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Low level arsenic contaminated water consumption and birth outcomes in Romania - an exploratory study

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Abstract

Women are exposed to drinking water with low arsenic concentrations (<10.0 µg/L) worldwide, yet little work has been done to assess the risk. To begin to address this data gap, we conducted an exploratory study of birth outcomes in Timis County, Romania. We prospectively followed 122 women with singleton deliveries, for whom we constructed individual exposure indicators using self-reported water consumption weighted by arsenic measured in drinking water sources. There were no overall confounder-adjusted effects for arsenic exposure on birth outcomes. Yet, higher average arsenic (10 µg/L) was associated with a -2.45 lower birth weight Z-score (P=0.021) and a -1.17 shorter birth length Z-score (P=0.029) among smokers. Higher average iAs (10 µg/L) was also associated with smaller ponderal index in boys (P=0.023). Our results suggest smoking may potentiate an otherwise benign arsenic exposure. A larger, more definitive biomarker-based study is needed to investigate the potential risks in conjunction with smoking.

Keywords

Arsenic; birth outcomes; birth length; birth weight; birth size; drinking water; gestational age; ponderal index

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1. Introduction

The extensive distribution of inorganic arsenic (iAs) in the earth's crust leads to local and regional contamination of ground drinking water supplies and widespread human exposure [1, 2]. Following ingestion, iAs is well-absorbed by the mammalian gastrointestinal tract, and myriad adverse health effects have been characterized in association with chronic exposure [3, 4]. Although less investigated to date, concern is growing with respect to an increased risk for adverse birth outcomes including preterm delivery and smaller neonatal size [3, 5, 6].

Inorganic arsenic crosses the human placenta, and accumulates in a developing fetus [7, 8]. It is not only genotoxic [9, 10] and anti-estrogenic [11, 12], but studies also indicate a generalized inflammatory response to long-term iAs exposure [13], and increased inflammation in newborns following exposure *in utero* [14, 15]; inflammation is a strong predictor of preterm delivery and fetal growth restriction [16]. Experimental and observational evidence also suggests that iAs accumulates in and disrupts placental function [15, 17]. Altered vasculogenesis leading to dysplastic placental development has been reported *in vivo* following iAs treatment [18], as have changes in placental levels of reactive oxygen species *in vitro* [10, 19]; abnormal placentation is also a strong predictor of preterm delivery and restricted fetal growth [20].

A growing body of epidemiologic evidence suggests increased risks for adverse birth outcomes in association with the use of highly iAs contaminated water sources by pregnant women ($> 10 \mu\text{g/L}$), including the delivery of lower birth weight babies at term [21, 22], in Argentina [23], Bangladesh [24, 25], India [26, 27], Mexico [28], and Taiwan [29]. Shorter birth length [28, 30] and smaller head and chest circumferences [25] have also been reported among mothers with high level iAs exposure. Studies in populations presumed to have low iAs exposure ($< 10 \mu\text{g/L}$ [31]) reported lower birth weight or smaller size at birth in association with higher total blood or urine arsenic [32–36], although others reported no association [37, 38]. However, the results of these prior studies were often limited by cross-sectional designs, small sample size and the absence of source data, or ill-timed exposure assessments that inappropriately incorporated less toxic organic arsenic compounds [39].

Millions of reproductively-aged women are likely to be exposed to low level drinking water iAs contamination ($< 10 \mu\text{g/L}$) worldwide [40, 41] and few data are available to characterize the risks [21, 42]. There is a critical need for additional epidemiologic studies of the health effects of low level iAs exposure to help guide prevention efforts. In an initial step to address this gap, we conducted a preliminary cohort study by following up pregnant women who had previously participated as controls in a study of drinking water iAs exposure and pregnancy loss [43].

2. Material and methods

2.1. Study sample

The sampling frame comprised 150 women with ongoing singleton pregnancies, and receiving prenatal care services at Bega Hospital in Timisoara, Romania, between December

2011 and January 2013. Prenatal care is provided at no cost and a minimum of three visits is compulsory in Romania. All of the women resided in Timis County, Romania, an area known for low level iAs contamination of drinking groundwater sources [2]. The women were initially recruited as controls participants for our recent study of pregnancy loss, which was previously described in detail [43]. In brief, we recruited women with incident spontaneous pregnancy loss of 5 – 20 weeks completed gestation as cases (n = 150), and women with ongoing pregnancies matched by gestational age (+ 1 week) as controls (n = 150). Participants completed a physician-administered questionnaire regarding demographic, socioeconomic, and lifestyle factors, as well as medical, gynecologic, residential, and occupational histories, and provided blood and urine specimens. We also inquired as to water consumed through various means (e.g., directly, bottled, tea, coffee, mixed beverages, soups) and used for cooking, and their sources and locations.

Shortly after participant enrollment, our team collected water samples from up to two residential drinking water sources reported by each study participant and transferred them on ice to the Environmental Health Center in Cluj-Napoca, Romania. We determined iAs in duplicate by hydride generation-atomic absorption spectrometry according to a strict quality control procedure as previously described [44]. The method detection limit was 0.5 µg/L. Focusing on periconception and early gestation as critical windows [45], we calculated three metrics for drinking water iAs exposure. The average iAs and peak iAs concentrations (µg/L) were defined as the mean iAs concentration measured in up to two residential drinking water sources and the higher of the two, respectively. The daily iAs exposure (µg/day) was defined as the average iAs concentration (µg/L) multiplied by the average water consumed from non-bottled sources daily (L/day), including direct consumption, mixed beverages, and soups.

