

Arsenic in Drinking-water and Reproductive Health Outcomes: A Study of Participants in the Bangladesh Integrated Nutrition Programme

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ABSTRACT

This study examined 2,006 pregnant women chronically exposed to a range of naturally-occurring concentrations of arsenic in drinking-water in three upazilas in Bangladesh to find out relationships between arsenic exposure and selected reproductive health outcomes. While there was a small but statistically significant association between arsenic exposure and birth-defects (odds ratio=1.005, 95% confidence interval 1.001-1.010), other outcomes, such as stillbirth, low birth-weight, childhood stunting, and childhood under-weight, were not associated with arsenic exposure. It is possible that the association between arsenic exposure from drinking-water and birth-defects may be a statistical anomaly due to the small number of birth-defects observed. Future studies should look more closely at birth-defects, especially neural tube defects, to elucidate any potential health effects associated with arsenic exposure from drinking-water. Further, given the knowledge that serious health effects can result from chronic arsenic exposure, efforts to find alternatives of safe drinking-water for the population must continue.

Key words: Arsenic; Arsenic exposure; Drinking-water; Reproductive health; Pregnancy outcomes; Bangladesh

INTRODUCTION

Arsenic occurs naturally in the Earth's crust and is a natural contaminant of groundwater in certain parts of the world (1). Most risk modelling has focused on arsenic-related human carcinogenic effects at high doses (>300 parts per billion [ppb], equivalent to 300 µg/L). Questions remain about the shape of the dose-response relationship at lower concentrations (<100 ppb) (2,3). In addition to certain cancers, some researchers have also reported associations between arsenic exposure and diabetes, hypertension, bronchitis, skin lesions, and other vascular diseases and conditions (4-7). Few studies have investigated the potential adverse effects of arsenic exposure on pregnancy

and perinatal outcomes in humans. Results of studies suggest positive associations of arsenic exposure via drinking-water with spontaneous abortion (8-10), stillbirth (8-11), preterm birth (10), low birth-weight (12), and infant mortality (11). Unfortunately, all but one of these studies used ecological designs and, thus, could not control for potential confounding variables (8,10-12); one study with individual-level information used a retrospective design that raises the potential for bias by recall errors (9). Improvements in study design, assessment of exposure, and statistical power may lead to more definitive conclusions about the relationships between arsenic-contaminated drinking-water and adverse reproductive health outcomes.

This study assessed the effect of arsenic-contaminated drinking-water on adverse pregnancy outcomes, such as stillbirth, birth-defects, and low birth-weight using individual-level exposure and health data on 2,006 pregnant women who were exposed to a range of drinking-water arsenic levels. The impact of arsenic exposure on early childhood growth, measured in terms of whether a child was stunted and underweight, was also assessed.

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MATERIALS AND METHODS

Study area

In total, 261 villages from three highly arsenic-contaminated upazilas (sub-districts) of two districts in Bangladesh were selected for this study (Fig.). The upazilas were Faridpur Sadar of Faridpur district and Matlab and Shahrasti of Chandpur district. Data were collected from 100 villages of Faridpur Sadar, 55 villages of Matlab, and 106 villages of Shahrasti.

The three study upazilas were chosen because these satisfied three criteria: (a) These had active Community Nutrition Centres (CNCs) during the study period (calendar year 2002) that had been in operation for at least one year; (b) The CNCs were administered by BRAC (Bangladesh Rural Advancement Committee), a non-governmental organization (NGO) that carried out the fieldwork, ensuring access to CNC data; and (c) The upazilas were known to have a high percentage of tubewells containing arsenic contamination (>50 ppb) identified in prior testing by World Vision.

The CNCs were developed under the Bangladesh Integrated Nutrition Programme (BINP), a government effort to improve maternal and child health outcomes by improving nutrition. In areas where CNCs were established, all pregnant women were enrolled, and tracking continued until the babies were two years old.

Data collection and sampling technique

BRAC developed the data-collection instruments and collected all data for this study during August-September 2003. The study goal was to include 2,200 pregnancies that were completed (by livebirth, stillbirth, or miscarriage) during 2002 for which a drinking-water sample could be obtained. A random number table was used for randomly selecting women into the study cohort from among all those listed in the CNC pregnancy records.

Data on maternal age, marital status, home address, and reproductive health outcomes for 2002 were collected from the CNC logbooks. These data included pregnancy outcomes, any complications during pregnancy or delivery, birth date of the newborn babies, date of the last menstrual period (LMP), dates of prenatal-care visits, body mass index (BMI) of the mother at four, seven, and nine months into the pregnancy, weight gained by the mother, supplementation of vitamin B and iron used, date of admission to CNC, date of delivery, gestational age at delivery, birth-weight

of the newborn, location of birth, type of birth attendant used, and receipt of antenatal care and antenatal health education.

