internal study validity caused by recall bias. Unmeasured covariates such as air, food, dust and soil arsenic contents may also affect the internal study validity. Analysis of the home and daycare facilities locations and proximity to the waste deposit would help understanding whether it may be the source of arsenic exposure in this population.

Toddler models only explained up to 37% of the response variable variation. Also, stratification analysis could be further performed to address potential confounders. Study may also suffer from information bias or differential misclassification as some sensitive information (smoking status, income, etc) were self-reported by study participants. Also, food portions and particularly breast milk portions are difficult to estimate and standardize. Although study data was overall complete, missing data may have affected study power and external validity of the results. Toddler models range from 125 to 167 study subjects from a total of 192 participants, implying a 13% to 35% observation loss, Backward elimination regression models resulted having different sample size at each step, which made each comparison unequal. To avoid that in the future the analysis could be done including multiple imputation methods and to compare the results. Also and adequate balance between variable significance and losing sample size should be made.

This study recommends that more work is needed to better understand dietary and environmental sources of arsenic species and their metabolism in this population. More studies with higher statistical power and targeted design will be required to confirm these findings.

Sampling of dust, soil, water, foods and other could help understand better the setting of the study to be able to generalize these results to other similar conditions. Airborne exposures, are overlooked by this study and would be interesting to explore in a future one. (Martin et al., 2014)

Despite these limitations, our findings suggest that regardless the multiple other sources of arsenic exposure extended breastfeeding is still beneficial protecting toddlers from water or formula arsenic and highlight the need to better understand other environmental arsenic sources (i.e soil and airborne)-

References

Abir, T., Rahman, B., D'Este, C., Farooq, A., & Milton, A. H. (2012). The Association between Chronic Arsenic Exposure and Hypertension: A Meta-Analysis. *J Toxicol, 2012*, 198793. doi:10.1155/2012/198793

Ahmad, S. A., Sayed, M. H., Barua, S., Khan, M. H., Faruquee, M. H., Jalil, A., et al. (2001). Arsenic in drinking water and pregnancy outcomes. *Environ Health Perspect, 109*(6), 629-631.

Argos, M., Kalra, T., Pierce, B. L., Chen, Y., Parvez, F., Islam, T., et al. (2011). A prospective study of arsenic exposure from drinking water and incidence of skin lesions in Bangladesh. *Am J Epidemiol, 174*(2), 185-194. doi:10.1093/aje/kwr062

Aylward, L. L., Ramasamy, S., Hays, S. M., Schoeny, R., & Kirman, C. R. (2014). Evaluation of urinary speciated arsenic in NHANES: issues in interpretation in the context of potential inorganic arsenic exposure. *Regul Toxicol Pharmacol, 69*(1), 49-54. doi:10.1016/j.yrtph.2014.02.011

Barr, D. B., Wilder, L. C., Caudill, S. P., Gonzalez, A. J., Needham, L. L., & Pirkle, J. L. (2005). Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. *Environ Health Perspect, 113*(2), 192-200.

Brima, E. I., Haris, P. I., Jenkins, R. O., Polya, D. A., Gault, A. G., & Harrington, C. F. (2006). Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of healthy volunteers from three unexposed ethnic groups in the United Kingdom. *Toxicol Appl Pharmacol, 216*(1), 122-130. doi:10.1016/j.taap.2006.04.004

Caldwell, K. L., Jones, R. L., Verdon, C. P., Jarrett, J. M., Caudill, S. P., & Osterloh, J. D. (2009). Levels of urinary total and speciated arsenic in the US population: National Health and Nutrition Examination Survey 2003-2004. *J Expo Sci Environ Epidemiol, 19*(1), 59-68. doi:10.1038/jes.2008.32

Carbonell-Barrachina, A. A., Wu, X., Ramirez-Gandolfo, A., Norton, G. J., Burlo, F., Deacon, C., et al. (2012). Inorganic arsenic contents in rice-based infant foods from Spain, UK, China and USA. *Environ Pollut, 163*, 77-83. doi:10.1016/j.envpol.2011.12.036

Carignan, C. C., Cottingham, K. L., Jackson, B. P., Farzan, S. F., Gandolfi, A. J., Punshon, T., et al. (2015). Estimated exposure to arsenic in breastfed and formula-fed infants in a United States cohort. *Environ Health Perspect, 123*(5), 500-506. doi:10.1289/ehp.1408789

Carignan, C. C., Karagas, M. R., Punshon, T., Gilbert-Diamond, D., & Cottingham, K. L. (2015). Contribution of breast milk and formula to arsenic exposure during the first year of life in a US prospective cohort. *J Expo Sci Environ Epidemiol*. doi:10.1038/jes.2015.69

Castro, F., Harari, F., Llanos, M., Vahter, M., & Ronco, A. M. (2014). Maternal-child transfer of essential and toxic elements through breast milk in a mine-waste polluted area. *Am J Perinatol, 31*(11), 993-1002. doi:10.1055/s-0034-1370343

Chowdhury, U. K., Rahman, M. M., Sengupta, M. K., Lodh, D., Chanda, C. R., Roy, S., et al. (2003). Pattern of excretion of arsenic compounds [arsenite, arsenate, MMA(V), DMA(V)] in urine of children compared to adults from an arsenic

exposed area in Bangladesh. *J Environ Sci Health A Tox Hazard Subst Environ Eng, 38*(1), 87-113.

Christakopoulos, A., Norin, H., Sandstrom, M., Thor, H., Moldeus, P., & Ryhage, R. (1988). Cellular metabolism of arsenocholine. *J Appl Toxicol, 8*(2), 119-127.

Concha, G. (1998a). Exposure to inorganic arsenic metabolites during early human development.

Concha, G. (1998b). Metabolism of Inorganic Arsenic in Children with Chronic High Arsenic Exposure in Northern Argentina

CR. (2012). Arsenic in your food: our findings show a real need for federal standards for this toxin. *Consum Rep, 77*(11), 22-27.

Dauphine, D. C., Ferreccio, C., Guntur, S., Yuan, Y., Hammond, S. K., Balmes, J., et al. (2011). Lung function in adults following in utero and childhood exposure to arsenic in drinking water: preliminary findings. *Int Arch Occup Environ Health, 84*(6), 591-600. doi:10.1007/s00420-010-0591-6

Davis, M. A., Mackenzie, T. A., Cottingham, K. L., Gilbert-Diamond, D., Punshon, T., & Karagas, M. R. (2012). Rice consumption and urinary arsenic concentrations in U.S. children. *Environ Health Perspect, 120*(10), 1418-1424. doi:10.1289/ehp.1205014

EFSA. (2010). Scientific Opinion on Arsenic in Food (Update). *EFSA Journal, 7*(10), 1351. doi:10.2903/j.efsa.2009.1351

EFSA. (2014). Dietary exposure to inorganic arsenic in the European population. *EFSA Journal, 12*(3), 3597. doi:10.2903/j.efsa.2014.3597

Fangstrom, B., Hamadani, J., Nermell, B., Grander, M., Palm, B., & Vahter, M. (2009). Impaired arsenic metabolism in children during weaning. *Toxicol Appl Pharmacol, 239*(2), 208-214. doi:10.1016/j.taap.2008.12.019

Fernandez, M. I., Lopez, J. F., Vivaldi, B., & Coz, F. (2012). Long-term impact of arsenic in drinking water on bladder cancer health care and mortality rates 20 years after end of exposure. *J Urol, 187*(3), 856-861. doi:10.1016/j.juro.2011.10.157

Ferreccio, C., Gonzalez, C., Milosavjlevic, V., Marshall, G., Sancha, A. M., & Smith, A. H. (2000). Lung cancer and arsenic concentrations in drinking water in Chile. *Epidemiology, 11*(6), 673-679.

Ferreccio, C., Smith, A. H., Duran, V., Barlaro, T., Benitez, H., Valdes, R., et al. (2013). Case-control study of arsenic in drinking water and kidney cancer in uniquely exposed Northern Chile. *Am J Epidemiol, 178*(5), 813-818. doi:10.1093/aje/kwt059

Ferreccio, C., Yuan, Y., Calle, J., Benitez, H., Parra, R. L., Acevedo, J., et al. (2013). Arsenic, tobacco smoke, and occupation: associations of multiple agents with lung and bladder cancer. *Epidemiology, 24*(6), 898-905. doi:10.1097/EDE.0b013e31829e3e03

Fowler, B. A. (1983). *Biological and environmental effects of arsenic*.