In early 2014, we re-enrolled 124 control participants, having delivered 126 infants (two sets of twins), who consented for access to the study pregnancy birth record. The response rate was 95%; we could not contact n = 15, n = 4 had losses or terminations, and n = 7 refused to participate. At delivery, data are handwritten by the attending obstetrician on a standardized form, following good practice guidelines established by the Neonatology Association of Romania [46]; data are also collected in the rare event of a home birth. The birth record is linked to the maternal health records, and stored by surname in a secure area of Bega Hospital. The current sample includes 122 mothers with singleton live births.

2.2 Birth outcomes

We abstracted birth information from the medical record. We considered gestational age at delivery as continuous and as dichotomized at < 37 weeks to indicate preterm delivery (PD) [47]. We expressed birth weight as Z-scores (BW-Z) relative to expected mean reference population values for gestational age [48], dichotomized at < 10th %tile for the reference population distribution (small for gestational age (SGA)), and also dichotomized at < 2,500 g [47]. We considered birth length (BL-Z) and head circumference (HC-Z) at delivery as Z-scores relative to expected mean reference population values for gestational age [49], and dichotomized at < 10th %tiles. We used ponderal index (PI-Z) as $100 \times (\text{birth weight/birth$

length³), expressed as Z-scores relative to an expected median reference population value for gestational age [50], and dichotomized at < 10th %tile.

2.3 Statistical analysis

We characterized distributions for birth outcomes, iAs exposure metrics, and for potential confounders. We natural log-transformed skewed distributions and identified outliers for further examination. We assessed unadjusted associations among predictors, outcomes, and potential confounders using ANOVA and linear correlation. We used Cox-proportional hazards models to accommodate medically-assisted deliveries indicated by Cesarean section, in confounder adjusted analyses of iAs exposure predicting gestational age at birth; exponentiated coefficients and their 95% CIs provided hazard ratios [51]. For other continuous outcomes we used multivariable linear regression to evaluate associations with iAs exposure, adjusted for confounders. For dichotomous outcomes, we used logistic regression; exponentiation of model coefficients and corresponding 95% CIs provided odds ratios [52]. We entered maternal age in years [53], self-reported pre-pregnancy body mass index (BMI) in kg/m² [54], self-reported cigarette smoking during pregnancy as a dichotomous variable [55, 56], and education as a trichotomous marker of socioeconomic status [57, 58], into regression models contingent on literature evidence of confounding coupled to selection using directed acyclic graphs (DAGs) [59]. Due to convergence issues, logistic regression models for PD were adjusted for only maternal age and cigarette smoking. We also considered residential location as a covariate in the models, defined as urban vs. rural; to serve as a proxy for air pollution [60–63] and for exposure to drinking water chlorine disinfection byproducts (DBPs) [64].

We included product terms in additional linear regression models to assess interactions for continuous iAs exposure metrics with cigarette smoking, infant sex, and prior LBW delivery; factors likely to modify associations with continuous birth size outcomes. Interactions were tested using likelihood ratios and expressed as individual and joint effects [65].

We considered drinking water iAs metrics as continuous (to maximize study power) and as tertiles (to accommodate potential non-linear associations). Extreme outliers, defined as observations > 3 interquartile ranges above the 75th % tile for the distribution were excluded from the analysis [66]. Observations with DFBetas > |1| for the exposure were excluded as influential and regression models repeated [67]. Effects were expressed for 10 unit increases in iAs. Statistical significance was defined as $P < 0.05$ for main effects and $P < 0.10$ for product terms. Consistent with the exploratory aim of our study we made no adjustments for type-1 error inflation due to multiple-comparisons, in an effort to maximize sensitivity for detecting modest associations [68]. SAS v.9.4 (SAS Institute Inc., Cary, NC) was used for the analysis.

3. Results

3.1 Univariate and bivariate analyses

Descriptive statistics for participants are provided in Table 1. Mothers were 19–42 years of age (mean = 29), with mean pre-pregnancy BMI = 22 kg/m², and almost 80% married or

living as married. Most women resided in an urban locale (66%), yet with a substantial number of rural residents (34%), and 46% reported completion of a university education. Twenty-nine (24%) women smoked cigarettes since learning of the study pregnancy (mean = 11 cigarettes/day), and $n = 13$ (11%) reported consumption of alcohol since learning of pregnancy. Fifty-one (42%) women were multiparous, with a mean of 1.24 prior live births, $n = 6$ (13%) of which were PD (13%), and $n = 3$ (6%) were LBW. The mean average iAs concentration was 4.11 $\mu\text{g/L}$, with a median = 1.28 $\mu\text{g/L}$ (1st tertile, 0 $\mu\text{g/L}$; 2nd tertile, 0.07 – 4.12 $\mu\text{g/L}$; 3rd tertile, 5.3 – 130.3 $\mu\text{g/L}$). The distribution is characterized graphically by Supplementary Figure 1. Similarly, mean peak iAs concentration was 4.82 $\mu\text{g/L}$, yet with a median = 2.27 $\mu\text{g/L}$ (1st tertile, 0 – 0.07 $\mu\text{g/L}$; 2nd tertile, 0.08 – 4.12 $\mu\text{g/L}$; 3rd tertile, 5.3 – 130.3 $\mu\text{g/L}$). The mean daily iAs exposure was 4.90 $\mu\text{g/day}$ with a median = 1.64 $\mu\text{g/day}$ (1st tertile, 0 $\mu\text{g/day}$; 2nd tertile, 0.04 – 3.2 $\mu\text{g/day}$; 3rd tertile, 3.3 – 65.2 $\mu\text{g/day}$).