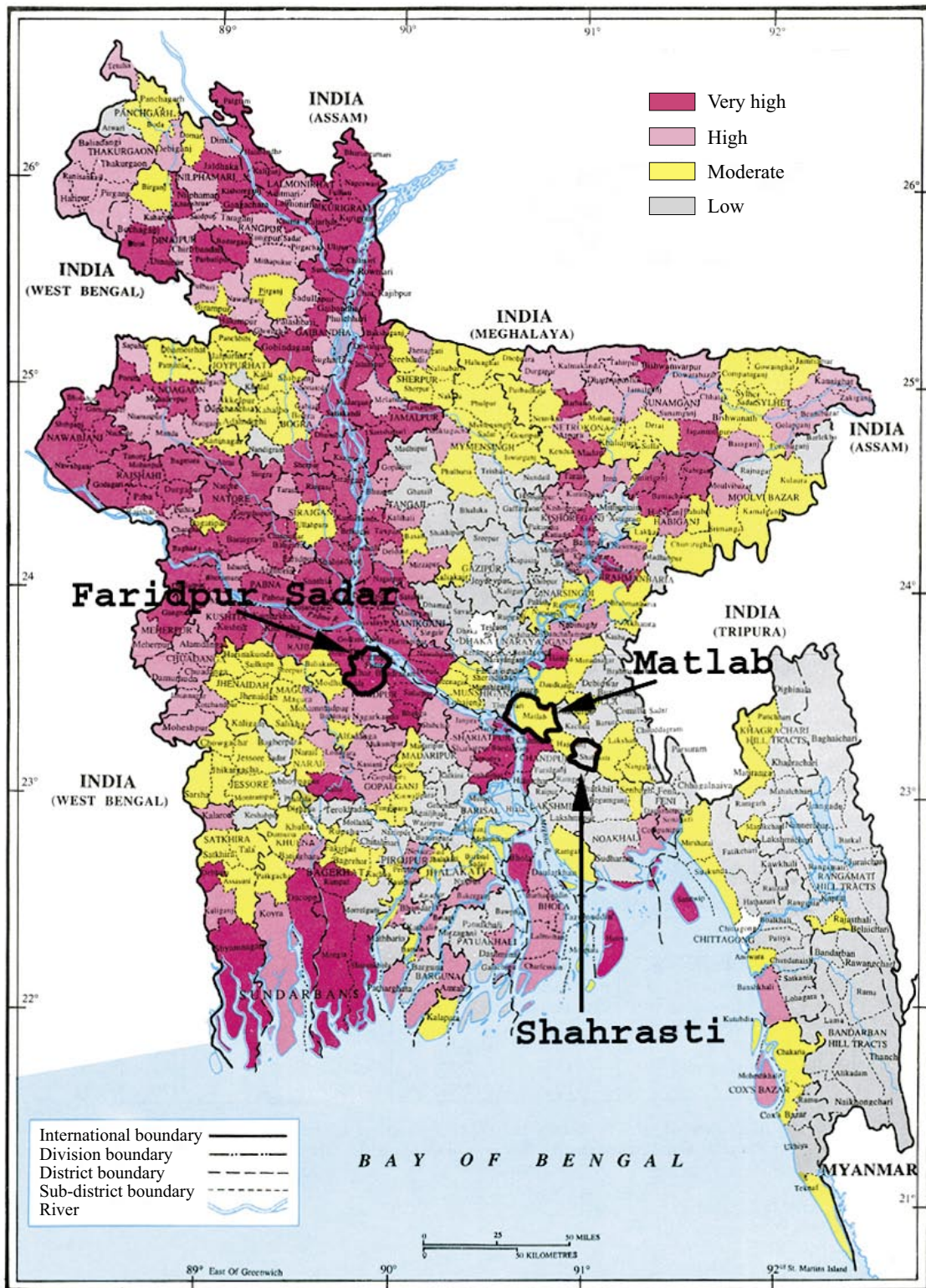
Following abstraction of the CNC records, the interviewers visited mothers at their homes. To ensure that the sample size remained at 2,200, women who were unavailable for any reason (for example, having moved or being away during the interviewer's visits) were replaced in the cohort by other randomly-selected women. Only one mother declined to participate in the home interview. As the field staff did not track information on mothers who were unavailable for the interview, a summary response rate could not be calculated.

During home-visits, mothers were interviewed to verify the information abstracted from the CNC record and to collect additional information on their pregnancy during 2002, prior reproductive history, and household factors, such as assets and monthly income, educational status of the mother and male head of household, exposure to cigarette smoke, tubewell-use, and information on cooking fuel and kitchen ventilation. The interviewers also collected information pertaining to any history of diabetes and hypertension of the respondent. If the mother could not answer a particular question about a past pregnancy, the interviewers sought assistance from any of the senior members of the household who were present at the time of delivery.

The interviewers recorded the current height and weight of the respondent mothers and the babies who were born in 2002. At the time of home-visits, all the surviving babies were aged 7-20 months. Weight was measured using a standard UNICEF-type maternal scale (using the weigh-reweigh method for children). Height was measured in the standing position; for those children who are unable to stand without assistance, height was measured in the supine position.

During the in-home interview, a water sample was collected from the main drinking-water source that each woman used during her pregnancy in 2002. The samples were collected in 100-mL polyethylene bottles with 1 mL of hydrochloric acid at 37% concentration for sample preservation. These samples were sent each week to the Intronics Laboratory in Dhaka for analysis of arsenic concentrations in drinking-water. As 2,003 water samples were collected, it is presumed that some wells were shared by multiple households. The study protocol was reviewed and approved by a human subjects ethics committee convened by the Bangladesh Medical Research Council.