Francesconi, K. A., Tanggaar, R., McKenzie, C. J., & Goessler, W. (2002). Arsenic metabolites in human urine after ingestion of an arsenosugar. *Clin Chem, 48*(1), 92-101.

Gamble, M. V., Liu, X., Ahsan, H., Pilsner, J. R., Ilievski, V., Slavkovich, V., et al. (2006). Folate and arsenic metabolism: a double-blind, placebo-controlled folic acid-supplementation trial in Bangladesh. *Am J Clin Nutr, 84*(5), 1093-1101.

Garcia-Esquinas, E., Pollan, M., Umans, J. G., Francesconi, K. A., Goessler, W., Guallar, E., et al. (2013). Arsenic exposure and cancer mortality in a US-based prospective cohort: the strong heart study. *Cancer Epidemiol Biomarkers Prev, 22*(11), 1944-1953. doi:10.1158/1055-9965.EPI-13-0234-T

George, C. M., Brooks, W. A., Graziano, J. H., Nonyane, B. A., Hossain, L., Goswami, D., et al. (2015). Arsenic exposure is associated with pediatric pneumonia in rural Bangladesh: a case control study. *Environ Health, 14*, 83. doi:10.1186/s12940-015-0069-9

Gundert-Remy, U., Damm, G., Foth, H., Freyberger, A., Gebel, T., Golka, K., et al. (2015). High exposure to inorganic arsenic by food: the need for risk reduction. *Arch Toxicol, 89*(12), 2219-2227. doi:10.1007/s00204-015-1627-1

Hall, M. N., Niedzwiecki, M., Liu, X., Harper, K. N., Alam, S., Slavkovich, V., et al. (2013). Chronic arsenic exposure and blood glutathione and glutathione disulfide concentrations in Bangladeshi adults. *Environ Health Perspect, 121*(9), 1068-1074. doi:10.1289/ehp.1205727

Hojsak, I., Braegger, C., Bronsky, J., Campoy, C., Colomb, V., Decsi, T., et al. (2015). Arsenic in rice: a cause for concern. *J Pediatr Gastroenterol Nutr, 60*(1), 142-145. doi:10.1097/MPG.0000000000000502

Hopenhayn, C., Huang, B., Christian, J., Peralta, C., Ferreccio, C., Atallah, R., et al. (2003). Profile of urinary arsenic metabolites during pregnancy. *Environ Health Perspect, 111*(16), 1888-1891.

Hopenhayn-Rich, C., Browning, S. R., Hertz-Picciotto, I., Ferreccio, C., Peralta, C., & Gibb, H. (2000). Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environ Health Perspect, 108*(7), 667-673.

Huang, M., Chen, X., Zhao, Y., Yu Chan, C., Wang, W., Wang, X., et al. (2014). Arsenic speciation in total contents and bioaccessible fractions in atmospheric particles related to human intakes. *Environ Pollut, 188*, 37-44. doi:10.1016/j.envpol.2014.01.001

Hughes, M. F. (2002). Arsenic toxicity and potential mechanisms of action. *Toxicol Lett, 133*(1), 1-16.

IARC. (2004). Some drinking-water disinfectants and contaminants, including arsenic. *IARC Monogr Eval Carcinog Risks Hum, 84*, 1-477.

Islam, R., Khan, I., Hassan, S. N., McEvoy, M., D'Este, C., Attia, J., et al. (2012). Association between type 2 diabetes and chronic arsenic exposure in drinking water: a cross sectional study in Bangladesh. *Environ Health, 11*, 38. doi:10.1186/1476-069X-11-38

Jackson, B. P., Taylor, V. F., Punshon, T., & Cottingham, K. L. (2012). Arsenic concentration and speciation in infant formulas and first foods. *Pure Appl Chem, 84*(2), 215-223. doi:10.1351/PAC-CON-11-09-17

James, K. A., Marshall, J. A., Hokanson, J. E., Meliker, J. R., Zerbe, G. O., & Byers, T. E. (2013). A case-cohort study examining lifetime exposure to inorganic arsenic in drinking water and diabetes mellitus. *Environ Res, 123*, 33-38. doi:10.1016/j.envres.2013.02.005

JECFA. (2014). Joint FAO/WHO food standards committee of the Codex Alimentarius.

Kippler, M., Skroder, H., Rahman, S. M., Tofail, F., & Vahter, M. (2016). Elevated childhood exposure to arsenic despite reduced drinking water concentrations - A longitudinal cohort study in rural Bangladesh. *Environ Int, 86*, 119-125. doi:10.1016/j.envint.2015.10.017

Kurzius-Spencer, M., Burgess, J. L., Harris, R. B., Hartz, V., Roberge, J., Huang, S., et al. (2014). Contribution of diet to aggregate arsenic exposures-an analysis across populations. *J Expo Sci Environ Epidemiol, 24*(2), 156-162. doi:10.1038/jes.2013.37

Kwon, E., Zhang, H., Wang, Z., Jhangri, G. S., Lu, X., Fok, N., et al. (2004). Arsenic on the hands of children after playing in playgrounds. *Environ Health Perspect, 112*(14), 1375-1380.

Lai, V. W., Sun, Y., Ting, E., Cullen, W. R., & Reimer, K. J. (2004). Arsenic speciation in human urine: are we all the same? *Toxicol Appl Pharmacol, 198*(3), 297-306. doi:10.1016/j.taap.2003.10.033

Laine, J. E., Bailey, K. A., Rubio-Andrade, M., Olshan, A. F., Smeester, L., Drobna, Z., et al. (2015). Maternal arsenic exposure, arsenic methylation efficiency, and birth outcomes in the Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Mexico. *Environ Health Perspect, 123*(2), 186-192. doi:10.1289/ehp.1307476

Lampe, J. W., & Peterson, S. (2002). Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. *J Nutr, 132*(10), 2991-2994.

Liaw, J., Marshall, G., Yuan, Y., Ferreccio, C., Steinmaus, C., & Smith, A. H. (2008). Increased childhood liver cancer mortality and arsenic in drinking water in northern Chile. *Cancer Epidemiol Biomarkers Prev, 17*(8), 1982-1987. doi:10.1158/1055-9965.EPI-07-2816

Ljung, K., Palm, B., Grander, M., & Vahter, M. (2011). High concentrations of essential and toxic elements in infant formula and infant foods - A matter of concern. *Food Chem, 127*(3), 943-951. doi:10.1016/j.foodchem.2011.01.062

Llorente-Mirandes, T., Calderon, J., Centrich, F., Rubio, R., & Lopez-Sanchez, J. F. (2014). A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by IC-ICPMS. *Food Chem, 147*, 377-385. doi:10.1016/j.foodchem.2013.09.138

Mandal, B. K., Ogra, Y., & Suzuki, K. T. (2001). Identification of dimethylarsinous and monomethylarsonous acids in human urine of the arsenic-affected areas in West Bengal, India. *Chem Res Toxicol, 14*(4), 371-378.

Marshall, G., Ferreccio, C., Yuan, Y., Bates, M. N., Steinmaus, C., Selvin, S., et al. (2007). Fifty-year study of lung and bladder cancer mortality in Chile related to arsenic in drinking water. *J Natl Cancer Inst, 99*(12), 920-928. doi:10.1093/jnci/djm004

Martin, R., Dowling, K., Pearce, D., Sillitoe, J., & Florentine, S. (2014). Health Effects Associated with Inhalation of Airborne Arsenic Arising from Mining Operations. *Geosciences, 4*(3), 128-175. doi:10.3390/geosciences4030128

Mass, M. J., Tennant, A., Roop, B. C., Cullen, W. R., Styblo, M., Thomas, D. J., et al. (2001). Methylated trivalent arsenic species are genotoxic. *Chem Res Toxicol, 14*(4), 355-361.

MINSAL. (2014). Reglamento Sanitario de los alimentos Dto. 117/14, Minsal D.OF. 17.09.14.