The distribution of birth outcomes among participants is described in Table 2. Mean gestational age at delivery was 38.7 weeks (standard deviation (SD) = 1.4), with range 34 – 42. There were $n = 32$ (26.2%) Caesarean births and $n = 7$ (5.7%) PDs. Mean BW was 3222.7 g (SD = 448.9) with range 1800 – 4190; $n = 6$ (4.9%) were LBW and $n = 10$ (8.2%) were SGA. BL was 51.4 cm on average (SD = 1.9) with range 47 – 58, and with no babies < 10th %tile of the reference distribution. PI was 23.6 kg/m^3 on average (SD = 2.4), with range 15.3 – 29.9, and with $n = 14$ (11.5%) < 10th %tile of the reference distribution. Mean HC was 32.4 cm (SD = 1.8), with range 29 – 39, and $n = 37$ (30.3%) < 10th %tile for the reference distribution. There were fewer boys (48.4%) than girls (51.6%).

3.2 Multivariable analyses

Table 3 describes the main effect of iAs exposure on continuous birth outcomes adjusted for maternal age, pre-pregnancy BMI, cigarette smoking during pregnancy, and education. No associations were suggested for gestational age at delivery or for birth size indicators as continuous outcomes. Including residential location as a covariate had no meaningful impact on the models (data not shown). Table 4 describes the main effect of iAs exposure on dichotomous birth outcomes adjusted for covariates. Point estimates were protective for tertiles of iAs exposure in association with PD, yet the confidence intervals were wide. We detected a protective effect for HC < 10th %tile in the 2nd tertile of average iAs exposure (OR = 0.31; 95% CI = 0.12, 0.83), but the effect was not significant for the 3rd tertile of exposure (OR = 0.33, 95% CI = 0.10, 1.13), and the effect was positive using continuous iAs exposure (OR = 1.23; 95% CI = 0.78, 2.01). We identified similar patterns for HC < 10th %tile when using peak iAs or daily iAs as the exposure.

Table 5 describes interaction models for continuous iAs exposure and birth size by self-reported cigarette smoking during pregnancy. We detected a –2.45 lower Z-BW (95% CI = –4.49, –0.42) among cigarette smokers exposed to 10 $\mu\text{g/L}$ drinking water average iAs, but there was little impact for non-smokers exposed to iAs, or for smokers unexposed to iAs ($P_{\text{Interaction}} = 0.021$). We also detected a –1.17 lower Z-BL (95% CI = –2.23, –0.001) among cigarette smokers exposed to 10 $\mu\text{g/L}$ average iAs in drinking water, again with little impact indicated for non-smokers exposed to iAs or for smokers unexposed to iAs ($P_{\text{Interaction}} = 0.029$). However, only $n=14$ and $n=15$ smokers had average drinking water iAs

> 0 µg/L in the final models for Z-BW and Z-BL, respectively (n=13 < 0 µg/L), and so the effect estimates were imprecise. Z-BW and Z-BL are shown as a function of average drinking water iAs concentration, stratified by cigarette smoking, in Supplementary Figures 2 and 3. We found similar results using peak iAs and daily iAs for the exposure, and when excluding n=2 mothers using drinking water sources contaminated by > 50 µg/L iAs on average (data not shown).

Table 6 describes interaction models for continuous iAs exposure and birth size by neonatal sex. We detected statistically significant interactions for PI, although effects were modest. Boys had higher PI than iAs unexposed girls overall; however, iAs exposure modified the effect, and so boys with gestational exposure to 10 µg/L iAs had a 0.23 smaller Z-PI than unexposed boys ($P_{\text{Interaction}} = 0.023$). Unexpectedly, girls with gestational iAs exposure also had higher PI than girls unexposed in utero. There were n=41 mothers of boys with average drinking water iAs > 0 µg/L (n=18 < 0 µg/L) and n=40 mothers of girls (n=22 < 0 µg/L) in the final model. Z-PI is shown as a function of average drinking water iAs concentration, stratified by infant sex, in Supplementary Figure 4. The results were similar using peak iAs and daily iAs consumption for exposure and when excluding mothers exposed to average drinking water iAs > 50 µg/L. We did not detect interactions for prior LBW delivery with birth size (data not shown).

4. Discussion

In this exploratory prospective investigation of birth outcomes we report no significant main effects for maternal iAs exposure on singleton live deliveries. Adjusted for confounding factors, maternal effect estimates were close to the null for three continuous metrics of maternal drinking water iAs intake and gestational age at delivery, as well as with BW, BL, PI, and HC standardized to reference populations as Z-scores, or dichotomized at the 10th %tiles. Considered as categorical, we detected a counterintuitive protective effect for the 2nd tertile of iAs exposure on HC < 10th %tile; however, with no evidence for a dose-response and without an effect for continuous exposure. We believe this result reflects a chance occurrence. We found modest evidence for maternal cigarette smoking and newborn sex as modifiers of relations between gestational iAs exposure with BW, BL, and PI, suggesting heightened vulnerability to iAs-associated toxicity among cigarette smokers and for boys. Our outcome measures may reflect heterogeneous processes *in utero* (e.g., birth weight reflects gestational age and fetal growth, [69]), yet the overall picture suggests specific subgroups may be predisposed to iAs-associated reproductive toxicity even at low levels of maternal exposure. Still, only a small number of smokers were exposed to drinking water iAs and so the results should be considered preliminary.