Fig. Government of Bangladesh/WFP resource-allocation map for food-assisted development



Dhaka, April 1997

Graphosman Tel 9552394

Laboratory analyses

Intronics Laboratory measured the drinking-water samples for arsenic following the U.S. Environmental Protection Agency (US EPA) Method 1632 for the determination of arsenic in groundwater (13). This method uses hydride generation-atomic absorption spectroscopy (HG-AAS) and has a detection limit of 0.5 ppb. In addition to Intronics' internal quality-control protocols, an independent audit was performed on the laboratory data by sending 60 water samples to the U.S. Geological Survey's National Water Quality Laboratory (NWQL) in Denver, Colorado (United States) for blinded re-analysis. The 60 samples were selected to cover a range of Intronics' analysis dates, chemists, and arsenic values. The laboratory validation showed good agreement in terms of absolute value of samples ($R^2=0.96$), although the NWQL result tended to be slightly higher than the Intronics' result.

Data analysis

Health outcomes

The main health outcomes of interest were stillbirth, birth-defects, low birth-weight, stunting (in early childhood), and under-weight (in early childhood). Information on these health outcomes was either obtained from the CNC health records, from the mother during the interview, or from measurements made at the time of interview.

A stillbirth is usually defined as a death of a foetus during birth or during the late stages of pregnancy when it would have been expected to survive. Pre-term birth is typically defined as any delivery after 20 weeks but before 37 weeks of gestation. A birth-defect is defined as an abnormality of structure, function, or metabolism (body chemistry) present at birth that results in physical or mental disability, or leads to death. Low birth-weight is typically defined as any birth-weight below 2.5 kg. For the purposes of data analysis, only full-term infants were considered in calculations relating to low birth-weight. In addition to noting the presence of these conditions, the CNC records included continuous values for gestational age and birth-weight. These values were not used in the analysis because, in some cases, the values were missing (5% and 8% respectively) and, apparently, were not always reliably recorded in that the numbers did not always match the categorical outcomes (7% of babies with gestational age <20 weeks were coded in the full-term category and 2% of babies with birth-weight of <2.5 kg were coded in the normal birth-weight category). Instead, the categorical health outcomes were

used in the analysis because these were deemed more reliable for these variables because less information was missing, and there was better agreement between participants' answers and the CNC records.

Several variables were located in the CNC records and also asked of the participant during health-related interviews. When discrepancies occurred between the CNC record and the participant's answer, the BRAC field investigators attempted to determine which was correct. In general, the field workers assumed that the CNC record was more likely to be correct in cases where recall of specific values might be questionable, unless the difference was large and the mother was certain that the CNC record was wrong. As it was possible that the mother may not remember some specific details, the RTI International data analysts used a variable that the BRAC investigators created to adjudicate some values, including weights of mothers and infants, dates of delivery and examination, prenatal use of vitamin B, prenatal use of iron supplement, birth abnormalities and complications during pregnancy, and birth attendants. The participant's response to sex of the child was used regardless of what was indicated on the CNC record. Overall, about 2% of data were adjudicated in this fashion. There were no differences in error rate between different upazilas.

The early childhood growth outcomes of stunting and under-weight were determined using z-scores by comparing the children's measurements of height-for-age and weight-for-age to those of the standard international reference population (14). Those children whose height-for-age z-scores were <-2 were considered stunted, and those whose weight-for-age z-scores were <-2 were considered under-weight.

Derived variables

Since mothers typically do not gain a substantial amount of pregnancy weight before the fourth month of pregnancy (15), the weight gained during pregnancy was calculated by subtracting the mother's four-month pregnancy weight from her nine-month pregnancy weight. BMI was also measured during home-interview, and this value was used as an approximation of the mother's usual or 'baseline' BMI. Since the interviews all occurred at least seven months after the pregnancy ended, it was assumed that the maternal weight had returned to normal.

In developing countries, income of the household does not necessarily present a full picture of the socio-economic status of the household because it does not reflect resources available through household-produced

food, bartering, and so forth. To overcome this obstacle, a household asset index was created. The approach used for developing the household asset index for this study was developed by Filmer and Pritchett (16) who showed that the asset index performs just as well as a more traditional measure, such as household size-adjusted consumption expenditure. The index is based on whether the household possesses certain assets, in this case the presence of a wardrobe, table, chair, bench, watch, cot or bed, radio, television, bicycle, electricity in the house, and high-quality housing materials for the roof, walls, and floor. Principal components analysis of the factors was used for producing a household asset score, which was classified into quintiles.

Additionally, a series of questionnaire items were tested for whether there were indicators of indoor air pollution in the home. Information was obtained on the type of fuel used for cooking, whether cooking was done outside or inside the home, if the stove had a chimney or some sort of vent to the outside, and whether the cooking room had a window or a wall to separate it from the living areas. These variables were coded dichotomously as yes/no and were added to the models independently. Because most households use biomass fuel, it was assumed that fuel used did not contain arsenic and that the only important sources of inorganic arsenic exposure in this population are via drinking-water.

The delivery attendant was coded according to comparisons between trained medical doctors and individuals with varying degrees of training: from less intensive (traditional birth attendants, family welfare visitors, or village doctors) to none (relatives). Similarly, the location of the birth was coded such that facilities with advanced medical care and equipment (government hospitals or private clinics) were compared with other sites, such as homes, local health centres, and community clinics.

Finally, to evaluate the effect of season by pregnancy trimester, the season in which the woman's last menstrual period occurred was coded as Spring (March-May), Summer (June-August), Fall (September-November), or Winter (December-February). Because seasonal fluctuations in groundwater levels may affect arsenic concentration of wells (17,18), and effects of arsenic may vary depending on the stage of pregnancy, a term measuring the interaction between the arsenic level and the season in which the woman's last menstrual period occurred was created.