Molin, M., Ulven, S. M., Dahl, L., Telle-Hansen, V. H., Holck, M., Skjegstad, G., et al. (2012). Humans seem to produce arsenobetaine and dimethylarsinate after a bolus dose of seafood. *Environ Res, 112*, 28-39. doi:10.1016/j.envres.2011.11.007

Moon, K. A., Guallar, E., Umans, J. G., Devereux, R. B., Best, L. G., Francesconi, K. A., et al. (2013). Association between exposure to low to moderate arsenic levels and incident cardiovascular disease. A prospective cohort study. *Ann Intern Med, 159*(10), 649-659. doi:10.7326/0003-4819-159-10-201311190-00719

Naujokas, M. F., Anderson, B., Ahsan, H., Aposhian, H. V., Graziano, J. H., Thompson, C., et al. (2013). The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. *Environ Health Perspect, 121*(3), 295-302. doi:10.1289/ehp.1205875

Navas-Acien, A., Francesconi, K. A., Silbergeld, E. K., & Guallar, E. (2011). Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. *Environ Res, 111*(1), 110-118. doi:10.1016/j.envres.2010.10.009

NRC. (2001). *Arsenic in Drinking Water: 2001 Update*. Washington, DC: The National Academies Press.

Punshon, T., Davis, M. A., Marsit, C. J., Theiler, S. K., Baker, E. R., Jackson, B. P., et al. (2015). Placental arsenic concentrations in relation to both maternal and infant biomarkers of exposure in a US cohort. *J Expo Sci Environ Epidemiol, 25*(6), 599-603. doi:10.1038/jes.2015.16

Rahman, A., Vahter, M., Smith, A. H., Nermell, B., Yunus, M., El Arifeen, S., et al. (2009). Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am J Epidemiol, 169*(3), 304-312. doi:10.1093/aje/kwn332

Rivara, M. I., Cebrian, M., Corey, G., Hernandez, M., & Romieu, I. (1997). Cancer risk in an arsenic-contaminated area of Chile. *Toxicol Ind Health, 13*(2-3), 321-338.

Rivera-Nunez, Z., Meliker, J. R., Meeker, J. D., Slotnick, M. J., & Nriagu, J. O. (2012). Urinary arsenic species, toenail arsenic, and arsenic intake estimates in a Michigan population with low levels of arsenic in drinking water. *J Expo Sci Environ Epidemiol, 22*(2), 182-190. doi:10.1038/jes.2011.27

Sancha, A. M., & Frenz, P. (1997). Estimate of the current exposure of the urban population of northern Chile to arsenic.

Schencke, V. (2010). Determinación de elementos traza (Pb, Hg, Cd, As, Cu, Mn, Zn, Ca, Mg y Fe) EN ARROZ (Oryza sativa) y trigo mote (Tritricum aestivum) de differentes marcas comerciales (Master's thesis). http://cybertesis.uach.cl/tesis/uach/2010/fcs324d/doc/fcs324d.pdf.

Schmidt, C. W. (2014). Low-dose arsenic: in search of a risk threshold. *Environ Health Perspect, 122*(5), A130-134. doi:10.1289/ehp.122-A130

Schoof, R. A., Yost, L. J., Eickhoff, J., Crecelius, E. A., Cragin, D. W., Meacher, D. M., et al. (1999). A market basket survey of inorganic arsenic in food. *Food Chem Toxicol, 37*(8), 839-846.

Signes-Pastor, A. J., Carey, M., & Meharg, A. A. (2016). Inorganic arsenic in rice-based products for infants and young children. *Food Chem, 191*, 128-134. doi:10.1016/j.foodchem.2014.11.078

Silbergeld, E. K., & Nachman, K. (2008). The environmental and public health risks associated with arsenical use in animal feeds. *Ann N Y Acad Sci, 1140*, 346-357. doi:10.1196/annals.1454.049

Smith, A. H., Ercumen, A., Yuan, Y., & Steinmaus, C. M. (2009). Increased lung cancer risks are similar whether arsenic is ingested or inhaled. *J Expo Sci Environ Epidemiol, 19*(4), 343-348. doi:10.1038/jes.2008.73

Smith, A. H., Marshall, G., Liaw, J., Yuan, Y., Ferreccio, C., & Steinmaus, C. (2012). Mortality in young adults following in utero and childhood exposure to arsenic in drinking water. *Environ Health Perspect, 120*(11), 1527-1531. doi:10.1289/ehp.1104867

Smith, A. H., Marshall, G., Yuan, Y., Ferreccio, C., Liaw, J., von Ehrenstein, O., et al. (2006). Increased mortality from lung cancer and bronchiectasis in young adults after exposure to arsenic in utero and in early childhood. *Environ Health Perspect, 114*(8), 1293-1296.

Steinmaus, C., Ferreccio, C., Yuan, Y., Acevedo, J., Gonzalez, F., Perez, L., et al. (2014). Elevated lung cancer in younger adults and low concentrations of arsenic in water. *Am J Epidemiol, 180*(11), 1082-1087. doi:10.1093/aje/kwu238

Sun, G. X., Williams, P. N., Carey, A. M., Zhu, Y. G., Deacon, C., Raab, A., et al. (2008). Inorganic arsenic in rice bran and its products are an order of magnitude higher than in bulk grain. *Environ Sci Technol, 42*(19), 7542-7546.

Vahter, M. (2000). Genetic polymorphism in the biotransformation of inorganic arsenic and its role in toxicity. *Toxicology Letters, 112-113*, 209-217. doi:10.1016/s0378-4274(99)00271-4

Wang, W., Xie, Z., Lin, Y., & Zhang, D. (2014). Association of inorganic arsenic exposure with type 2 diabetes mellitus: a meta-analysis. *J Epidemiol Community Health, 68*(2), 176-184. doi:10.1136/jech-2013-203114

WHO. (2012). Arsenic in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. *WHO/SDE/WSH/03.04/75/Rev/1*

Xue, J., Zartarian, V., Wang, S. W., Liu, S. V., & Georgopoulos, P. (2010). Probabilistic Modeling of Dietary Arsenic Exposure and Dose and Evaluation with 2003-2004 NHANES Data. *Environ Health Perspect, 118*(3), 345-350. doi:10.1289/ehp.0901205

Yager, J. W., Greene, T., & Schoof, R. A. (2015). Arsenic relative bioavailability from diet and airborne exposures: Implications for risk assessment. *Sci Total Environ, 536*, 368-381. doi:10.1016/j.scitotenv.2015.05.141

Yehiayan, L., Pattabiraman, M., Kavallieratos, K., Wang, X., Boise, L. H., & Cai, Y. (2009). Speciation, formation, stability and analytical challenges of human arsenic metabolites. *J Anal At Spectrom, 24*(10), 1397-1405. doi:10.1039/B910943A

Yu, H. S., Liao, W. T., & Chai, C. Y. (2006). Arsenic carcinogenesis in the skin. *J Biomed Sci, 13*(5), 657-666. doi:10.1007/s11373-006-9092-8

Yuan, Y., Marshall, G., Ferreccio, C., Steinmaus, C., Liaw, J., Bates, M., et al. (2010). Kidney cancer mortality: fifty-year latency patterns related to arsenic exposure. *Epidemiology, 21*(1), 103-108. doi:10.1097/EDE.0b013e3181c21e46

Zagury, G. J., & Pouschat, P. (2005). Arsenic on children's hands after playing in playgrounds. *Environ Health Perspect, 113*(8), A508; author reply A508-509.

