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<http://dx.doi.org/10.1289/ehp.1307476>

Received: 7 August 2013

Accepted: 15 October 2014

Advance Publication: 17 October 2014

This article will be available in its final, 508-conformant form 2–4 months after Advance Publication. If you require assistance accessing this article before then, please contact [Dorothy L. Ritter](#), *EHP* Web Editor. *EHP* will provide an accessible version within 3 working days of request.



National Institute of
Environmental Health Sciences

Maternal Arsenic Exposure, Arsenic Methylation Efficiency, and Birth Outcomes in the Biomarkers of Exposure to ARsenic (BEAR) Pregnancy Cohort in Mexico

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Running title: Maternal arsenic methylation and birth outcomes

Acknowledgments: This research was supported in part by grants from the National Institute of Environmental Health Sciences (P30ES010126, P42ES005948, R01ES019315 and T32ES07018).

Competing financial interests: The authors claim no competing financial interests.

Abstract

Background: Exposure to inorganic arsenic (iAs) from drinking water is a global public health problem yet much remains unknown about the extent of exposure in susceptible populations.

Objectives: To establish the Biomarkers of Exposure to ARsenic (BEAR) prospective pregnancy cohort in Gómez Palacio, Mexico, to better understand the effects of iAs exposure on pregnant women and their children.

Methods: Two hundred pregnant women were recruited for this study. Concentrations of iAs in drinking water (DW-iAs) and maternal urinary concentrations of iAs and its monomethylated and dimethylated metabolites (MMAs and DMAs, respectively) were determined. Birth outcomes were analyzed for their relationship to DW-iAs and to the concentrations and proportions of maternal urinary arsenicals.

Results: DW-iAs for the study subjects ranged from <0.5 to 236 µg As/L. More than half of the women (53%) had DW-iAs that exceeded the World Health Organization's recommended guideline of 10 µg As/L. DW-iAs was significantly associated with the sum of the urinary arsenicals (U-tAs). Maternal urinary concentrations of MMAs were negatively associated with newborn birth weight and gestational age. Maternal urinary concentrations of iAs were associated with lower mean gestational age and newborn length.

Conclusions: Biomonitoring results demonstrate that pregnant women in Gómez Palacio are exposed to potentially harmful levels of DW-iAs. The data support a relationship between iAs metabolism in pregnant women and adverse birth outcomes. The results underscore the risks associated with iAs exposure in vulnerable populations.

Introduction

The World Health Organization (WHO) has established guidelines suggesting levels of arsenic (As) in drinking water should not exceed 10 µg/L (WHO 1993). Several countries including the United States of America (US), India, Taiwan, Vietnam, and Japan have adopted the WHO's guidelines in the establishment of their maximum contaminant levels (MCL) for As in drinking water, yet others have a higher MCL including Mexico at 25 µg As/L and Bangladesh at 50 µg As/L (Chen and Chiou 2011; Nordstrom 2002; Salud 2000). Even with these guidelines in place, there are currently millions of people worldwide who are drinking water with levels of As that greatly exceed the WHO standard or national standards (Centeno et al. 2007; Uddin and Huda 2011).

Exposure to elevated inorganic arsenic (iAs) in drinking water is a major public health concern as it has been linked with numerous adverse health outcomes (Kapaj et al. 2006; M Rahman et al. 2009). The detrimental effects from long-term exposure include skin lesions, cardiovascular disease, peripheral vascular disease, diabetes mellitus, and cancers of the urinary bladder, skin, lung, liver and prostate (Del Razo et al. 2011; IARC 2004; Maull et al. 2012; M Rahman et al. 2009; Tseng 2007b). It is also evident that certain populations, including pregnant women and their unborn children, are particularly susceptible to the adverse effects of iAs exposure (Vahter 2009). For instance, prenatal iAs exposure has been associated with a greater risk of adverse pregnancy outcomes such as preterm birth, low birth weight, and/or fetal loss (as reviewed in (Vahter 2009)). In addition, exposure to high levels of iAs *in utero* or in early childhood has been associated with multiple adverse health outcomes, including both cancer and non-cancer endpoints, later in life (Smith et al. 2006). The biological mechanisms that determine the nature and

severity of adverse effects in iAs-exposed populations involve multiple factors such as the dose and duration of iAs exposure, efficiency of iAs metabolism, genetic background, nutritional status, and co-exposure to other toxicants (Gebel 2000; Kapaj et al. 2006; Vahter and Concha 2001).