Statistical analysis

First, bivariate analyses were performed to determine if the reproductive health outcomes and select covariates varied across upazila or arsenic-exposure category. For these analyses, the quantity of arsenic in water samples was categorized as below the limit of detection of the test (BLD) to 10 ppb, 11-50 ppb, 51-100 ppb, 101-200 ppb, 201-300 ppb, and >300 ppb. The presence of differences in the mean and median values across upazilas was assessed using a one-way analysis of variance (ANOVA) for continuous variables or an extended Mantel-Haenszel (MH) general association (chi-square) statistic for categorical variables. A Kendall's tau-b correlation statistic was used for determining if there was a dose-response trend between concentration of arsenic and selected covariates (19). Furthermore, differences in birth outcomes were studied between geographical location (upazilas) and whether or not there were differences in the mean arsenic concentrations within these upazilas.

Logistic regression models were used for assessing whether any associations existed between arsenic exposure and selected outcomes of interest. First, models with a single independent variable were created to determine if there was a crude association between arsenic in drinking-water and each of these health outcomes. These models were then refined by adding in other variables that may have accounted for health outcomes. Variables known to be independently associated with reproductive health outcomes of interest, such as maternal age, household assets, smoking in the household, parity, baseline BMI, and duration (number of years) of tubewell-use were forced into all models. Other potential covariates were initially placed in the models and then dropped if they did not change the adjusted odds ratio (OR) of the exposure of interest (arsenic) by more than 5%, or if keeping them worsened the model according to a goodness-of-fit statistic. These covariates included prenatal care, birth-location and attendants used, weight gained during pregnancy, household ventilation, cooking fuels used, prenatal use of vitamin B and iron, the interaction between arsenic concentrations and the season the woman became pregnant, and number of years using the well.

Adverse pregnancy outcomes have been associated with elevated arsenic exposure and to diabetes and hypertension (9,20,21). Analyses were performed to determine whether any association existed in the study dataset between arsenic exposure and current or prior diabetes or hypertension, as diagnosed by a

medical professional. Separate analyses were then performed to assess the impact of arsenic on health outcomes of interest when women with these diagnoses were alternately included in the dataset, and then excluded, and results were compared. Separate analyses were also performed on a subset of data where the CNC record and the participant's response exactly corresponded. This was done to determine whether potential misclassification affected the study results.

Multiple births were excluded from the dataset because multiple pregnancies are more likely to experience adverse outcomes (22). These selection criteria may have made it easier to determine whether the effects of arsenic truly exist by limiting competing causes of poor outcomes that could potentially introduce more variability into analysis. Furthermore, only full-term births were used in the analyses studying low birth-weight since gestational age is an obvious confounder for low birth-weight.

RESULTS

Of the 2,200 pregnancies, 11 were twin pregnancies, which were excluded from the dataset (there were no higher-order pregnancies). In total, 2,189 women were eligible for this study. Within the study, the number of individuals located in each upazila was equal in Faridpur Sadar and Shahrasti (n=707), but slightly lower in Matlab (n=582). All the sample women used tubewells during their pregnancy in 2002. Arsenic levels in well-water ranged from below the limit of detection (BLD) to 668 ppb, with median concentrations of arsenic of 73 ppb in Faridpur Sadar (range BLD-528 ppb), 139 ppb in Matlab (range BLD-635 ppb), and 24 ppb in Shahrasti (range BLD-668 ppb) (p<0.001 for difference of medians).

Basic demographic characteristics of the study population were stratified by the arsenic-exposure category (Tables 1 and 2). Most correlation coefficients, while sometimes statistically significant, were less than 0.10, indicating very slight relationships. Positive correlation coefficients indicated a positive association between the covariate of interest and the increase in the arsenic category. Several covariates displayed a statistically significant dose-response trend as concentrations of arsenic increased, including smoking by the mother, primary education only, receiving antenatal care, receiving health education, cooking without electricity inside the home, no window in the cooking room, age, BMI, parity, household income, and weight gained during pregnancy. Generally, it seemed that factors relating to lower socioeconomic status

Table 1. Maternal characteristics of women in Faridpur Sadar, Matlab and Shahrasti upazilas, overall, and by arsenic-exposure category, 2002