Tables

Table 1a. Categorical characteristics of participants and non-participants (FU interview)

		Participant		Non-participants		
		n=210	%	n=47	%	*p-value*
Characteristics						
Education						
	Not Highschool graduate	42	*20.0%*	8	*17.02%*	
	Highschool graduate	87	*41.43%*	23	*48.94%*	
	Superior education	81	*38.57%*	16	*34.04%*	*0.6410**
Civil status						
	Married	46	*21.90%*	12	*25.53%*	
	Living with partner	39	*18.57%*	12	*25.53%*	
	Single	121	*57.62%*	21	*44.68%*	
	Separated	4	*1.9%*	1	*2.13%*	
	Other	0	*0.0%*	1	*2.13%*	*0.1591†*
Ethnic origin						
	Caucasian	28	*59.57%*	28	*59.57%*	
	Aymara	15	*31.91%*	15	*31.91%*	
	Other	4	*8.51%*	4	*8.51%*	*0.5443†‡*
	Missing	1	*0.48%*	0	*0.0%*	
Income						
	Less or equal than CL$200,000	83	*39.52%*	22	*46.81%*	
	Between CL$200,001 and CL$400,000	88	*41.90%*	14	*29.79%*	
	More than CL$400,000	39	*18.57%*	11	*23.40%*	*0.3041**
Nationality						
	Other South American	19	*9.05%*	6	*12.77%*	
	Chilean	191	*90.95%*	41	*87.23%*	*0.4368**
Home street type						
	Private	104	*49.52%*	19	*40.43%*	*0.0941**
	Open with light/moderate traffic	56	*26.67%*	20	*42.55%*	
	Open with heavy traffic or highroad	50	*23.81%*	8	*17.02%*	
Asphalted street						
	No	34	*16.19%*	9	*19.15%*	*0.6233**
	Yes	176	*83.81%*	38	*80.85%*	

* Chi-square test

† Fisher's exact test (counts less than 5)

‡ Fisher's exact test excludes missing data

Table 1b. Continous characteristics of participants and non-participants (FU interview)

	Participant (n=210)					Non-participant (n=47)					
	n	Mean	SD	*Median*	*Range*	n	Mean	SD	*Median*	*Range*	*p-value**
Maternal characteristics at initial interview											
Urine AsIII (ug/L)	205	0.87	1.07	0.70	*0-9,30*	37	0.95	0.77	0.80	*0.05-3,60*	0.2057
Urine AsV (ug/L)	205	0.50	0.54	0.40	*0-4,20*	37	0.62	0.55	0.60	*0.05-2,70*	0.1669
Urine DMA (ug/L)	205	14.84	9.90	12.10	*1,4-61,30*	37	16.15	7.73	15.60	*5,30-39,10*	0.0977
Urine MMA (ug/L)	205	1.44	1.03	1.20	*0-10,00*	37	1.50	0.83	1.20	*0,40-4,70*	0.4681
AsIN (AsIII + AsV + DMA + MMA) (ug/L)	205	17.65	11.24	14.30	*2,00-69,30*	37	19.20	8.48	18.20	*8,20-41,40*	0.0985
Age (in years)	210	25.92	6.21	25.00	*16-44*	47	25.64	5.55	25.00	*17-36*	0.9567
Infant characteristics at birth											
Height (in cms)	200	49.88	2.27	50.00	*38,00-54,50*	37	49.55	2.40	49.50	*42,00-53,00*	0.4137
Weight (in grs)	200	3389.65	459.39	3400.00	*1540,00-4700,00*	38	3336.58	492.04	3385.00	*2210,00-4490,00*	0.5796

* Mann–Whitney–Wilcoxon Test

Table 2a, Categorical characteristics of participants with and without urine sample

		With urine sample		Without urine sample		
		n=192	%	n=18	%	*p-value†*
Characteristics						
Education						
	Not Highschool graduate	39	*20.3%*	3	*16.7%*	
	Highschool graduate	78	*40.6%*	9	*50.0%*	
	Superior education	75	*39.1%*	6	*33.3%*	*0.8091*
Civil status						
	Married	41	*21.4%*	5	*27.8%*	
	Living with partner	36	*18.8%*	3	*16.7%*	
	Single	112	*58.3%*	9	*50.0%*	
	Separated	3	*1.6%*	1	*5.6%*	*0.4256*
Ethnic origin						
	Caucasian	131	*59.6%*	8	*59.6%*	
	Aymara	50	*31.9%*	8	*31.9%*	
	Other	11	*8.5%*	1	*8.5%*	*0.1397*
Income						
	Less or equal than CL$200,000	76	*39.6%*	7	*38.9%*	
	Between CL$200,001 and CL$400,000	82	*42.7%*	6	*33.3%*	
	More than CL$400,000	34	*17.7%*	5	*27.8%*	*0.5237*
Nationality						
	Other South American	17	*8.9%*	2	*11.1%*	
	Chilean	175	*91.2%*	16	*88.9%*	*0.6699*
Home street type						
	Private	97	*50.5%*	7	*38.9%*	*0.4486*
	Open with light/moderate traffic	49	*25.5%*	7	*39.9%*	
	Open with heavy traffic or highroad	46	*24.0%*	4	*22.2%*	
Asphalted street						
	No	30	*15.6%*	4	*22.2%*	*0.502*
	Yes	162	*84.4%*	14	*77.8%*	

† Fisher's exact test (counts less or equal than 5)

Table 2b. Continous characteristics of participants with and without urine sample

	With urine sample (n=192)					Without urine sample (n=18)					
	n	Mean	SD	*Median*	*Range*	n	Mean	SD	*Median*	*Range*	*p-value**
Maternal characteristics at initial interview											
Urine AsIII (ug/L)	188	0.83	0.91	0.70	*0-8.1*	17	1.34	2.15	0.70	*0,05-9.3*	0.5831
Urine AsV (ug/L)	188	0.51	0.55	0.40	*0-4.2*	17	0.36	0.38	0.20	*0,05.1.3*	0.1614
Urine DMA (ug/L)	188	14.49	9.89	12.10	*1.4-61.3*	17	18.72	9.42	18.10	*7.1-36.3*	0,0473**
Urine MMA (ug/L)	188	1.46	1.06	1.20	*0-10,00*	17	1.25	0.68	1.30	*0,15-2.6*	0.6261
AsIN (AsIII + AsV + DMA + MMA) (ug/L)	188	17.29	11.20	14.00	*2,00-69,30*	17	21.63	11.16	19.00	*7.9-46.3*	0.0775
Age (in years)	192	25.96	6.24	25.00	*16-44*	18	25.50	6.11	25.50	*17-38*	0.8137
Infant characteristics at birth											
Height (in cms)	184	49.83	2.30	50.00	*38,00-54,50*	16	50.41	1.84	50.00	*48-54*	0.5859
Weight (in grs)	184	3376.20	458.73	3395.00	*1540,00-4700,00*	16	3544.38	452.55	3485.00	*2910-4420*	0.342

* Wilcoxon Two Sample Test

** Significant at alpha < 0.05

Table 3. Urine arsenic concentrations of participants

	Participants (n=192)							
	n	Mean	SD	%	Median	*Min*	*Max*	%<LOD*
AsB in Infant Urine (ug/L)	192	1.20	3.07	9.2**%	0.27	0.07	25.96	15.1%
AsC in Infant Urine (ug/L)†	192	0.17	0.38	---	0.07	3.92	0.07	80.7%
AsIII in Infant Urine (ug/L)	192	0.51	0.60	4.3%	0.28	0.07	3.23	26.0%
AsV in Infant Urine (ug/L)	192	1.31	1.17	11.1%	0.88	0.07	6.61	1.6%
DMA in Infant Urine (ug/L)	192	8.71	6.39	74.0%	7.22	1.02	28.11	---
MMA in Infant Urine (ug/L)	192	1.25	1.14	10.6%	0.84	0.12	7.66	---
Total iAs (AsIII+DMA+MMA+AsV) in infant urine (ug/L)	192	11.78	8.56	100.0%	9.32	1.55	38.24	---
AsIII in Mother Urine (ug/L)	188	0.84	0.90	4.9%	0.70	0.07	8.10	18.6%
AsV in Mother Urine (ug/L)	188	0.53	0.53	3.1%	0.40	0.07	4.20	25.0%
DMA in Mother Urine (ug/L)	188	14.49	9.89	83.8%	12.00	1.40	61.30	---
MMA in Mother Urine (ug/L)	188	1.46	1.05	8.4%	1.20	0.15	10.00	
Total iAs (AsIII+DMA+MMA+AsV) in mother urine (ug/L)	188	17.29	11.20	100.0%	14.00	2.00	69.30	1.1%

* Values below LOD (i.e. 0.1 ug/L) were set to LOD/sqrt(2)