Previous studies also evaluated associations between low level arsenic exposure and birth outcomes, although the results were inconsistent. In a cross-sectional study of 125 Chinese mothers sampled at delivery [32], 1 µg/L higher total blood arsenic was associated with 20 g lower BW (95% CI = -39.60, -0.40) and 1 mm shorter BL (95% CI = -1.78, -0.22), with no effect for HC. Conversely, a cross-sectional study of 76 Tenerife newborns reported that babies with detectable meconium arsenic were 223.8 g heavier than babies without [70], with no effects for BL or HC. A small prospective cohort study of 78 Japanese women

reported no associations for urine total arsenic sampled in the 9–40th week of gestation and BW, HC, or BL [38]. More recently, a prospective study of 299 Taiwanese women reported no associations between maternal urine iAs and gestational age, or for BW, BL or HC [9]. The prospective MIREC Study also recently reported no associations between low level maternal urine iAs and SGA births in Canada [37]. A prospective U.S. investigation of 133 women with low drinking water iAs exposure reported a –1.30 g lower birth weight per 1 µg/L higher total maternal urine iAs [36]. A larger U.S. study of 693 mother-infant pairs with low-level exposure from a nearby Superfund site [34] also reported an inverse association between blood total arsenic and Z-BW although not for BL or HC. Finally, seven women with urine iAs > 9 µg/L had a 57% higher prevalence of a prior LBW delivery than 12 women with urine iAs ≤ 9 µg/L ($P = 0.019$), in a cross-sectional study from nearby Arad County, Romania; yet without an association for drinking water iAs concentration [35].

To our knowledge, this is the first report of smaller size at birth associated with maternal co-exposure to cigarette smoke and low level drinking water iAs in early pregnancy. In our earlier work, we reported an analogous interaction for spontaneous pregnancy loss [71]. Higher levels of oxidative stress (OS) and specific inflammatory markers have been reported for cigarette smokers [72], and increased placental OS was induced by iAs in experimental [73] and observational studies [9, 15, 74]. Excess OS may inhibit placental development and function [75] and it is increased among growth restricted infants [76, 77]. Higher urine iAs concentration was associated with higher levels of 8-hydroxydeoxyguanosine and N⁷-methylguanosine, biomarkers of OS induced DNA damage, in the aforementioned Taiwanese study [9]. Co-administration of suspended iAs and cigarette smoke depleted glutathione, a critical antioxidant molecule, and synergistically increased OS damage in hamsters [78, 79]. Observational evidence also indicates lower glutathione levels among women with long-term iAs exposure [80] and similar decrements in levels of nutritional components important for iAs metabolism have been observed in cigarette smokers [81, 82]. It is thus tempting to speculate that a reduced capacity to detoxify iAs and to moderate iAs-induced OS predisposes cigarette smokers to reproductive toxicity, even at low levels of iAs exposure. Yet, this result may have occurred by chance. A comprehensive investigation integrating relevant biomarkers will be necessary to more thoroughly assess the plausibility of OS as a biologic mechanism.

We also report lower PI among boys delivered to mothers with exposure to iAs, compared to boys delivered to unexposed mothers. PI is an indicator of growth proportionality; lower values indicate asymmetric development and are associated with a greater risk for adverse health outcomes [50]. The apparent vulnerability in boys is unique for low levels of maternal drinking water iAs exposure, although similar observations have been reported for high levels of maternal drinking water iAs exposure [30, 83]. We also identified an anomalous higher PI among exposed girls, which we suspect is a chance finding. Still, given the novelty of this result additional confirmation is necessary and so we are reluctant to speculate with respect to biologic mechanism. U.S. investigators recently reported that smaller head size on ultrasound was associated with higher maternal urine iAs for girls, but not for boys, in a

New Hampshire population with exposure to 0 – 9.3 µg/L drinking water iAs [84]. Thus sex-specific vulnerabilities may be important to the impact of iAs on growth *in utero*.

Drinking water iAs intake levels were very low for most participants in our study, an average of 4.9 µg/day. The European Food Safety Authority (EFSA) recently reported a mean adult iAs intake of 0.11–0.17 µg/kg body weight/day from dietary sources including drinking water, which translates to 7.7–11.9 µg/day for a 70 kg adult; grain-based processed products (non rice-based) was the largest contributor [85]. In our study we used environmental sampling weighted by questionnaire data to reconstruct residential drinking water iAs exposure during pregnancy, rather than a biomarker of iAs exposure. This may have misclassified some women. We were unfortunately unable to measure urine iAs in all participants, although we reported a moderate, positive correlation between urine total iAs and average drinking water iAs concentration ($r = 0.35$; $P = 0.13$) in a recent substudy of $n = 20$ control women [86]. These results suggested that the origin of urine total iAs must have been not only iAs in residential drinking water but also some other source, possibly dietary. Still, the impact for this type of misclassification is likely to be towards the null hypothesis and our exposure assessment was prospective in nature.