Variable	Arsenic category (ppb)					Overall (% of column)	Kendall Tau-b correlation coefficient	Test for dose- response trend across arsenic concentrations, p value
	BLD-10	11-50	51-100	101-200	201-300			
Number of individuals	326	230	219	499	496	2,006		
Primiparous	106 (32.5)	71 (30.9)	75 (34.2)	123 (24.6)	132 (26.6)	559 (27.9)	0.0	0.002
Mother who smoked	8 (2.5)	4 (1.7)	1 (0.5)	5 (1.0)	0 (0.0)	18 (0.9)	0.0	<0.0001
Smoking in household	186 (57.1)	128 (55.7)	138 (63.0)	322 (64.5)	293 (59.1)	1,196 (59.6)	2.1	0.925
Primary education only	207 (63.5)	134 (58.3)	140 (63.9)	343 (68.7)	351 (70.8)	1,337 (66.7)	0.0	0.002
Vitamin supplementation	321 (98.5)	222 (96.5)	216 (98.6)	490 (98.2)	490 (98.8)	1,974 (98.4)	0.7	0.129
Received antenatal care	288 (88.3)	215 (93.5)	186 (84.9)	386 (77.4)	353 (71.2)	1,711 (72.5)	1.2	<0.001
Received health education	318 (97.5)	220 (95.7)	206 (94.1)	493 (98.8)	488 (98.4)	1,957 (97.6)	0.0	0.015
Cooking without electricity inside home	205 (62.9)	149 (64.8)	162 (74.0)	379 (76.0)	364 (73.4)	1,428 (71.2)	0.0	0.007
No ventilation to outside on stove	3 (0.9)	1 (0.4)	0 (0.0)	3 (0.6)	1 (0.2)	8 (0.4)	0.0	-0.027
No window in cooking room	193 (59.2)	143 (62.2)	161 (73.5)	390 (78.2)	424 (85.5)	1,511 (75.3)	0.0	<0.0001
No wall separating cooking room	159 (48.8)	121 (52.6)	106 (48.4)	230 (46.1)	225 (45.4)	943 (47.0)	0.0	-0.033
BLD=Below limit of detection; ppb=Parts per billion								

Table 2. Maternal characteristics of women in Faridpur Sadar, Matlab and Shahrasti upazilas, overall, and by arsenic-exposure category, 2002

Variable	Arsenic category (ppb)						Overall mean (SD)	Overall mean (SD)	% missing information	Kendall Tau-b coefficient	Test for dose response trend across-arsenic p value†
	BLD-10 Mean (SD)	11-50 Mean (SD)	51-100 Mean (SD)	101-200 Mean (SD)	201-300 Mean (SD)	>300 Mean (SD)					
Age (years)	26.0 (5.2)	26.2 (5.5)	25.5 (5.3)	26.4 (5.5)	26.5 (5.4)	27.4 (6.1)	26.4 (5.5)	0.0	0.043	0.005	
Body mass index at interview†	19.8 (3.1)	19.5 (2.5)	18.9 (2.3)	19.0 (2.5)	18.7 (2.6)	18.6 (2.4)	19.1 (2.7)	0.4	-0.097	<0.001	
Body mass index at 4th month of pregnancy†	19.6 (2.5)	19.1 (2.3)	18.6 (2.0)	18.7 (2.2)	18.6 (2.4)	18.6 (2.2)	18.8 (2.3)	7.2	-0.080	<0.001	
Parity	2.3 (1.4)	2.5 (1.4)	2.4 (1.4)	2.7 (1.5)	2.8 (1.6)	2.9 (1.6)	2.6 (1.5)	0.0	0.076	<0.001	
Week of first prenatal-care visit	15.0 (6.4)	14.3 (5.1)	14.5 (6.2)	15.9 (8.7)	15.8 (8.1)	15.4 (7.4)	15.3 (7.5)	7.0	0.029	0.070	
Household income (Taka per month)	4512.6 (4827.1)	4044.3 (3357.5)	3710.0 (4190.6)	3911.9 (4376.0)	3905.4 (4761.1)	4065.0 (4583.8)	4019.1 (4454.9)	0.0	-0.040	0.010	
Household asset quintiles	3.2 (1.5)	3.1 (1.5)	2.8 (1.5)	2.9 (1.4)	3.1 (1.4)	3.0 (1.4)	3.0 (1.4)	0.0	-0.003	0.869	
Weight gained during pregnancy (kg)	7.1 (2.4)	7.1 (2.3)	6.9 (2.3)	6.9 (2.5)	6.9 (2.4)	6.6 (2.3)	6.9 (2.4)	0.0	-0.037	0.018	
Years tubewell used	4.7 (3.5)	5.5 (4.0)	5.4 (3.7)	6.2 (4.2)	5.9 (3.8)	6.1 (4.1)	5.7 (4.0)	0.0	0.095	<0.001	

BLD=Below limit of detection; ppb=Parts per billion; SD=Standard deviation

†Calculated as weight (kg)/height (m) * height (m); ‡Source of p value: Kendall's tau-b correlation test for trend statistic

(low household income, primary education only, no electricity or window in cooking room, low BMI, etc.) were associated with increased arsenic exposure. Given this fact, it is possible that these covariates were potential confounders between arsenic exposure and adverse birth outcomes and should be assessed for confounding in data analyses.

Most (95.2%) pregnancies resulted in a livebirth (Table 3). Low birth-weight was the predominant adverse pregnancy outcome within all three upazilas, followed by stillbirth and birth-defects. Only full-term births were included in data analysis for low birth-weight (n=194). In total, 27 women were excluded from the low birth-weight analysis because, while they did have a low-birth-weight infant, they did not have a full-term birth. The prevalence of adverse growth outcomes in early childhood (ages 7 to 20 months) was 55.2% for stunting and 48.0% for under-weight. Stunting was less frequent among children aged 7-11 months (42.8%) than those aged 12-20 months (64.4%). Similarly, under-weight was less frequent among children aged 7-11 months (40.6%) than among those aged 12-20 months (53.5%). The birth-defects included cleft lip and cleft palate (n=1), anencephaly (n=1), hydrocephalus (n=1), club feet (n=3), congenital heart diseases (n=1), laryngomalacia (n=1), neural tube defects or meningocele (n=2), and missing a hand (n=1).