** of Total iAS + AsB

† Excluded from further analyses

Table 4. Correlation Coefficients between mother and child urine arsenic species, Prob > |r| under H0: Rho=0 & Number of Observations

	logAsB in Infant Urine ((log)ug/L)	logAsIII in Infant Urine ((log)ug/L)	logAsV in Infant Urine ((log)ug/L)	logDMA in Infant Urine ((log)ug/L)	logMMA in Infant Urine ((log)ug/L)	Total logiAs (logAsIII+logDMA+logMMA+logAsV) in infant urine ((log)ug/L)	logAsIII in Mother Urine ((log)ug/L)	logAsV in Mother Urine ((log)ug/L)	logDMA in Mother Urine ((log)ug/L)	logMMA in Mother Urine ((log)ug/L)	Total logiAs (logAsIII+logAsV+logDMA+logMMA) in mother urine ((log)ug/L)
logAsB in Infant Urine ((log)ug/L)		0.0318 0.6615 192	**0.27566** **0.0001** **192**	**0.29101** **<.0001** **192**	**0.18582** **0.0099** **192**	**0.29192** **<.0001** **192**	-0.07309 0.3189 188	**-0.17501** **0.0163** **188**	-0.06423 0.3812 188	**-0.14719** **0.0438** **188**	-0.07575 0.3015 188
logAsIII in Infant Urine ((log)ug/L)	0.0318 0.6615 192		0.05507 0.448 192	**0.45732** **<.0001** **192**	**0.42783** **<.0001** **192**	**0.45527** **<.0001** **192**	-0.11122 0.1286 188	-0.01459 0.8424 188	-0.04164 0.5705 188	-0.01988 0.7865 188	-0.04614 0.5295 188
logAsV in Infant Urine ((log)ug/L)	**0.27566** **0.0001** **192**	0.05507 0.448 192		**0.67197** **<.0001** **192**	***0.66823*** ***<.0001*** ***192***	**0.72504** **<.0001** **192**	-0.02877 0.6951 188	0.05794 0.4297 188	*-0.06231* *0.3956* *188*	*-0.05651* *0.4411* *188*	*-0.05815* *0.428* *188*
logDMA in Infant Urine ((log)ug/L)	**0.29101** **<.0001** **192**	**0.45732** **<.0001** **192**	**0.67197** **<.0001** **192**		**0.90543** **<.0001** **192**	**0.99393** **<.0001** **192**	-0.02635 0.7196 188	-0.0197 0.7884 188	-0.05102 0.4869 188	-0.03076 0.6752 188	-0.0458 0.5326 188
logMMA in Infant Urine ((log)ug/L)	**0.18582** **0.0099** **192**	**0.42783** **<.0001** **192**	***0.66823*** ***<.0001*** ***192***	**0.90543** **<.0001** **192**		**0.92552** **<.0001** **192**	0.01042 0.8871 188	0.022 0.7644 188	*-0.03418* *0.6415* *188*	*0.0387* *0.598* *188*	*-0.02275* *0.7566* *188*
Total logiAs (logAsIII+logDMA+logMMA+logAsV) in infant urine ((log)ug/L)	**0.29192** **<.0001** **192**	**0.45527** **<.0001** **192**	**0.72504** **<.0001** **192**	**0.99393** **<.0001** **192**	**0.92552** **<.0001** **192**		-0.02044 0.7807 188	-0.00799 0.9133 188	-0.05516 0.4521 188	-0.02279 0.7562 188	-0.04787 0.5142 188
logAsIII in Mother Urine ((log)ug/L)	-0.07309 0.3189 188	-0.11122 0.1286 188	-0.02877 0.6951 188	-0.02635 0.7196 188	0.01042 0.8871 188	-0.02044 0.7807 188		**0.17918** **0.0139** **188**	**0.3655** **<.0001** **188**	**0.52402** **<.0001** **188**	**0.45061** **<.0001** **188**
logAsV in Mother Urine ((log)ug/L)	**-0.17501** **0.0163** **188**	-0.01459 0.8424 188	0.05794 0.4297 188	-0.0197 0.7884 188	0.022 0.7644 188	-0.00799 0.9133 188	**0.17918** **0.0139** **188**		**0.16436** **0.0242** **188**	**0.1985** **0.0063** **188**	**0.23606** **0.0011** **188**
logDMA in Mother Urine ((log)ug/L)	-0.06423 0.3812 188	-0.04164 0.5705 188	*-0.06231* *0.3956* *188*	-0.05102 0.4869 188	*-0.03418* *0.6415* *188*	-0.05516 0.4521 188	**0.3655** **<.0001** **188**	**0.16436** **0.0242** **188**		**0.65085** **<.0001** **188**	**0.988** **<.0001** **188**
logMMA in Mother Urine ((log)ug/L)	**-0.14719** **0.0438** **188**	-0.01988 0.7865 188	*-0.05651* *0.4411* *188*	-0.03076 0.6752 188	*0.0387* *0.598* *188*	-0.02279 0.7562 188	**0.52402** **<.0001** **188**	**0.1985** **0.0063** **188**	**0.65085** **<.0001** **188**		**0.71621** **<.0001** **188**
Total logiAs (logAsIII+logDMA+logMMA+logAsV) in mother urine ((log)ug/L)	-0.07575 0.3015 188	-0.04614 0.5295 188	*-0.05815* *0.428* *188*	-0.0458 0.5326 188	*-0.02275* *0.7566* *188*	-0.04787 0.5142 188	**0.45061** **<.0001** **188**	**0.23606** **0.0011** **188**	**0.988** **<.0001** **188**	**0.71621** **<.0001** **188**	

Italic: Pearson Correlation Coefficient

Bold: P < 0.05

Table 5b. Toddler Urine DMA, MMA and total arsenic concentrations and distributions by significant characteristics