Our study design introduced several limitations and so our results should be considered preliminary in nature. The small number of study outcomes diminished the precision of effect estimates and is likely to have undermined our ability to detect modest effects. Ultrasound is not used routinely in Romania to date gestational age; 9% of our participants had an ultrasound to augment the report of last menstrual period. Misclassification of gestational age may have biased our results, most likely towards the null hypothesis. Smoking is a well-recognized risk factor for PD and LBW, yet we did not identify an association [87]. Misclassification may have been introduced by our use of self-reported smoking early in pregnancy. However, smoking prevalence in our study (24%) was similar to 27% reported for Romanian women of reproductive age [88]. Still, use of a biomarker, such as cotinine, would allow for more precise effect estimates as well as integration of indirect exposure [89].

The nature of the Romanian health care system and high participation rates allowed us to capture what we believe to be a representative population sample. Point estimates for the incidence of PD (5.7%; 95% CI = 1.6, 9.0) and LBW deliveries (4.9%; 95% CI = 1.0, 8.8) were lower in our sample than reported for Romania overall, 7.3% and 8.0%, respectively [90, 91]. However, the overlapping 95% CIs suggest these are chance deviations, or alternately they may reflect regional variation. A reference population for Romania was unavailable and so we employed birth size measures for gestational age as reported in the literature to standardize measures of birth size [48–50]. Differences in population size for gestational age might have biased our results, in particular when using dichotomized endpoints for SGA and at $< 10^{\text{th}}$ %tiles. However, the impact is likely modest as the population differences would have been unrelated to iAs exposure *per se*.

5. Conclusion

In conclusion, our results should re-assure mothers that low level residential drinking water iAs exposure has little effect on birth outcomes. However, our results also suggest smoking may potentiate an otherwise benign iAs exposure. Given the widespread exposure of women to low levels of drinking water iAs worldwide, and the exquisite vulnerability of a developing fetus to iAs toxicity, these preliminary results merit confirmation. At low levels of drinking water iAs contamination, women may receive about the same or higher iAs exposure from sources other than drinking water, and our study included only a limited number of smokers with drinking water iAs exposure. Thus, a larger prospective investigation is needed, using urine iAs and cotinine as biomarkers of exposure, and with a sample size sufficiently powered to definitively assess the interactions suggested here.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We prospectively followed 122 women with singleton live births residing in Romania.
- We measured arsenic in drinking water and abstracted data from delivery records.
- Women were exposed to low levels of drinking water arsenic, mostly $<10 \mu\text{g/L}$.
- Higher arsenic associated with lower birth weight and shorter length among smokers.
- Higher arsenic was associated with lower ponderal index in boys only.

Distribution of demographic factors, health-related behaviors, reproductive histories, and drinking water arsenic exposure among Romanian mothers with singleton live births (n = 122).

Table 1

Characteristics	n	Mean (%)	SD	Min.	25 th %tile	Median	75 th %tile	Max.
Demographic								
Maternal age (years)	122	28.85	5.14	18.79	24.65	28.21	33.19	41.94
BMI, pre-pregnancy (kg/m ²)	121	22.24	3.56	15.06	19.84	21.51	24.09	34.48
Married, living as married	97	(79.51)	-	-	-	-	-	-
Residence	-	-	-	-	-	-	-	-
Urban	80	(65.57)	-	-	-	-	-	-
Rural	42	(34.43)	-	-	-	-	-	-
Education	-	-	-	-	-	-	-	-
No High School diploma	26	(21.31)	-	-	-	-	-	-
High School diploma	40	(32.79)	-	-	-	-	-	-
University diploma	56	(45.90)	-	-	-	-	-	-
Health-related behaviors								
Smoked during pregnancy ^a	29	(23.77)						
Average daily cigarettes ^{a,b}	29	11.38	6.83	1	5	10	20	20
Alcohol during pregnancy	13	(10.66)	-	-	-	-	-	-
Reproductive history								
Prior live births	51	1.24	0.55	1.0	1.0	1.0	1.0	3.0
Prior preterm births ^{c,d}	6	(12.77)	-	-	-	-	-	-
Prior low weight births ^{c,e}	3	(6.12)	-	-	-	-	-	-
Drinking water iAs exposure								
Daily water consumption (L) ^f	122	1.31	0.64	0.00	0.80	1.20	1.80	4.00
Average iAs (µg/L) ^g	122	4.11	12.99	<MDL	<MDL	1.28	3.58	130.30
Peak iAs (µg/L) ^h	122	4.82	13.28	<MDL	<MDL	2.27	3.75	130.30
Daily iAs (µg/day) ⁱ	122	4.90	9.77	<MDL	<MDL	1.64	5.35	65.15

^a Reported during the baseline study interview at 5–20 weeks completed gestation;

^b Among n = 29 cigarette smokers only;

^c Among n = 51 reporting a prior live birth;

^d n = 4 missing;

^e n = 2 missing;

^f average water consumed from non-bottled sources, including direct consumption, teas, coffees, mixed beverages, and soups;

^g mean iAs level measured in up to two residential drinking water sources;

^h the highest iAs concentration measured in up to two residential drinking water sources;

ⁱ product of 'average daily water consumption' multiplied by 'average iAs.'

BMI, body mass index; iAs, inorganic arsenic; Max., maximum reported value; MDL, method detection limit (0.50 µg/L); Min., minimum reported value; SD, standard deviation.

Table 2

Distribution of birth outcomes among Romanian mothers with singleton live births (n = 122).