Several adverse health outcomes differed across the three upazilas—Faridpur Sadar, Matlab, and Shahrasti. Stillbirth (2.5%, 0.8%, and 4.2% respectively), low birth-weight (6.6%, 16.4%, and 10.9% respectively), stunting (46.7%, 48.9%, and 69.5% respectively) and under-weight (40.7%, 44.9%, and 58.3% respectively) had differing rates across the three upazilas—Faridpur Sadar, Matlab, and Shahrasti. The rate of birth-defects did not differ across the three upazilas—Faridpur Sadar, Matlab, and Shahrasti (0.6%, 1.0%, and 0.1% respectively). The large majority (81.9%) of women gave birth at home, while births in a government hospital or other locations had significantly lower percentages (14.5%, 3.6% respectively). Of the births at home, the majority of women used a traditional birth attendant (TBA) versus a relative (untrained) or a doctor (69.6%, 27.1%, and 3.3% respectively). This is in contrast to a government hospital where most (97.9%) births were delivered by a trained nurse or a doctor. Despite these differences, an ANOVA demonstrated that there were no statistically significant differences between birth-weights in the three birth locations (p=0.3987).

Table 3. Health outcomes in Faridpur Sadar, Matlab and Shahraști upazilas, overall, and by arsenic exposure category, 2002

Variable	Arsenic category (ppb)						Overall (% of column)	% missing information	Kendall tau-b correlation coefficient	Test for dose- response trend across arsenic concentrations, p value*
	BLD-10 Number (% of column)	11-50 Number (% of column)	51-100 Number (% of column)	101-200 Number (% of column)	201-300 Number (% of column)	>300 Number (% of column)				
Number of individuals	326	230	219	499	496	236	2,006			
Stillbirth	8 (2.5)	5 (2.2)	6 (2.7)	14 (2.8)	17 (3.4)	3 (1.3)	53 (2.6)	0.0	-0.003	0.876
Birth-defects	2 (0.6)	1 (0.4)	1 (0.5)	1 (0.2)	2 (0.4)	4 (1.7)	11 (0.5)	0.0	0.021	0.245
Low birth-weight [†]	40 (12.3)	18 (7.8)	17 (7.8)	44 (8.8)	53 (10.7)	22 (9.3)	194 (9.7)	0.0	-0.006	0.768
Stunting	146 (44.8)	102 (44.3)	89 (40.6)	256 (51.3)	265 (53.4)	146 (61.9)	1,004 (55.2)	9.3	0.097	<0.001
Under-weight	126 (38.7)	86 (37.4)	88 (40.2)	227 (45.5)	223 (45.0)	124 (52.5)	874 (48.0)	9.2	0.008	<0.001

BLD=Below limit of detection; ppb=Parts per billion

*Source of p value: Kendall's tau-b correlation test for trend statistic; [†]Calculated only for full-term pregnancies

The prevalence of adverse outcomes was examined for any dose-response trends across the arsenic-exposure category (Table 3). There were positive, statistically significant arsenic dose-response trends only for stunting and under-weight, and these correlations were very weak. The other outcomes, such as stillbirth, birth-defects, and low birth-weight, did not exhibit any association across the arsenic categories.

In total, 183 women were identified as either having diabetes (n=29), or hypertension (n=147), or both (n=7). Because having diabetes or hypertension could affect the reproductive health outcomes of interest, logistic regression models to assess crude (bivariate) associations between arsenic exposure and the outcomes were employed twice, both including and excluding women with these diagnoses; the results were then compared. In these models, both prior and current diagnoses were combined. The inferences about the associations between arsenic and adverse reproductive health outcomes from the two sets of models were identical. Therefore, women with hypertension and diabetes were excluded from the main analyses.

Multivariate logistic regression models, adjusting for potential risk factors, were used for the main health outcomes. These models controlled for potential confounding effects of factors were thought likely to influence health outcomes.

No association was found between arsenic concentrations in drinking-water and the odds of experiencing a stillbirth (Table 4). However, while no association with arsenic concentrations in drinking-water was found (odds ratio [OR]=0.999, 95% confidence interval [CI] 0.996-1.002), there was a protective association found in women who gained more weight during their pregnancy and the risk of stillbirth.

Studying only full-term births, no association was observed between concentration of arsenic and low birth-weight (Table 4, OR=0.999, 95% CI 0.997-1.000). A woman's higher baseline BMI was shown to be protective against low birth-weight (OR=0.871, 95% CI 0.813-0.934). A similar reduction in odds of low birth-weight was seen for weight gained during pregnancy (kg) (OR=0.878, 95% CI 0.818-0.944) and maternal height (cm) (OR=0.965, 95% CI 0.937-0.995). Female infants were more likely to be of low birth-weight than male infants (OR=1.626, 95% CI 1.170-2.261). There were also differences in low birth-weight between upazilas. Women who lived in Matlab were more likely to experience a low birth-weight outcome than women who lived in Shahrasti

Table 4. Adjusted odds of selected outcomes relating to arsenic exposure through drinking-water in Faridpur Sadar, Matlab and Shahrasti upazilas, 2002