Along with life stage at time of exposure, the efficiency of iAs metabolism is a well-documented risk factor for the development of several iAs-associated diseases (as reviewed in (Tseng 2007a)). In humans, iAs has a biological half-life of approximately 24 hours (Buchet et al. 1981) and is metabolized to produce monomethylated and dimethylated arsenicals (MMAs and DMAs, respectively). Six major arsenicals associated with iAs exposure and metabolism have been detected in human urine, namely arsenite (iAs^{III}), arsenate (iAs^V), monomethylarsonous acid (MMA^{III}), monomethylarsonic acid (MMA^V), dimethylarsinous acid (DMA^{III}) and dimethylarsinic acid (DMA^V) (Thomas et al. 2001; Vahter 2002). Generally, total urinary arsenic in iAs-exposed individuals is composed of 10-20% total (trivalent + pentavalent) iAs, 10-20% total MMAs, and 60-80% total DMAs (Vahter 1999, 2002). In contrast to iAs, organic arsenicals such as arsenobetaine, arsenocholine, and arsenosugars are derived from food sources, primarily seafood (Choi et al. 2010). As arsenosugars can be metabolized to DMAs, the presence of DMAs in urine may reflect exposure to both iAs and organic arsenic compounds (Choi et al. 2010). 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 including urinary bladder cancer, non-melanoma skin cancers, carotid atherosclerosis, and chromosomal aberrations (as reviewed in (Tseng 2007a)). It is

known that pregnancy can alter the metabolism efficiency of iAs (Gardner et al. 2012; Hopenhayn et al. 2003b). However, the impact of this altered metabolism on pregnant women's or children's health is not well established.

We set out to understand the effects of iAs exposure and iAs metabolism during pregnancy on maternal/fetal health with the ultimate aim of increasing awareness and reducing exposures to iAs. To this end, we established the Biomarkers of Exposure to ARsenic (BEAR) prospective pregnancy cohort. Women recruited to participate in the BEAR project reside in the Gómez Palacio area, which is located in the state of Durango in the Lagunera region of Northern Mexico. It is believed that more than 450,000 people are exposed to levels of iAs in drinking water that exceed 50 µg/L in Mexico, including residents of Lagunera and Durango (Bundschuh et al. 2012). Multiple adverse health outcomes have been associated with high iAs exposure in Lagunera, including skin lesions (Del Razo et al. 1997) and diabetes mellitus (Del Razo et al. 2011). The effects of iAs exposure on pregnant women and their newborn children have not been studied in this region. This study investigates the association between iAs exposure during pregnancy, iAs metabolism efficiency, and birth outcomes in Gómez Palacio, Mexico.

Materials and Methods

Study design

BEAR participants, adult women, were recruited during the time frame of August 2011 to March 2012 at the General Hospital of Gómez Palacio. Recruitment took place prior to delivery, usually within 24 hours of birth. The mean gestational age at birth was 39 weeks with a range of 34-42 weeks. All procedures associated with this study were

approved by the Institutional Review Boards of Universidad Juarez del Estado de Durango (UJED), Gómez Palacio, Durango, Mexico, and the University of North Carolina at Chapel Hill (UNC), Chapel Hill, North Carolina, U.S.A. For each woman participation requirements at the time of recruitment included: (i) one year minimum residence in the Gómez Palacio region, which included urban locations of Gómez Palacio and surrounding rural locations, (ii) confirmation of a pregnancy without complications such as eclampsia or preeclampsia, and (iii) good overall health status (i.e. no signs of chronic or acute disease). A total of 221 women were approached for the study. Of those, 93% (n=206) provided informed consent for participation in the study. Six women were not included in the study as a result of confirmation of a twin pregnancy (n=1; 0.5%) or sample collection failure (n=5; 2.4%). A social worker administered questionnaires to the study participants to collect the following information: age, education, occupation, time living at residence, smoking status and alcoholic beverage consumption during pregnancy (both defined as yes or no and frequency), daily prenatal supplement intake (yes or no), residence location (urban or rural), seafood consumption (yes or no), source and daily consumption of drinking and cooking water and source of bathing water. In addition, information on previous pregnancy outcomes including number of pregnancies and number of previous pregnancy losses was gathered from questionnaires. Information on birth outcomes/measures including newborn birth weight, newborn length, gestational age, head circumference, placental weight, and five minute Appearance, Pulse, Grimace, Activity, Respiration (APGAR) score was gathered at time of delivery by the physician (Montgomery 2000). Data on adverse outcomes were collected including preterm birth (gestational age <37 weeks), low birth weight (LBW; <2500 g); small for gestational age

(SGA; birth weight <10th percentile); large for gestational age (LGA; birth weight ≥90th percentile). SGA and LGA categories were based on newborn data collected from northern regions of Mexico (Montes-Nunez et al. 2011; Rios et al. 2008).