Outcome	n	Mean (%)	SD	Min.	25 th %tile	Median	75 th %tile	Max.
Gestational age (wks.)	122	38.74	1.36	34	38	39	40	42
Caesarean birth	32	(26.23)	-	-	-	-	-	-
Preterm delivery (< 37 wks.)	7	(5.74)	-	-	-	-	-	-
BW (g)	122	3222.70	448.87	1800	2960	3200	3500	4190
LBW(< 2500 g)	6	(4.92)	-	-	-	-	-	-
SGA	10	(8.20)	-	-	-	-	-	-
BL (cm)	122	51.43	1.88	47	50	52	53	58
PI (kg/m ³)	122	23.61	2.37	15.30	22.15	23.57	24.95	29.86
PI < 10 th %tile	14	(11.48)	-	-	-	-	-	-
HC (cm)	122	32.39	1.81	29	31	32	34	39
HC < 10 th %tile	37	(30.33)	-	-	-	-	-	-
Male baby	59	(48.36)	-	-	-	-	-	-

BL, birth length; BW, birth weight; HC, head circumference; LBW, low birth weight; Max., maximum reported value; Min., minimum reported value; PI, ponderal index; SD, standard deviation; SGA, small for gestational age; wks., weeks of gestation.

Adjusted associations between drinking water arsenic exposure metrics and continuous birth outcomes among Romanian mothers with singleton live births (n = 122).

Table 3

Outcome	β 2 nd tertile (95% CI) ^a	β 3 rd tertile (95% CI) ^a	β continuous (95% CI) ^b
Average iAs ($\mu\text{g/L}$) ^c			
Gestational age	-0.27 (-0.67, 0.33)	-0.42 (-1.04, 0.20)	0.002 (-0.01, 0.02)
Z-BW	0.02 (-0.41, 0.45)	-0.15 (-0.70, 0.40)	-0.06 (-0.21, 0.08)
Z-BL ^d	0.10 (-0.29, 0.49)	-0.17 (-0.66, 0.32)	0.08 (-0.32, 0.48) ^e
Z-PI	0.10 (-0.32, 0.53)	0.26 (-0.28, 0.79)	0.12 (-0.31, 0.56) ^e
Z-HC	0.12 (-0.29, 0.54)	0.25 (-0.27, 0.78)	-0.05 (-0.19, 0.09)
Peak iAs ($\mu\text{g/L}$) ^f			
Gestational age	-0.20 (-0.71, 0.30)	-0.34 (-0.95, 0.26)	-0.0003 (-0.02, 0.02)
Z-BW	0.01 (-0.42, 0.45)	-0.12 (-0.66, 0.41)	-0.07 (-0.21, 0.08)
Z-BL ^d	0.11 (-0.28, 0.50)	-0.17 (-0.65, 0.31)	0.03 (-0.28, 0.34) ^e
Z-PI	0.09 (-0.33, 0.52)	0.27 (-0.25, 0.79)	0.11 (-0.23, 0.45) ^e
Z-HC	0.14 (-0.28, 0.56)	0.20 (-0.32, 0.71)	0.05 (-0.18, 0.09)
Daily iAs ($\mu\text{g/day}$) ^g			
Gestational age	-0.16 (-0.70, 0.38)	-0.33 (-0.86, 0.21)	-0.003 (-0.03, 0.02)
Z-BW	0.06 (-0.41, 0.52)	-0.12 (-0.59, 0.36)	-0.05 (-0.24, 0.14)
Z-BL ^d	0.04 (-0.38, 0.46)	0.01 (-0.42, 0.43)	-0.02 (-0.21, 0.16) ^h
Z-PI	0.21 (-0.25, 0.66)	0.08 (-0.38, 0.54)	0.02 (-0.18, 0.23) ^h
Z-HC	0.21 (-0.24, 0.66)	0.10 (-0.35, 0.56)	-0.08 (-0.26, 0.11)

^a Linear (or Cox proportional hazards for gestational age) regression coefficient (β) and 95% confidence intervals (CI) with iAs as tertiles (reference = 1st tertile) adjusted for maternal age (years), pre-pregnancy body mass index (kg/m^2), cigarette smoking during pregnancy (no/yes), and education (< High School, High School, University);

^b Linear (or Cox proportional hazards for gestational age) regression coefficient (β) and 95% CI with iAs as continuous and expressed as a 10 units increase, adjusted for maternal age (years), cigarette smoking during pregnancy (no/yes), pre-pregnancy body mass index (kg/m^2), and education (< High School, High School, University);

^c mean iAs level measured in up to two residential drinking water sources (1st tertile, 0 $\mu\text{g/L}$ iAs; 2nd tertile, 0.07 – 4.12 $\mu\text{g/L}$ iAs; 3rd tertile, 5.3 – 130.3 $\mu\text{g/L}$ iAs);

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p $n = 1$ extreme observation excluded;
 e $n = 2$ influential observations excluded;
 f the highest iAs level measured in up to two residential drinking water sources (1st tertile, 0 – 0.07 µg/L iAs; 2nd tertile, 0.08 – 4.12 µg/L iAs; 3rd tertile, 5.3 – 130.3 µg/L iAs);
 g product of ‘average daily water consumption’ multiplied by ‘average iAs’ (1st tertile, 0 µg/day iAs; 2nd tertile, 0.04 – 3.2 µg/day iAs; 3rd tertile, 3.3 – 65.2 µg/day iAs);
 h $n = 1$ influential observation excluded.
iAs, inorganic arsenic; Z-BL, birth length for gestational age standardized to a reference population; Z-BW, birth weight for gestational age standardized to a reference population; Z-HC, head circumference for gestational age standardized to a reference population; Z-PI, ponderal index for gestational age standardized to a reference population.