Effect	Odds ratio (95% CI)				
	Stillbirth	Birth-defect	Low birth-weight [†]	Stunting	Under-weight
Arsenic concentration (ppb)	0.999 (0.996-1.002)	1.005 (1.001-1.010)	0.999 (0.997-1.000)	1.000 (1.000-1.001)	1.000 (0.999-1.001)
Maternal age (years)	1.038 (0.973-1.107)	0.958 (0.814-1.128)	0.989 (0.953-1.026)	1.012 (0.988-1.037)	1.016 (0.992-1.041)
Household asset index (quintile)	0.966 (0.768-1.215)	0.762 (0.472-1.231)	0.941 (0.831-1.065)	0.859 (0.794-0.929)	0.815 (0.755-0.880)
Smoking in household	1.111 (0.583-2.117)	0.502 (0.143-1.764)	1.035 (0.739-1.449)	1.002 (0.807-1.245)	1.201 (0.970-1.487)
Primiparous	0.995 (0.418-2.364)	1.818 (0.365-9.048)	0.992 (0.636-1.546)	0.998 (0.748-1.331)	0.958 (0.721-1.273)
Baseline BMI	1.084 (0.968-1.214)	1.163 (1.015-1.332)	0.871 (0.813-0.934)	0.939 (0.900-0.980)	0.909 (0.870-0.950)
Years tubewell used	0.971 (0.891-1.058)	1.030 (0.880-1.207)	0.968 (0.922-1.015)	0.971 (0.945-0.999)	0.929 (0.962-1.017)
Birth attendant	-	-	-	-	2.128
Relative/others vs Dr.	(-)	(-)	(-)	(-)	(1.068-4.243)
Birth attendant	-	-	-	-	1.646
TBA/VD and FWV vs Dr.	(-)	(-)	(-)	(-)	(0.852-3.178)
Birth location	0.246	-	-	1.497	-
Home/health centre vs hospital	(0.114-0.530)	(-)	(-)	(1.100-2.039)	(-)
Iron tablet supplementation	-	-	-	1.001	-
Height of mother (cm)	(-)	(-)	0.965 (0.937-0.995)	0.95 (0.932-0.968)	0.947 (0.929-0.965)
Season—fall vs winter	(-)	(-)	-	1.415 (1.068-1.876)	1.396 (1.056-1.847)
Season—spring vs winter	(-)	(-)	-	2.436 (1.819-3.261)	1.773 (1.336-2.354)
Season—summer vs winter	(-)	(-)	-	1.922 (1.424-2.595)	1.773 (1.328-2.368)
Sex—female vs male	(-)	(-)	1.626 (1.170-2.261)	-	0.789 (0.642-0.970)
Upazila—Faridpur Sadar vs Shahrasti	0.473 (0.202-1.108)	-	0.589 (0.352-0.984)	0.38 (0.279-0.517)	0.496 (0.365-0.675)
Upazila—Matlab vs Shahrasti	0.099 (0.033-0.297)	-	1.857 (1.243-2.775)	0.473 (0.356-0.627)	0.726 (0.546-0.967)
Week of first prenatal-care visit	-	0.817 (0.689-0.968)	1.004 (0.985-1.025)	-	1.002 (0.988-1.016)
Weight gained during pregnancy (kg)	0.814 (0.706-0.938)	-	0.878 (0.818-0.944)	-	-

BMI=Body mass index; CI=Confidence interval; Dr=Medical Doctor; ppb=Parts per billion; VD=Village doctor; TBA=Traditional birth attendant; [†]Calculated only for full-term pregnancies

(OR=1.857, 95% CI 1.243-2.775), whereas women who lived in Faridpur Sadar were less likely to experience a low birth-weight outcome than women who lived in Shahrasti (OR=0.589, 95% CI 0.352-0.984). This analysis was repeated in the subset of women whose responses exactly corresponded to the CNC records. The results from this analysis did not vary significantly between arsenic concentration and low

birth-weight (OR=0.999, 95% CI 0.997-1.002), stillbirth (OR=0.999, 95% CI 0.996-1.004), birth-defect (OR=1.005, 95% CI 1.000-1.012), stunting (OR=1.000, 95% CI 1.000-1.001), or under-weight (OR=1.000, 95% CI 0.999-1.002).

No association was found between stunting in early childhood (low height-for-age) and concentration of arsenic (Table 4, OR=1.000, 95% CI 0.999-

1.001). However, there was an association between stunting and the season in which the pregnancy began. Compared to the winter, women who had their first trimester beginning in the spring (OR=2.436, 95% CI 1.819-3.261) and summer (OR=1.922, 95% CI 1.424-2.595) exhibited elevated odds for their child experiencing stunting. Additionally, babies born at home or in a village health centre or clinic, compared to a hospital, experienced elevated odds of stunting. Those babies whose mothers had received iron tablets were also slightly more likely to be stunted. Protective associations were found for higher household asset index, greater baseline BMI, greater mother's height, more years of using tubewell, and living in Faridpur Sadar or Matlab compared to living in Shahrasti upazila.

No association was found between under-weight in early childhood (low weight-for-age) and concentration of arsenic (Table 4, OR=1.000, 95% CI 0.999-1.001). There was a positive association between the seasons in which the last menstrual period occurred. Compared to the winter, first trimester beginning in the spring or summer both exhibited elevated odds for experiencing stunting. Additionally, children born without the attendance of a trained medical doctor experienced elevated odds of stunting. Protective associations for stunting were found for higher quintile of the household asset index, higher baseline BMI, greater height of the mother, female sex, and living in Faridpur Sadar compared to living in Shahrasti.

DISCUSSION

This study found a small association between arsenic exposure and all birth-defects combined but did not find an association between arsenic exposure and separate outcomes of stillbirth, low birth-weight, stunting, or under-weight.