Sample collection

Within four weeks of newborn delivery, a drinking water sample was collected by the research team at the homes of each of the study participants. Drinking water samples were collected based on the subjects' primary drinking water source (i.e. tap or bottled water). Maternal spot urine samples were collected at the hospital prior to birth, immediately transferred to cryovials and placed in liquid nitrogen. Aliquots of urine samples were shipped on dry ice to UNC-Chapel Hill, NC, and immediately stored at -80°C. It was not possible to inform the women of the levels of iAs in their drinking water during their pregnancies as these results were not available prior to the birth of their children. However, the women were informed within three months of delivery.

Detection of arsenic in drinking water and urine

The concentrations of iAs in drinking water ($\mu\text{g As/L}$; DW-iAs) were measured at UJED, Mexico, using hydride generation-atomic absorption spectrometry (HG-AAS) supported by a FIAS-100 flow injection accessory system as described previously (Devesa et al. 2004; Le and Ma 1998). The Trace Elements in Water standard reference material (SRM 1643e) (National Institute of Standards and Technology, Gaithersburg, MD) was used for quality control. The limit of detection (LOD) for iAs in drinking water by HG-AAS was 0.46 $\mu\text{g As/L}$.

All urine analyses were conducted at UNC-Chapel Hill, NC. Due to the capacity of trivalent arsenicals to oxidize to pentavalent forms in urine, the accurate measurements of urinary iAs^{III} , MMA^{III} , DMA^{III} were not possible after transport to the U.S.A. (Valenzuela et al. 2005). Concentrations of U- iAs , U-MMAs, and U-DMAs were determined by HG-AAS with cryotrapping (Devesa et al. 2004; Hernandez-Zavala et al. 2008; Hernandez-Zavala et al. 2009). Five-point calibration curves were prepared using pentavalent iAs , MMAs, DMAs standards ($> 98\%$ pure) as described previously (Hernandez-Zavala et al. 2008), and the Standard Reference Material (SRM 2669), Arsenic Species in Frozen Human Urine (National Institute of Standards and Technology), was used for quality control (Del Razo et al. 2011). The LODs for U- iAs , U-MMAs and U-DMAs were 0.2, 0.1, and 0.1 $\mu g As/L$, respectively.

The specific gravity (SG) of each urine sample was measured using a handheld refractometer (Reichert TX 400 #13740000; Reichert Inc., Depew, NY). To account for differences in water intake/differential hydration, concentrations of U- iAs , U-MMAs, and U-DMAs in each urine sample were adjusted using the following equation: $iAs \times (\text{mean SG}-1)/(\text{individual SG}-1)$ as previously described (Nermell et al. 2008; Yassine et al. 2012). Urinary concentrations of U-tAs (sum of iAs , MMAs, DMAs) were reported as SG-adjusted values ($\mu g As/L$ urine).

Statistical analysis

Data were analyzed using the statistical package SAS 9.3 (SAS Institute Inc., Cary, North Carolina). Arsenical concentrations in drinking water and urine that were below their respective LODs were assigned a value equal to $LOD/\sqrt{2}$. To determine the efficiency of iAs metabolism, the proportion of total urinary As comprising the individual arsenicals

iAs, MMAs, and DMAs (e.g. metabolism indicators) was calculated for each study subject. A Spearman rank correlation was calculated to quantify the relationship between DW-iAs and U-tAs.

Differences in DW-iAs were analyzed based on maternal characteristics (i.e. reported drinking and cooking water source as tap or bottle, reported seafood consumption, location of residence, maternal smoking status and alcohol consumption). The nonparametric Wilcoxon Rank Sum Test was used due to failure of DW-iAs to achieve normality in transformations. Differences in log transformed U-tAs were analyzed based on maternal characteristics using a Student's two sample T-test. Age and education-based comparisons of levels of DW-iAs and U-tAS were analyzed using a Kruskal-Wallis test and ANOVA, respectively