			DMA in Infant Urine (ug/L)				MMA in Infant Urine (ug/L)				AsIn in Infant Urine (ug/L)			
		n	Mean	SD	%	p-value*	Mean	SD	%	p-value*	Mean	SD	%	p-value*
Overall			8.7133	6.3862	74.0%		1.2506	1.1353	10.6%		11.7824	8.5631	100.0%	
Time of the interview/sampling	AM	85	8.3597	5.8675	75.4%	0.7017	1.2747	1.2487	11.5%	0.8394	11.0807	7.9220	100.0%	0.4802
	PM	107	8.9943	6.7841	72.9%		1.2314	1.0420	10.0%		12.3398	9.0380	100.0%	
Daycare attendance	No	144	**8.1916**	**5.8957**	**74.3%**	**0.0048**	**1.1649**	**1.0309**	**10.6%**	**0.0050**	**11.0242**	**7.8918**	**100.0%**	**0.0035**
	Yes	21	**12.6576**	**7.7285**	**74.0%**		**1.9433**	**1.7004**	**11.4%**		**17.0966**	**10.1241**	**100.0%**	
Asphalted street	No	30	**11.3100**	**6.3750**	**75.2%**		**1.6257**	**1.4290**	**10.8%**		**15.0432**	**8.5875**	**100.0%**	
	Yes	162	**8.2325**	**6.2907**	**73.6%**	**0.0073**	**1.1811**	**1.0631**	**10.6%**	**0.0397**	**11.1786**	**8.4474**	**100.0%**	**0.0104**
Cereal in the last 48 hours	No	99	8.2481	5.8141	74.5%	0.6366	1.1338	1.0099	10.2%	0.2140	11.0734	7.8602	100.0%	0.4135
	Yes	74	9.3133	7.1465	73.0%		1.4418	1.3368	11.3%		12.7612	9.5625	100.0%	
Formula in the last 48 hours	No	37	7.5510	5.4718	72.8%		1.1385	1.0930	11.0%		10.3702	7.5778	100.0%	
	Yes	138	8.9605	6.6087	74.0%	0.3895	1.2903	1.1825	10.7%	0.3310	12.1060	8.8577	100.0%	0.3915
Formula portions	No formula	37	7.5510	5.4718	72.8%	0.5988	1.1385	1.0930	11.0%	0.7573	10.3702	7.5779	100.0%	0.6924
	1-5 portions	39	8.5804	6.7499	71.9%		1.4275	1.5341	12.0%		11.9409	9.5354	100.0%	
	6-9 portions	68	9.3079	6.4673	75.1%		1.2190	0.8877	9.8%		12.3997	8.4944	100.0%	
	10+ portions	24	9.4123	7.5359	75.3%		1.3623	1.3804	10.9%		12.5026	9.8837	100.0%	
Tangerine in the last 48 hours	No	149	8.9310	6.7000	73.5%	0.2707	**1.3316**	**1.2189**	**11.0%**	**0.0121**	12.1503	9.0321	100.0%	0.1612
	Yes	8	5.6188	1.6506	78.2%		**0.5078**	**0.2641**	**7.1%**		7.1817	2.2318	100.0%	
Watermelon in the last 48 hours	No	**147**	**8.4268**	**6.3239**	**73.5%**	**0.0266**	**1.2364**	**1.1507**	**10.8%**	**0.0489**	**11.4630**	**8.5325**	**100.0%**	**0.0374**
	Yes	**10**	**13.6935**	**8.4825**	**74.9%**		**2.0712**	**1.6899**	**11.3%**		**18.2794**	**11.6971**	**100.0%**	
Water source for drinking	Bottled	84	**8.0631**	**6.8024**	**73.6%**	**0.0185**	1.2646	1.3927	11.5%	0.0643	**10.9501**	**9.2808**	**100.0%**	**0.0126**
	Tap	76	**10.0119**	**6.5958**	**74.3%**		1.3310	0.9662	9.9%		**13.4761**	**8.6765**	**100.0%**	
	Both	24	**6.7716**	**3.5231**	**73.3%**		0.9940	0.6511	10.8%		**9.2321**	**4.7047**	**100.0%**	
Breastmilk in the last 48 hours	No	69	9.0712	6.5614	75.5%	0.4273	1.1892	0.9321	9.9%	0.7624	12.0224	8.5490	100.0%	0.5588
	Yes	106	8.3965	6.3040	72.7%		1.3031	1.2933	11.3%		11.5545	8.6877	100.0%	
Breastmilk portions	0 to 5 portions	95	9.4073	6.5126	74.4%	0.2360	1.2733	0.9460	10.1%	0.2273	12.6472	8.5693	100.0%	0.2302
	6+ portions	52	7.6420	5.5660	73.1%		1.1615	1.3496	11.1%		10.4504	7.9001	100.0%	
	Ad-libitum	20	8.4829	7.5817	73.8%		1.4063	1.4778	12.2%		11.5014	10.3141	100.0%	
Meat in the last 48 hours	No	92	8.2915	5.8720	74.5%	0.6157	1.2007	1.1435	10.8%	0.6746	11.1330	7.8742	100.0%	0.4929
	Yes	83	9.0738	6.9447	73.1%		1.3219	1.1874	10.7%		12.4107	9.3642	100.0%	
Chicken/Turkey portions	No chicken/turkey	26	**8.1246**	**7.4541**	**73.0%**	**0.0407**	**1.3109**	**1.2879**	**11.8%**	**0.0336**	**11.1267**	**10.0186**	**100.0%**	**0.0334**
	1 portion	46	**8.0411**	**5.5775**	**74.6%**		**1.0754**	**0.8696**	**10.0%**		**10.7739**	**7.4367**	**100.0%**	
	2 portions	52	**10.7239**	**7.2042**	**74.3%**		**1.5205**	**1.2035**	**10.5%**		**14.4254**	**9.4360**	**100.0%**	
	3+ portions	41	**7.0745**	**4.8886**	**73.2%**		**1.0302**	**1.2419**	**10.7%**		**9.6703**	**7.1471**	**100.0%**	
Rice in the last 48 hours	No	40	7.1265	5.1118	72.3%	0.0833	1.0580	0.9334	10.7%	0.1149	9.8504	7.3276	100.0%	0.0760
	Yes	110	9.3949	6.8815	74.3%		1.3269	1.1467	10.5%		12.6425	9.1093	100.0%	
Seafood in the last 48 hours	No	151	8.4782	6.2763	73.7%	0.4337	1.2400	1.1463	10.8%	0.9550	11.4984	8.4730	100.0%	0.4161
	Yes	24	9.8219	7.1417	74.1%		1.3730	1.2815	10.4%		13.2529	9.4900	100.0%	
Cauliflower in the last 48 hours	No	165	**8.7353**	**6.4334**	**73.9%**	**0.0247**	**1.2700**	**1.1778**	**10.7%**	**0.0406**	**11.8195**	**8.6412**	**100.0%**	**0.0222**
	Yes	3	**2.5500**	**0.6022**	**74.4%**		**0.3789**	**0.1720**	**11.1%**		**3.4264**	**0.5798**	**100.0%**	
Corn in the last 48 hours	No	162	8.5269	6.2945	74.0%	0.6292	1.2397	1.1610	10.8%	0.5411	11.5157	8.4085	100.0%	0.5932
	Yes	6	11.2683	9.7836	71.2%		1.6441	1.5464	10.4%		15.8264	13.8818	100.0%	

*Wilcoxon-Mann-Whitney test if two levels, Kruskal Wallis test if 3 or more levels.
** of Total iAS + AsB
Bold p-value < 0.05

Table 5a. Toddler Urine AsB, AsIII and AsV concentrations and distributions by significant characteristics

			AsB in Infant Urine (ug/L)				AsIII in Infant Urine (ug/L)				AsV in Infant Urine (ug/L)			
		n	Mean	SD	%**	p-value*	Mean	SD	%	p-value*	Mean	SD	%	p-value*
Overall		192	1.1995	3.0736	9.2%		0.5104	0.6012	4.3%		1.3081	1.1657	11.1%	
Time of the interview/sampling	AM	85	1.0717	3.1850	8.8%	0.0691	0.4725	0.5311	4.3%	0.7596	**0.9739**	**0.9269**	8.8%	**0.0002**
	PM	107	1.3009	2.9933	9.5%		0.5406	0.6524	4.4%		**1.5736**	**1.2677**	12.8%	
Daycare attendance	No	144	1.1748	2.7765	9.6%	0.3612	**0.4710**	**0.5537**	**4.3%**	**0.0405**	**1.1966**	**1.0611**	**10.9%**	**0.0237**
	Yes	21	2.2170	5.6502	11.5%		**0.7576**	**0.7368**	**4.4%**		**1.7381**	**1.2899**	**10.2%**	
Asphalted street	No	30	0.6377	0.8979	4.1%		**0.7403**	**0.6549**	**4.9%**		1.3671	0.8848	9.1%	
	Yes	162	1.3035	3.3154	10.4%	0.3600	**0.4679**	**0.5830**	**4.2%**	**0.0031**	1.2972	1.2126	11.6%	0.1827
Cereal in the last 48 hours	No	99	1.2330	3.6759	10.0%	0.3367	0.5209	0.5880	4.7%	0.1077	**1.1705**	**1.1001**	**10.6%**	**0.0337**
	Yes	74	1.1883	2.3479	8.5%		0.4833	0.6490	3.8%		**1.5227**	**1.2705**	**11.9%**	
Formula in the last 48 hours	No	37	**1.9361**	**4.2314**	**15.7%**		**0.2221**	**0.2368**	**2.1%**		1.4585	1.4025	14.1%	
	Yes	138	**1.0045**	**2.7805**	**7.7%**	**0.0065**	**0.5793**	**0.6561**	**4.8%**	**<.0001**	1.2758	1.1159	10.5%	0.9301
Formula portions	No formula	37	**1.9361**	**4.2314**	**15.7%**	**0.0425**	**0.2221**	**0.2368**	**2.1%**	**0.0002**	1.4585	1.4025	14.1%	0.8853
	1-5 portions	39	**0.8388**	**1.9302**	**6.6%**		**0.5232**	**0.6586**	**4.4%**		1.4098	1.4355	11.8%	
	6-9 portions	68	**1.1307**	**3.4815**	**8.4%**		**0.6181**	**0.6950**	**5.0%**		1.2548	1.0309	10.1%	
	10+ portions	24	**0.8913**	**1.8404**	**6.7%**		**0.6780**	**0.6057**	**5.4%**		1.0498	0.8305	8.4%	
Tangerine in the last 48 hours	No	149	1.0395	2.5061	7.9%	0.4666	0.5205	0.6410	4.3%	0.9679	1.3672	1.2393	11.3%	0.1244
	Yes	8	2.8915	4.5473	28.7%		0.3369	0.2071	4.7%		0.7182	0.3885	10.0%	
Watermelon in the last 48 hours	No	147	1.1507	2.7350	9.1%	0.2863	0.4869	0.5935	4.2%	0.3805	1.3129	1.2266	11.5%	0.2124
	Yes	10	0.8868	0.9171	4.6%		0.8678	0.9761	4.7%		1.6470	1.0989	9.0%	
Water source for drinking	Bottled	84	1.5779	4.0899	12.6%	0.5935	0.4676	0.5958	4.3%	0.1206	**1.1548**	**1.2036**	**10.5%**	**0.0102**
	Tap	76	0.9112	2.1609	6.3%		0.5873	0.6505	4.4%		**1.5460**	**1.2389**	**11.5%**	
	Both	24	0.8192	0.9241	8.2%		0.3686	0.3402	4.0%		**1.0979**	**0.7162**	**11.9%**	
Breastmilk in the last 48 hours	No	69	1.1579	3.4402	8.8%	0.2856	**0.5529**	**0.5905**	**4.6%**	**0.0485**	1.2092	0.9609	10.1%	0.9976
	Yes	106	1.2298	2.9666	9.6%		**0.4719**	**0.6228**	**4.1%**		1.3830	1.3033	12.0%	
Breastmilk portions	0 to 5 portions	95	1.0648	2.9948	7.8%	0.7243	**0.6093**	**0.6630**	**4.8%**	**0.0363**	1.3573	1.1314	10.7%	0.2579
	6+ portions	52	1.3505	3.7462	11.4%		**0.4064**	**0.5774**	**3.9%**		1.2406	1.2417	11.9%	
	Ad-libitum	20	1.5229	2.7105	11.7%		**0.3101**	**0.2483**	**2.7%**		1.3022	1.4019	11.3%	
Meat in the last 48 hours	No	92	1.2085	3.1263	9.8%	0.2858	0.5170	0.5732	4.6%	0.5068	**1.1239**	**1.0708**	**10.1%**	**0.0132**
	Yes	83	1.1936	3.1999	8.8%		0.4892	0.6513	3.9%		**1.5257**	**1.2640**	**12.3%**	
Chicken/Turkey portions	No chicken/turkey	26	1.0118	2.0557	8.3%	0.6495	0.4785	0.6855	4.3%	0.4892	1.2127	1.1167	10.9%	0.1797
	1 portion	46	1.7314	4.3377	13.8%		0.5452	0.6866	5.1%		1.1122	0.9067	10.3%	
	2 portions	52	1.2427	3.3438	7.9%		0.5509	0.5497	3.8%		1.6301	1.4626	11.3%	
	3+ portions	41	0.7584	1.9805	7.3%		0.4527	0.5961	4.7%		1.1129	1.0603	11.5%	
Rice in the last 48 hours	No	40	1.3800	3.9809	12.3%	0.6200	0.5637	0.7404	5.7%	0.6899	**1.1022**	**1.0966**	**11.2%**	**0.0307**
	Yes	110	1.2105	2.9600	8.7%		0.4734	0.5489	3.7%		**1.4473**	**1.2683**	**11.4%**	
Seafood (fish and shelfish) in the last 48 hours	No	151	**0.9711**	**3.1235**	**7.8%**	**<.0001**	0.5051	0.6283	4.4%	0.4382	1.2751	1.1195	11.1%	0.3356
	Yes	24	**2.6505**	**3.0019**	**16.7%**		0.4959	0.4890	3.7%		1.5622	1.5138	11.8%	
Cauliflower in the last 48 hours														
	No	165	1.1957	3.1843	9.2%	0.4533	0.5026	0.6088	4.3%	0.0754	1.3116	1.1670	11.1%	0.0531
	Yes	3	0.2211	0.1314	6.1%		0.0958	0.0435	2.8%		0.4017	0.2022	11.7%	
Corn in the last 48 hours														
	No	162	**1.0378**	**2.5494**	**8.3%**	**0.0269**	0.4879	0.5889	4.2%	0.7593	1.2612	1.0892	11.0%	0.4941
	Yes	6	**4.9699**	**10.2950**	**23.9%**		0.6964	1.0203	4.4%		2.2176	2.4311	14.0%	