Adjusted associations between drinking water arsenic exposure metrics and dichotomous birth outcomes among Romanian mothers with singleton live births (n = 122).

Table 4

Outcome	OR 2 nd tertile (95% CI) ^a	OR 3 rd tertile (95% CI) ^a	OR continuous (95% CI) ^b
Average iAs (µg/L) ^c			
Preterm delivery	0.12 (0.01, 1.13)	0.31 (0.03, 3.05)	0.79 (0.22, 2.78)
SGA	0.69 (0.12, 3.89)	2.78 (0.50, 15.28)	0.91 (0.44, 1.91)
PI <10 th %tile	1.83 (0.42, 8.08)	1.61 (0.26, 9.93)	0.85 (0.38, 1.91)
HC <10 th %tile	0.31 (0.12, 0.83) *	0.33 (0.10, 1.13)	1.23 (0.78, 2.01)
Male baby	1.68 (0.70, 3.99)	0.69 (0.23, 2.13)	0.66 (0.32, 1.37)
Peak iAs (µg/L) ^d			
Preterm delivery	0.13 (0.01, 1.15)	0.30 (0.03, 2.87)	0.72 (0.18, 2.88)
SGA	0.71 (0.12, 4.02)	2.49 (0.46, 13.60)	0.96 (0.54, 1.73)
PI <10 th %tile	1.96 (0.44, 8.70)	1.38 (0.23, 8.34)	0.74 (0.27, 2.03)
HC <10 th %tile	0.33 (0.12, 0.87) *	0.30 (0.09, 1.01)	1.18 (0.80, 1.74)
Male baby	1.59 (0.67, 3.82)	0.85 (0.29, 2.50)	0.74 (0.44, 1.25)
Daily iAs (µg/day) ^e			
Preterm delivery	0.18 (0.02, 1.63)	0.18 (0.02, 1.54)	0.84 (0.34, 2.11)
SGA	1.00 (0.18, 5.63)	1.40 (0.26, 7.50)	0.95 (0.46, 1.98)
PI <10 th %tile	1.76 (0.36, 8.65)	1.79 (0.37, 8.70)	0.81 (0.37, 1.82)
HC <10 th %tile	0.25 (0.08, 0.75) *	0.39 (0.14, 1.11)	1.17 (0.78, 1.76)
Male baby	1.65 (0.65, 4.22)	1.05 (0.41, 2.70)	0.80 (0.52, 1.23)

* P < 0.05;

^a Odds ratio (OR) and 95% confidence intervals (CI) from logistic regression models with iAs as tertiles (reference = 1st tertile) adjusted for maternal age (years), pre-pregnancy body mass index (kg/m²), cigarette smoking during pregnancy (no/yes), and education (< High School, High School, University), or for preterm delivery adjusted for only maternal age (years) and cigarette smoking during pregnancy (no/yes);

^b OR for a 10 units increase in continuous arsenic exposure from logistic regression models adjusted for maternal age (years), pre-pregnancy body mass index (kg/m²), cigarette smoking during pregnancy (no/yes), and education (< High School, High School, University), or for preterm delivery adjusted for only maternal age (years) and cigarette smoking during pregnancy (no/yes);

^c mean iAs level measured in up to two residential drinking water sources (1st tertile, 0 µg/L iAs; 2nd tertile, 0.07 – 4.12 µg/L iAs; 3rd tertile, 5.3 – 130.3 µg/L iAs);

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^d the highest iAs level measured in up to two residential drinking water sources (1st tertile, 0 – 0.07 µg/L iAs; 2nd tertile, 0.08 – 4.12 µg/L iAs; 3rd tertile, 5.3 – 130.3 µg/L iAs);
^e product of ‘average daily water consumption’ multiplied by ‘average iAs’ (1st tertile, 0 µg/day iAs; 2nd tertile, 0.04 – 3.2 µg/day iAs; 3rd tertile, 3.3 – 65.2 µg/day iAs).
iAs, inorganic arsenic; HC, head circumference; PI, ponderal index; SGA, small for gestational age.

Adjusted effects of average drinking water arsenic exposure on birth outcomes, by cigarette smoking in pregnancy (n = 93 non-smokers, n = 29 smokers), among Romanian mothers with singleton live births (n = 122). ^a