Multivariable adjusted regression models were used to assess the relationships between exposure or metabolism indicators (modeled as non-transformed, urinary SG-adjusted, continuous variables) and birth outcomes (modeled as continuous variables). Regression assumptions of linearity and the homogeneity of residuals were evaluated by examination of appropriate residual plots. Covariates were selected if associated with both exposure and outcome or based on their *a priori* status as known confounders using a directed acyclic graph (DAG). Additionally, if covariates changed the estimate by at least 10% they were included in the model. Model I included the following covariates: maternal age at delivery as a continuous variable, education level as a measure for socioeconomic status (SES) (<high school, high school, college, >college), smoking status (dichotomized as yes/no) and alcoholic beverage consumption during pregnancy (dichotomized as yes/no). Gestational age was not included in Model I because of its

potential role as a mediator of birth weight which can result in biased estimates (Wilcox et al. 2011). Model II included gestational age as a covariate for comparative purposes. Model III was run as part of a sensitivity analysis examining the effects of seafood consumption as a variation of Model I that excluded seafood consumers. Regression results are presented as a change in birth outcomes/measures with a one unit change in exposure indicators or metabolism indicators. The relationships between covariates, exposure indicators, metabolism indicators and sex of newborn were assessed using a Wilcoxon Rank Sum Test. Statistical significance for all tests was set at an alpha of less than 0.05.

Results

Maternal and newborn characteristics

All maternal and birth characteristics are presented in Table 1. Women in this cohort were relatively healthy and free of disease with no major adverse birth outcomes observed. All pregnancies resulted in a singleton live birth. The ages of the participants ranged from 18 to 41 years with an average age of 24 years and all were of Hispanic ethnicity. Of the study cohort, ~75% had at least a high school education and all had lived in their current residence for at least one year with an average of 20 years. Most women (97%) reported taking prenatal vitamins daily and most women (78%) did not consume seafood during their pregnancy. For 35% of the women, the index pregnancy was their first pregnancy. A previous pregnancy loss was reported by 12% of all women. Among all pregnancies, 59% were delivered by vaginal delivery and 41% were delivered by Caesarean section, a rate common in Mexico (Boyle and Reddy 2012).

The average gestational age of the newborns was 39 weeks. Of all births, 1.5% were preterm (<37 weeks). Of the newborns, 104 (52%) were male and 96 (48%) were female. The average weight of the newborns was 3339 g (SD +/- 500g); males had a significantly ($p=0.0003$) higher birth weight (3453 g) relative to females (3215 g). Among newborns, 2% were of low birth weight (LBW), 14% were small for gestational age (SGA) and ~10% were large for gestational age (LGA). The average placental weight was 648 g. The newborns had an average crown to heel length of 50 cm, an average head circumference of 35 cm, and an average APGAR score of 9 (see Table 1).

Levels of inorganic As in drinking water and urinary samples

Levels of iAs in study subjects' drinking water ranged from below the LOD (0.46 $\mu\text{g As/L}$) to 236.0 $\mu\text{g As/L}$ with a mean of 24.6 $\mu\text{g As/L}$ (see Table 1). Out of the 200 women, 76% had detectable levels of iAs in drinking water. In total, 107 drinking water samples (53%) exceeded the WHO standard of 10 $\mu\text{g As/L}$ and 56 (28%) were above Mexico's MCL of 25 $\mu\text{g As/L}$ (see Figure 1).

Arsenic was detected in the urine of all women. Specifically, out of the 200 women, DMAs were detected in all women's urine and 95% of the women had levels of iAs and MMAs that were above the LOD for these arsenicals (0.2 $\mu\text{g As/L}$ and 0.1 $\mu\text{g As/L}$, respectively). Across the entire cohort, there was a significant positive relationship between U-tAs and DW-iAs ($r = 0.51, p < 0.0001$) (see Figure 1). The unadjusted sum of the urinary arsenicals (U-tAs) ranged from 1.9 to 448.2 $\mu\text{g/L}$ (mean of 35.5 $\mu\text{g As/L}$) (data not shown). To control for differential hydration, SG-adjusted concentrations of U-tAs were determined and ranged from 4.3-319.7 $\mu\text{g As/L}$ (mean of 37.5 $\mu\text{g As /L}$) (see Table 1). The mean urinary concentrations of individual SG-adjusted arsenicals were 2.1,

2.3 and 33.1 $\mu\text{g As/L}$ for iAs, MMAs and DMAs respectively (see Table 1). This corresponds to the proportions of U-tAs comprising 6.1%, 6.4%, and 87.6 % for U-iAs, U-MMAs and U-DMAs respectively (see Table 1).