*Wilcoxon-Mann-Whitney test if two levels, Kruskal Wallis test if 3 or more levels.
** of Total iAS + AsB
Bold p-value < 0.05

Table 6a. Estimated percent change (95% CI) in toddler urinary arsenic metabolites (AsB, AsIII and AsV) concentration according to covariates from a multiple linear regression model.

		Multivariate† (n=167; R2=0.2125) logAsB in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Seafood in the last 48 hours	No	0.0 (ref)						
	Yes	1.05649	0.4518	1.6611	2.8762	1.5712	5.2653	0.0007
Formula	No	0	.	.				.
	Yes	-0.5209	-1.0232	-0.0187	0.5940	0.3594	0.9815	0.0422
Corn in the last 48 hours	No	0	.	.				.
	Yes	1.43054	0.3961	2.465	4.1810	1.4860	11.7637	0.007

		Multivariate† (n=167; R2=0.1902) logAsIII in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Formula portions	No formula	0.0 (ref)						
	1-5 portions	0.7105	0.1977	1.2233	2.0350	1.2186	3.3983	0.0069
	6-9 portions	0.93979	0.4699	1.4096	2.5594	1.5999	4.0945	0.0001
	10+ portions	1.218	0.6323	1.8037	3.3804	1.8820	6.0719	<.0001
Asphalted street	No							
	Yes	-0.3793	-0.8739	0.1153	0.6843	0.4173	1.1222	0.1318

		Multivariate† (n=130; R2=0.3669) logAsV in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Time of sampling	AM	0.0 (ref)						
	PM	0.42469	0.1115	0.7378	1.5291	1.1180	2.0914	0.0083
Water source for drinking	Bottled	0	.	.				.
	Tap	0.61229	0.3018	0.9228	1.8446	1.3523	2.5162	0.0002
	Both	0.05399	-0.4163	0.5243	1.0555	0.6595	1.6893	0.8204
Rice in the last 48 hours	No	0	.	.				.
	Yes	0.49121	0.1752	0.8072	1.6343	1.1915	2.2416	0.0026
Meat portions	No meat	0	.	.				.
	1 portion	0.1106	-0.2363	0.4575	1.1169	0.7895	1.5801	0.5288
	2+ portions	0.59975	0.2141	0.9854	1.8217	1.2387	2.6790	0.0026
Home street type	Private	0	.	.				.
	Light/moderate traffic	-0.2045	-0.5692	0.1601	0.8150	0.5660	1.1736	0.2687
	Heavy traffic or highroad	-0.4294	-0.7854	-0.0734	0.6509	0.4560	0.9292	0.0185
Cereal portions in the last 48 hours	No cereal	0	.	.				.
	1-3 portions	0.33659	0.0024	0.6708	1.4002	1.0024	1.9558	0.0484
	4+ portions	-0.1382	-0.5727	0.2962	0.8709	0.5640	1.3447	0.5296

*Multivariate model was adjusted by toddler age and sex; and maternal age, education, income, civil status and ethnic origin.

Table 6b. Estimated percent change (95% CI) in toddler urinary arsenic metabolites (DMA, MMA and AsIN) concentration according to covariates from a multiple linear regression model.

		Multivariate† (n=125; R2=0.3304)						
		logDMA in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Daycare attendance	No	0.0 (ref)						
	Yes	0.66367	0.2635	1.0638	1.9419	1.3015	2.8974	0.0014
Breastmilk portions	0-5 portions	0	.	.				.
	6+ portions	-0.3164	-0.6088	-0.024	0.7288	0.5440	0.9763	0.0342
	Ad-libitum	-0.074	-0.4802	0.3321	0.9286	0.6186	1.3940	0.7185
Watermelon in the last 48 hours	No	0	.	.				.
	Yes	0.76718	0.2392	1.2952	2.1537	1.2702	3.6517	0.0048
Cauliflower in the last 48 hours	No	0	.	.				.
	Yes	-0.8746	-1.7362	-0.013	0.4170	0.1762	0.9871	0.0467
Chicken or turkey portions	No chicken/turkey	0	.	.				.
	1 portion	0.05984	-0.3487	0.4684	1.0617	0.7056	1.5974	0.772
	2 portions	0.48507	0.0803	0.8899	1.6243	1.0836	2.4348	0.0193
	3+ portions	0.06991	-0.3568	0.4966	1.0724	0.6999	1.6432	0.7459