Table 5

Interaction model	Exposure factors	Z-BW		Z-BL		Z-PI		Z-HC	
		Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI
Average iAs	Non-smoker								
	0.0 µg/L iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/L iAs	-0.07	-0.21, 0.07	0.13	-0.30, 0.55	0.10	-0.38, 0.57	0.14	-0.34, 0.62
	Smoker								
	0.0 µg/L iAs	0.27	-0.24, 0.78	0.21	-0.24, 0.65	0.20	-0.32, 0.72	-0.06	-0.56, 0.44
	10.0 µg/L iAs	-2.45	-4.49, -0.42	-1.17	-2.33, -0.001	-1.15	-3.17, 0.87	-0.23	-1.54, 1.09
	P-value interaction	0.021 ^b		0.029 ^{c, d}		0.204 ^e		0.691 ^d	
Peak iAs	Non-smoker								
	0.0 µg/L iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/L iAs	-0.07	-0.21, 0.07	0.05	-0.28, 0.38	0.10	-0.26, 0.46	-0.06	-0.20, 0.08
	Smoker								
	0.0 µg/L iAs	0.19	-0.31, 0.70	0.14	-0.34, 0.62	0.06	-0.43, 0.56	-0.10	-0.60, 0.40
	10.0 µg/L iAs	-1.42	-2.67, -0.17	-0.61	-2.22, 1.01	0.25	-0.65, 1.14	-0.37	-1.62, 0.87
	P-value interaction	0.034 ^f		0.393 ^{c, e}		0.868 ^b		0.763 ^f	
Daily iAs	Non-smoker								
	0.0 µg/day iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/day iAs	-0.03	-0.23, 0.16	0.11	-0.15, 0.36	0.02	-0.20, 0.23	-0.09	-0.29, 0.10
	Smoker								
	0.0 µg/day iAs	0.28	-0.23, 0.79	0.21	-0.25, 0.66	0.05	-0.43, 0.53	-0.16	-0.65, 0.32
	10.0 µg/day iAs	-1.16	-2.21, -0.12	-0.48	-1.40, 0.45	0.13	-0.47, 0.74	-0.05	-0.67, 0.56
	P-value interaction	0.021 ^b		0.146 ^{c, e}		0.848 ^f		0.550	

NOTE: P < 0.05 in bold typeface; exposure was continuous but expressed as a 10 units iAs increase.

^aIndependent and joint effects generated using multivariable linear regression models incorporating a product term for maternal cigarette smoking during pregnancy (dichotomous) and iAs exposure (continuous), and adjusted for maternal age (years), pre-pregnancy body mass index (kg/m²), and education (< High School, High School, University);

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- b* n=2 influential observations excluded;
- c* n = 1 extreme value excluded;
- d* n = 3 influential observations excluded;
- e* n = 4 influential observations excluded;
- f* n = 1 influential observation excluded.

CI, confidence interval; iAs, inorganic arsenic; Z-BL, birth length for gestational age standardized to a reference population; Z-BW, birth weight for gestational age standardized to a reference population; Z-HC, head circumference for gestational age standardized to a reference population; Z-P1, ponderal index for gestational age standardized to a reference population.

Adjusted effects of average drinking water arsenic exposure on birth outcomes, by neonatal sex (n=63 girls, n=59 boys), among Romanian mothers with singleton live births (n = 122).^a

Table 6

Interaction model	Exposure factors	Z-BW		Z-BL		Z-PI		Z-HC	
		Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI
Average iAs	Girl								
	0.0 µg/L iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/L iAs	-0.04	-0.19, 0.10	-0.04	-0.58, 0.51	0.58	0.25, 0.91	-0.03	-0.17, 0.11
	Boy								
	0.0 µg/L iAs	0.48	0.09, 0.88	0.38	0.01, 0.75	0.43	0.04, 0.83	0.23	-0.17, 0.62
Peak iAs	10.0 µg/L iAs	0.61	0.01, 1.22	0.65	0.10, 1.19	0.20	-0.39, 0.79	0.67	-0.09, 1.42
	P-value interaction		0.593		0.441 <i>b, c</i>		0.023 <i>d</i>		0.254 <i>d</i>
	Girl								
	0.0 µg/L iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/L iAs	-0.05	-0.19, 0.09	-0.14	-0.54, 0.27	0.37	-0.09, 0.82	-0.04	-0.18, 0.10
Daily iAs	Boy								
	0.0 µg/L iAs	0.47	0.07, 0.87	0.32	-0.06, 0.69	0.41	-0.01, 0.82	0.20	-0.19, 0.60
	10.0 µg/L iAs	0.59	0.07, 1.10	0.60	0.14, 1.07	0.21	-0.31, 0.73	0.58	0.01, 1.16
	P-value interaction		0.535		0.175 <i>b, c</i>		0.107 <i>c</i>		0.173 <i>d</i>
	Girl								
Daily iAs	0.0 µg/day iAs	0.00	Reference	0.00	Reference	0.00	Reference	0.00	Reference
	10.0 µg/day iAs	-0.06	-0.28, 0.16	-0.15	-0.51, 0.21	0.38	-0.02, 0.79	-0.06	-0.27, 0.16
	Boy								
	0.0 µg/day iAs	0.48	0.08, 0.88	0.33	-0.04, 0.69	0.43	0.02, 0.83	0.23	-0.17, 0.63
	10.0 µg/day iAs	0.54	0.11, 0.98	0.50	0.11, 0.89	0.28	-0.15, 0.71	0.46	-0.06, 0.97
Daily iAs	P-value interaction		0.540		0.179 <i>b, c</i>		0.049 <i>c</i>		0.311 <i>d</i>

NOTE: P < 0.05 in bold typeface; exposure was continuous but expressed as a 10 units iAs increase.

^aIndependent and joint effects generated using multivariable linear regression models incorporating a product term for neonatal sex (dichotomous) and iAs exposure (continuous), and adjusted for maternal age (years), pre-pregnancy body mass index (kg/m²), cigarette smoking during pregnancy (no/yes), and education (< High School, High School, University);

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$n = 1$ extreme value excluded;
 $n = 2$ influential observations excluded;
 $n = 1$ influential observation excluded.

CI, confidence interval; iAs, inorganic arsenic; Z-BL, birth length for gestational age standardized to a reference population; Z-BW, birth weight for gestational age standardized to a reference population; Z-HC, head circumference for gestational age standardized to a reference population; Z-PI, ponderal index for gestational age standardized to a reference population.