DW-iAs and U-tAs levels were analyzed in relation to demographic characteristics. Most women (55%) reported that their primary drinking and cooking water source was from the tap (see Supplemental Material, Table S1). DW-iAs was higher ($p < 0.0001$) for those women who reportedly drank and cooked with municipally-supplied water (i.e. tap) (medians of 23.3 and 18.1 $\mu\text{g As/L}$, respectively) than those who reportedly drank and cooked with bottled water (medians of 0.40 and 0.65 $\mu\text{g As/L}$, respectively) (see Supplemental Material, Table S1). Similarly, U-tAs levels were higher in women who reportedly drank and cooked with municipally-supplied water (means of 49.5 and 42.9 $\mu\text{g As/L}$, respectively) than those who drank and cooked with bottled water (means of 22.9 and 18.5 $\mu\text{g As/L}$, respectively) (see Supplemental Material, Table S1). There were no statistically significant differences in U-tAs (see Supplemental Material, Table S1), U-iAs, or U-MMAs (data not shown) between the women who reported consuming seafood versus those who did not; however, women who consumed seafood had higher mean levels of U-DMAs than those who did not (42.5 $\mu\text{g As/L}$ versus 30.6 $\mu\text{g As/L}$, respectively). There was no statistical difference in U-tAs associated with a woman's age, however women with less than a high school education had significantly higher ($p = 0.0173$) U-tAs (mean of 47.70 $\mu\text{g As/L}$) than women with a high school education (mean of 37.2 $\mu\text{g As/L}$), college (mean of 32.1 $\mu\text{g As/L}$), and greater than college education (mean of 19.4 $\mu\text{g As/L}$). There was no statistical difference in U-tAs based on residence

status (e.g. urban or rural environment), smoking status, nor alcohol consumption (data not shown).

Relationship of DW-iAs and urinary arsenicals with birth outcomes

Multivariable regression models were used to examine the relationships between non-transformed DW-iAs, SG-adjusted urinary levels and proportions of iAs, MMAs, and DMAs and birth outcomes. The primary model (Model I) adjusted for maternal age, education, smoking, and alcohol consumption where all measured relationships represent analysis for a one unit changes in exposure measures in association with birth outcomes/measures. DW-iAs, U-tAs, U-DMAs, and %iAs were not significantly associated with any of the birth outcomes (see Table 2; see Supplemental Material, Figure S1).

There was a significant negative relationship between U-iAs and mean gestational age with an estimated decrease of 0.069 weeks in gestational age for every one unit change in U-iAs (95% CI: -0.13, -0.0043, $p=0.03$). Similarly, there was an estimated decrease in mean newborn length of 0.16 cm in relationship to U-iAs (95% CI: -0.31,-0.019, $p=0.02$). There was no significant association between U-iAs and birth weight, head circumference, placental weight, and APGAR score.

There was a significant negative association between U-MMAs and both mean gestational age (weeks) ($\beta= -0.067$, 95% CI: -0.13, -0.0092, $p=0.02$) and mean birth weight (g) ($\beta= -24.4$, 95% CI: -46.8,-2.0, $p=0.03$). There were no significant associations between U-MMAs and mean head circumference, placental weight, and APGAR score. There was a significant negative relationship between %MMAs and mean birth weight,

with an estimated 24g decrease in birth weight for every unit increase in %MMAs (95% CI: -45.6, -3.4, $p=0.02$). There was no significant association with %MMAs and mean head circumference or APGAR score. Placental weight (g) was also negatively associated with %MMAs ($\beta= -9.8$, 95% CI: -15.8, -3.8, $p=0.001$) and positively associated with %DMAs ($\beta= 3.5$, 95% CI: 0.53, 6.5, $p=0.021$).

Gestational age was included in Model II for comparative purposes (see Supplemental Material, Table S2). The inclusion of gestational age did influence the estimates, in general reducing the magnitude of the relationships observed in Model I. It is important to consider, however, the potential role of gestational age as a mediator of birth outcomes. A sensitivity analysis that excluded seafood consumers ($n=43$, 22% of all subjects) (Model III) showed consistent directions and significance of associations between exposure and metabolism indicators and birth outcomes as observed with the Model I (see Supplemental Material, Table S2). To note, maternal urinary means of iAs and urinary arsenical proportions were not statistically different ($p < 0.05$) based on the sex of the infant (data not shown).

Discussion

The BEAR prospective pregnancy cohort in Mexico was established with a long-term goal of examining the effects of environmental exposure to iAs on the health of women and children and providing information to protect individuals from preventable exposure. The results of the present study demonstrate that pregnant women in the BEAR cohort are exposed to potentially harmful levels of iAs in their drinking water, ranging up to 236 $\mu\text{g As/L}$. Over half of the samples exceeded the WHO-established safe drinking water

guideline of 10 µg As/L. Elevated levels of As have been observed in the drinking water of other exposed populations in Mexico. For instance, previously reported DW-iAs from central-eastern regions of Mexico ranged up to 1504 µg As/L (Valenzuela et al. 2005) and up to 215 µg/L (Del Razo et al. 2011) in Hidalgo and Zimapán, respectively.