		Multivariate† (n=130; R2=0.2989)						
		logMMA in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Daycare attendance	No	0.0 (ref)						
	Yes	0.63031	0.2005	1.0601	1.8782	1.2220	2.8868	0.0044
Breastmilk portions	0-5 portions	0	.	.				.
	6+ portions	-0.3832	-0.7063	-0.06	0.6817	0.4935	0.9417	0.0206
	Ad-libitum	0.02561	-0.4309	0.4821	1.0259	0.6499	1.6195	0.9117
Watermelon in the last 48 hours	No	0	.	.				.
	Yes	0.74733	0.1523	1.3424	2.1114	1.1645	3.8281	0.0143
Tangerine in the last 48 hours	No	0	.	.				.
	Yes	-0.8153	-1.463	-0.1676	0.4425	0.2315	0.8457	0.0141
Cauliflower in the last 48 hours	No	0	.	.				.
	Yes	-1.0438	-2.0131	-0.0745	0.3521	0.1336	0.9283	0.0351

		Multivariate† (n=125; R2=0.3419)						
		logASIN in toddler Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Daycare attendance	No	0.0 (ref)						
	Yes	0.65238	0.2624	1.0424	1.9201	1.3000	2.8359	0.0013
Breastmilk portions	0-5 portions	0	.	.				.
	6+ portions	-0.305	-0.59	-0.02	0.7371	0.5543	0.9802	0.0362
	Ad-libitum	-0.0632	-0.459	0.3327	0.9388	0.6319	1.3948	0.7524
Watermelon in the last 48 hours	No	0	.	.				.
	Yes	0.71378	0.1992	1.2284	2.0417	1.2204	3.4158	0.007
Cauliflower in the last 48 hours	No	0	.	.				.
	Yes	-0.8852	-1.7249	-0.0454	0.4126	0.1782	0.9556	0.039
Chicken or turkey portions	No chicken/turkey	0	.	.				.
	1 portion	0.00972	-0.3885	0.4079	1.0098	0.6781	1.5036	0.9615
	2 portions	0.45178	0.0572	0.8463	1.5711	1.0589	2.3311	0.0252
	3+ portions	0.0398	-0.3761	0.4557	1.0406	0.6865	1.5773	0.8499

*Multivariate model was adjusted by toddler age and sex; and maternal age, education, income, civil status and ethnic origin.

Table 7a. Estimated percent change (95% CI) in maternal AsIII, ASV and DMA urinary arsenic species concentration according to covariates from univariate and multiple linear regression models.

		Multivariate† (n=192, R2=0.0688) logAsIII in Mother Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Water source for drinking	Bottled							
	Tap	0.3996	0.05306	0.74624	1.4913	1.0545	2.1091	0.0241
Ethnic origin	Caucasian							
	Aymara	-0.3771	-0.7611	0.00696	0.6859	0.4671	1.0070	0.0543
	Other	-0.3260	-1.0454	0.39349	0.7218	0.3515	1.4821	0.3726
Civil status	Married							
	Living with partner	-0.2920	-0.8102	0.22614	0.7467	0.4448	1.2538	0.2676
	Single	-0.5249	-0.9419	-0.1078	0.5916	0.3899	0.8978	0.0139
	Separated	-0.6140	-1.9732	0.74517	0.5412	0.1390	2.1068	0.3739

		Multivariate† (n=192, R2=0.0948) logAsV in Mother Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Water source for drinking	Bottled	0.38788	0.07408	0.70168	1.4739	1.0769	2.0171	0.0157
	Tap							
Home street type	Private	-0.4031	-0.7673	-0.039	0.6682	0.4643	0.9618	0.0302
	Open with light/moderate traffic	-0.2621	-0.6362	0.11202	0.7695	0.5293	1.1185	0.1686
	Open with heavy traffic or highroad							
Mother's age		-0.0304	-0.0548	-0.006	0.9701	0.9467	0.9940	0.0149

		Multivariate† (n=192, R2=0.1584) logDMA in Mother Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Water source for drinking	Bottled	0.0 (ref)						
	Tap	0.38634	0.1063	0.66639	1.4716	1.1122	1.9472	0.0071
Asphalted street	No	0.0 (ref)						
	Yes	-0.3662	-0.7364	0.00407	0.6934	0.4788	1.0041	0.0525
Home street type	Private	0.0 (ref)						
	Open with light/moderate traffic	-0.3119	-0.6302	0.00641	0.7321	0.5325	1.0064	0.0547
	Open with heavy traffic or highroad	-0.0911	-0.4164	0.23406	0.9129	0.6594	1.2637	0.5809
Education	Not Highschool graduate	0.0 (ref)						
	Highschool graduate	0.18235	-0.1738	0.53854	1.2000	0.8404	1.7135	0.3138
	Superior education	0.39339	0.02692	0.75987	1.4820	1.0273	2.1380	0.0355
Ethnic origin	Caucasian	0.0 (ref)						
	Aymara	0.30188	-0.0068	0.61059	1.3524	0.9932	1.8415	0.0552
	Other	-0.0545	-0.629	0.51995	0.9469	0.5331	1.6819	0.8517
Civil status	Married	0.0 (ref)						
	Living with partner	-0.2933	-0.7096	0.12302	0.7458	0.4918	1.1309	0.1662
	Single	-0.5006	-0.8334	-0.1678	0.6061	0.4346	0.8455	0.0034
	Separated	-1.0169	-2.1129	0.07907	0.3617	0.1209	1.0823	0.0688

Table 7b. Estimated percent change (95% CI) in maternal MMA and total urinary arsenic species concentration according to covariates from univariate and multiple linear regression models.

		Multivariate† (n=192, R2=0.1381) logMMA in Mother Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Water source for drinking	Bottled	0.0 (ref)						
	Tap	0.2802	0.06141	0.49899	1.3234	1.0633	1.6471	0.0124
Home street type	Private	0.0 (ref)						
	Open with light/moderate traffic	-0.339	-0.5919	-0.0861	0.7125	0.5533	0.9175	0.0089
	Open with heavy traffic or highroad	-0.2939	-0.553	-0.0348	0.7454	0.5752	0.9658	0.0264
Education	Not Highschool graduate	0.0 (ref)						
	Highschool graduate	0.0888	-0.1952	0.37277	1.0929	0.8227	1.4517	0.538
	Superior education	0.26087	-0.0271	0.54881	1.2981	0.9733	1.7312	0.0755
Civil status	Married	0.0 (ref)						
	Living with partner	-0.3376	-0.6681	-0.0071	0.7135	0.5127	0.9929	0.0453
	Single	-0.3917	-0.6552	-0.1283	0.6759	0.5193	0.8796	0.0038
	Separated	-1.0454	-1.9156	-0.1751	0.3516	0.1472	0.8393	0.0188

		Multivariate† (n=188, R2=0.09668) logAsIN in Mother Urine (log(ug/L))						
		Estimate	Lower (95% CI)	Upper (95% CI)	exp (Estimate)	Lower (95% CI)	Upper (95% CI)	p-value
Water source for drinking	Bottled	0.0 (ref)						
	Tap	0.21887	0.04671	0.39103	1.2447	1.0478	1.4785	0.013
Civil status	Married	0.0 (ref)						
	Living with partner	-0.2731	-0.5333	-0.0129	0.7610	0.5867	0.9872	0.0398
	Single	-0.2814	-0.4908	-0.0721	0.7547	0.6122	0.9305	0.0087
	Separated	-1.0307	-1.7116	-0.3498	0.3568	0.1806	0.7048	0.0032

Appendix A

EMORY UNIVERSITY | Institutional Review Board

December 22, 2015

Maureen Hitschfeld
Emory University
Atlanta, GA 30322

RE: Determination: No IRB Review Required
Title: *Arsenic exposure determinants on one year old infants in Arica, Chile*
PI: Maureen Hitschfeld

Dear Maureen:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definitions of research with "human subjects" or "clinical investigation" as set forth in Emory policies and procedures and federal rules, if applicable. The purpose of this thesis is to explore determinants of acute arsenic concentration on urine samples obtained from children at 12 months of age by analyzing data collected in a previous study without access to identifiers (secondary analysis). Specifically, you will receive a data set that contains no identifying information from a previously conducted study. The study was conducted by a research team based in Chile with no affiliation with Emory. Neither you nor anyone on your thesis committee will have access to identifiers or the original dataset.

Please note that this determination does not mean that you cannot publish the results. If you have questions about this issue, please contact me.

This determination could be affected by substantive changes in the study design, subject populations, or identifiability of data. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Carolyn Sims, MPA
Research Protocol Analyst

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