The biomonitoring data further confirmed that pregnant women in the cohort are exposed to iAs, with SG-adjusted U-tAs ranging from 4.3-319.7 µg As/L. Levels of U-tAs were positively correlated with DW-iAs, consistent with drinking water being a source of iAs exposure in the BEAR cohort. The level of correlation between DW-iAs and U-tAs is similar to other iAs-exposed populations in Mexico (Del Razo et al. 2011). The levels of U-tAs in the BEAR cohort are generally lower than levels observed in pregnant women in Bangladesh (SG-adjusted medians: 23.3 µg As/L versus 80 µg As/L) (Vahter et al. 2006). However, the levels of U-tAs in this cohort are higher than levels observed in US women (SG-adjusted mean of 37.5 µg As/L versus creatinine adjusted geometric mean of 7.3 µg As/L) (Caldwell et al. 2009). The drinking water samples from the present study were collected postpartum and represent a single exposure measure, thus there is the potential that exposure varied over time. However, it has been demonstrated in prior research that iAs levels in drinking water can vary little temporally (Slotnick et al. 2006; Steinmaus et al. 2005). Additionally, the women in the study had lived in their current residences for at least one year, suggesting minimal variability in the source of iAs in drinking water and potentially for iAs exposure as well. Taken together, the data from the present study highlight a new area of concern for elevated levels of iAs in drinking water in Mexico.

When analyzed for relationships to birth outcomes, overall DW-iAs and the sum of the urinary arsenicals (U-tAs) showed no significant associations. Interestingly, there were significant relationships between levels and proportions of iAs, MMAs, and DMAs in maternal urine and selected birth outcomes. Specifically increases in U-iAs were associated with a lower means in gestational age and newborn length. Increases in U-MMAs were associated with a lower mean gestational age and birth weight. Similarly, increases in %MMAs were negatively associated with both lower mean placental weight and birth weight with an estimated ~24g decrease per unit increase in %MMAs. As total amounts of urinary arsenic as well as DMAs can be attributed in part to seafood consumption (Navas-Acien et al. 2011), a sensitivity analysis was performed on non-seafood consumers. The point estimates between U-MMAs and %MMAs with birth weight were similar between seafood and non-seafood consumers, suggesting these associations are not impacted by seafood consumption. While it is currently unknown whether the later-life health of the infants in this study will be influenced by the observed differences in birth weight, gestational age, and newborn length these relationships will be important given global world-wide exposure to arsenic. Thus, follow-up of these children is warranted as infants born with decreased gestational age, and changes to birth size are at risk for later life health effects.

Our data highlight the important finding that U-tAs alone was not significantly associated with birth outcomes suggesting that the metabolism of iAs by a pregnant woman may be a better predictor of impact on both birth and placental weight, and newborn length than exposure level alone. Of note, the mean proportions of total urinary arsenicals from pregnant women in Gómez Palacio were ~6% for iAs, 6% for MMAs and 88% for DMAs.

These data indicate increased iAs methylation capacity (i.e. a shift to higher proportions of DMAs) relative to the proportions generally observed in exposed populations around the world, with observed ranges of ~10-20% for iAs, 10-20% for MMAs and 60-80% for DMAs (Vahter 2002). It is important to note that U-tAs and the individual urinary metabolites were measured once during pregnancy; nevertheless, all samples represented urinary collection during the end of pregnancy, specifically immediately prior to delivery. Therefore, shifts in iAs metabolism that occur differentially between trimesters of pregnancy (Hopenhayn et al. 2003a) should not influence these findings. The results in the present study highlight a relative increase in proportions of DMAs, consistent with other studies where later stages of pregnancy were associated with proportions of DMAs exceeding 80% (Concha et al. 1998; Gardner et al. 2011; Gardner et al. 2012; Hopenhayn et al. 2003b). It has been suggested that higher DMAs/MMAs ratios indicate an increased iAs methylation capacity and may have a protective effect for both mother and fetus as it could reflect reduced levels of the highly toxic trivalent MMAs^{III} (Vahter 2002, 2009).

An inverse relationship between DW-iAs and birth weight has been observed previously (Hopenhayn et al. 2003a; Yang et al. 2003) as well as U-tAs and size at birth (A Rahman et al. 2009). However, some studies have found no association between prenatal iAs exposure and size at birth (Kwok et al. 2006; Myers et al. 2010). These differences may be attributed to variation in study design and methodology (e.g. bias, misclassification and/or random error), as well as inherent differences in population characteristics (e.g. exposure/consumption of iAs contaminated drinking water, genetic polymorphisms, and nutritional factors). Furthermore, studies that do not address inter-individual differences in metabolism to iAs would not capture the associations observed in the present study. To

our knowledge, the results from the BEAR cohort are the first to demonstrate that levels and proportions of MMAs, and levels of U-iAs in maternal urine are associated with poorer birth outcomes. These findings are supportive of a growing body of literature detailing that urinary proportions of MMAs are positively correlated with detrimental health effects of iAs (as reviewed in (Tseng 2007a).

While the data in the present study are suggestive of an association between maternal methylation of iAs and infant birth weight, gestational age, and newborn length this study is not without limitations. In addition to iAs exposure and metabolism, other factors that may influence birth outcomes include: co-exposure to other contaminants, gestational age, and maternal genotype, as well as others. Additionally, residual confounding may remain based on maternal socioeconomic status and potentially dietary sources of iAs. Together these complex factors may influence the relationships between iAs exposure during pregnancy and newborn health outcomes and should be examined in larger cohort studies. It will be important in future research to examine the links between early life exposure to iAs and postnatal development in the children through prospective analysis. Taken together, these data will enable a better understanding of the etiology of poorer birth outcomes associated with iAs exposure in early life.

Conclusions

In summary, there are three major findings from this study. First, more than half of the drinking water samples tested in Gómez Palacio exceeded the WHO's guidelines for acceptable limits of iAs in drinking water indicating that women drinking municipal water are at risk for elevated exposure to iAs. Second, biomonitoring data confirmed that

many pregnant women had elevated levels of iAs in their urine. Third, individual differences in iAs metabolism in the pregnant women were associated with birth outcomes, where elevated levels and proportions of MMAs in maternal urine were negatively associated with birth weight and gestational age and urinary iAs was negatively associated with gestational age and newborn length. Given that these exposures to elevated iAs are occurring during pregnancy, there may also be concerns about later life health effects for the children of the cohort. The results of this study suggest that a follow-up examination of the impacts of iAs exposure on the health of the women and their children is warranted.

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Table 1. Selected demographic characteristics and levels of iAs in drinking water and urinary arsenicals of the participants of the BEAR study.

Characteristic	<i>n</i>^a (%) or Mean, Median [Range]
Maternal Age at Delivery (years)	24, 23 [18-41]
Race	
Hispanic	199 (99.5%)
Education	
< High School	50 (25.1%)
High School	95 (47.7%)
College	41 (20.6%)
Post-College	13 (6.5%)
Time living at Residence (years)	20, 21 [1-41]
Smoking Status	
Non-smokers	186 (93.0%)
Current smokers	13 (7.0%)
Alcohol Consumption	
None	159 (79.5%)
Some	41 (20.5%)
Prenatal Vitamin Daily Intake	192 (97.0%)
Seafood Consumption	
None	155 (78.3%)
Some	43 (21.7%)
Previous Pregnancies (Gravidity)	
0 (Primigravidity)	70 (35.0%)
1 (Multigravidity)	50 (25.0%)
≥2 (Multigravidity)	80 (40.0%)
Previous Pregnancy Loss	
0	176 (88.0%)
1	18 (9.0%)
≥2	6 (3.0%)
Method of Delivery	
Vaginal	118 (59.0%)
Caesarean section	82 (41.0%)
Gestational Age (weeks)	
All	39, 40 [34-42]
<37 weeks	3 (1.5%)
≥37 weeks	197 (98.5%)
Newborn sex	
Male	104 (52.0%)
Female	96 (48.0%)
Birth weight (g)	
All	3339, 3355 [1800-5120]
Male	3453, 3490 [2100-5120]*
Female	3215, 3150 [1800-4200]
Low Birth Weight (LBW)	4 (2.0%)
Small for Gestational Age (SGA)	28 (14.0%)
Large for Gestational Age (LGA)	19 (9.5%)
Placental Weight (g)	648, 640 [390-1070]
Newborn Length (cm)	50, 50 [40-59]

Characteristic	<i>n</i> ^a (%) or Mean, Median [Range]
Head Circumference (cm)	35, 35 [31-38]
APGAR Score	9, 9 [8-10]
Exposure measures	
DW-iAs (µg As/L)	24.6, 13.0 [<LOD ^b - 236.0]
Urinary arsenicals ^c	
U-tAs (µg/L)	37.5, 23.3 [4.3-319.7]
U-iAs (µg/L)	2.1, 1.3 [0.14-23.0]
U-MMAs (µg/L)	2.3, 1.4 [0.12-18.2]
U-DMAs (µg/L)	33.1, 20.6 [1.4-292.5]
iAs (%)	6.1, 5.3 [0.77-45.1]
MMAs (%)	6.4, 6.0 [0.68-24.9]
DMAs (%)	87.6, 88.5 [32.7-96.7]
MMAs/iAs	1.2, 1.2 [0.13-5.5]
MMAs/DMAs	0.077, 0.069 [0.0072-0.68]
DMAs/MMAs	17.6, 14.6 [1.5-140.0]
MMAs+DMA/iAs	19.6, 18.1 [1.2-129.9]

^aDifferences in *n* based on missing demographic data. ^bLimit of detection (LOD) for DW-iAs=0.456 µg As/L. ^c All urinary values were adjusted by specific gravity.

* Significant difference in means (*p*=0.0003) between males and females.

Table 2. Multivariable regression analyses^a between a one unit increase in exposure and metabolism indicators (DW-iAs, SG-Adjusted U-tAs, U-iAs, U-MMAs, U-DMAs, %iAs, %MMAs, and %DMAs) and birth outcomes.

Variable	Gestational age (wks)	Birth weight (g)	Newborn length (cm)	Head circumference (cm)	Placental weight (g)	APGAR score
DW-iAs						
β (CI)	-0.0025 (-0.0065, 0.0061)	-0.1 (-1.7, 1.4)	-0.0051 (-0.014, 0.0038)	-0.0026 (-0.0077, 0.0026)	0.12 (-0.32, 0.57)	0 (-0.0005, 0.0005)
p-value	0.22	0.89	0.26	0.33	0.58	0.98
U-tAs						
β (CI)	-0.0036 (-0.0075, 0.003)	-0.58 (-2.1, 0.93)	-0.0055(-0.014, 0.0033)	-0.0022 (-0.0074, 0.0029)	0.31(-0.14, 0.76)	0 (-0.0004, 0.0005)
p-value	0.07	0.44	0.22	0.39	0.17	0.85
U-iAs						
β (CI)	-0.069 (-0.13, -0.0043)	-21.7(-46.8, 3.4)	-0.16 (-0.31, -0.019)	-0.061 (-0.15, 0.030)	0.73 (-7.1, 8.5)	-0.0013(-0.0097,0.0072)
p-value	0.03*	0.09	0.02*	0.18	0.85	0.76
U-MMAs						
β (CI)	-0.067 (-0.13, -0.0092)	-24.4 (-46.8, -2.0)	-0.11 (-0.24, 0.018)	-0.067 (-0.14, 0.011)	-1.3(-8.0, 5.5)	0.0004 (-0.0069, 0.0070)
p-value	0.02*	0.03*	0.08	0.09	0.71	0.91
U-DMAs						
β (CI)	-0.0037 (-0.0081, 0.0006)	-0.49 (-2.2, 1.2)	-0.0054 (-0.015, 0.0043)	-0.0021 (-0.0079, 0.0036)	0.38 (-0.11, 0.88)	0.0001 (-0.0005, 0.0006)
p-value	0.09	0.56	0.27	0.46	0.12	0.82
%iAs						
β (CI)	-0.0006 (-0.043, 0.042)	-6.4 (-22.9, 10.0)	-0.054 (-0.15, 0.041)	0.01(-0.044, 0.065)	-3.2 (-7.9, 1.6)	-0.0002(-0.0054, 0.0049)
p-value	0.97	0.44	0.26	0.71	0.18	0.92
%MMAs						
β (CI)	-0.043 (-0.098, 0.012)	-24.5(-45.6, -3.4)	-0.082 (-0.21, 0.041)	-0.03 (-0.10, 0.041)	-9.8(-15.8, -3.8)	0.011 (-0.0056, 0.078)
p-value	0.12	0.02*	0.19	0.41	0.001*	0.73
%DMAs						
β (CI)	0.011 (-0.017, 0.38)	8.9 (-1.5, 19.3)	0.041 (-0.020, 0.10)	0.0025(-0.032, 0.037)	3.5 (0.53, 6.5)	-0.0001 (-0.0034, 0.003)
p-value	0.44	0.09	0.17	0.88	0.02*	0.93

*Association between birth outcomes and arsenic exposure indicators where $p < 0.05$.

^aModel I adjusted for the following covariates: maternal age, education, smoking, and alcohol consumption.

Figure Legend

Figure 1. Relationship between DW-iAs and U-tAs (SG-adjusted) across the BEAR cohort ($r = 0.51, p < 0.0001$).

Figure 1.