The large number of women participating in the study and the quantity and quality of information about health and exposure, distinguish this study from similar studies. The larger sample size allows for greater statistical power to detect potential health effects, especially in a rural, largely poor population where there are many competing causes of poor health outcomes. At 80% statistical power, the sample size is sufficient to detect differences of at least 60% for stillbirth, 50% for low birth-weight, and 30% for stunting and under-weight within the study. However, for very rare outcomes, such as birth-defects, the study only had a sufficient sample size to detect differences of 100% at 80% statistical power.

The strength of the study design comes from the large amount of prospective pregnancy-related data collected at CNCs, which provided quantitative information to verify questions that may be subject to recall bias in studies that are solely based on retrospective interviews. Many important variables collected during health interviews, such as birth-weight, weight gain during pregnancy, and vitamin supplementation, were, in fact, verified with records from the CNCs, leading to improved quality of data. At the same time, the interviews provided an opportunity for the study subjects to correct any information that was misrecorded at the CNCs or by the interviewers during data abstraction.

Within the study population, drinking-water was thought to be the primary source of arsenic exposure regardless of socioeconomic status or other lifestyle factors, which limits potential measurement error due to multiple sources of arsenic exposure. The wide range of arsenic concentrations in the wells of the study areas eliminated the need to extrapolate health effects to higher or lower doses within the range typically seen in Bangladesh. Also, the wide range of arsenic exposure in this study provides an improvement upon animal models and other arsenic studies that have only a limited population for exposure, that have a population exclusively exposed to very high or low levels of arsenic without a gradient of exposure, or that lumped all exposures over 50 ppb into a single 'exposed' category.

This study design has several limitations. First, nutritional deficiencies are suspected to increase susceptibility to at least some effects of arsenic (6,7,23). This study was able to control for BMI of mothers in early pregnancy, weight gain during pregnancy, and use of vitamin supplement. These variables provided some indication of nutritional status of mothers during pregnancy. However, the data collected did not allow for explicit examination of whether the Bangladesh Integrated Nutrition Programme, through CNC-provided food supplements or other means, provided a protective factor against arsenic effects during pregnancy that would have otherwise occurred. Although it is yet unknown how, or whether, diet and nutritional status has an effect on toxicity of arsenic, it has been hypothesized that certain dietary elements may affect metabolism and toxicity of arsenic (24-26).

Second, although the assessment of arsenic exposure was conducted at the individual level, individual consumption and biomarker information was not collected. Furthermore, the water arsenic samples were

collected after the pregnancy, which could be problematic if arsenic levels within a given well vary much over time. Thus, the actual dosages (total quantity of arsenic ingested) could not be calculated. However, available evidence indicates that arsenic levels are stable within at least a two- to three-year period (27), which covers the longest possible time range from conception to assay in this study. Furthermore, a study performed in Bangladesh found a moderate positive correlation (correlation coefficient=0.50) between arsenic concentrations in well-water and urinary arsenic concentration, which reflects actual total intake and metabolic variation in individuals (28), indicating that arsenic in well-water is a reasonable proxy for quantity of exposure. Given the limited mobility of the study population and the limited use of other sources of water, it is reasonable to employ the strategy of assuming that the study population was exposed to the measured levels of arsenic in drinking-water from their wells to estimate individual arsenic exposure.

There are also some limitations to data items that were collected from the CNCs. As discussed above, actual weeks of gestation and birth-weight were not reliably recorded in all cases, leading to the possibility of some misclassification. Such errors are likely to have occurred because women typically did not receive early antenatal medical care or give birth at a medical facility; this could have led to errors in the recall of LMP, use of imperfect scales, or late weighing of newborn babies. The authors attempted to limit this type of error by only looking at categorically-defined full-term babies for low birth-weight analysis. The authors used the categorical variable for gestational age because it had less missing information, was less likely to be in error because it is easier to remember a 'yes' or 'no' answer than to remember an exact number of weeks, and had better agreement with the mother's recollection than the numeric variable. This validation approach used the subset of data where the medical records were internally consistent and where the medical records and the responses of the subjects exactly matched. As there were no appreciable differences between the full dataset and this subset, the authors felt confident that using the full dataset would be a valid approach.

Nevertheless, there are some challenges in even using the validated data. For instance, looking at low-birth-weight infants, there is a correlation between gestational age and low birth-weight. Full-term birth is considered to be at 38-42 weeks of gestation. There could be wide variations in birth-weight between a 38-week old newborn versus a 42-week old new-

born, although both would be considered full-term. Unfortunately, there would be no way to put the low-birth-weight 38-week versus the 42-week old babies into a separate analysis, and the reader must take this limitation into account when interpreting the findings of this study. Nevertheless, low-birth-weight infants have considerably more health risks than normal-weight infants regardless of gestational age, and it is possible that arsenic exposure may be mechanistically associated with this outcome through the restriction of nutrients to the foetus by vasoconstriction. Further research into this outcome is needed to determine the impact of arsenic versus nutrition in low-birth-weight outcomes.

It is possible that a lack of antenatal medical attention could have led to non-diagnosis or misdiagnosis of maternal medical conditions. This was evident in the overwhelming number of women who gave birth at home (81.9%) versus a hospital (14.5%). These differences in birth-location are magnified when looking at the birth attendants used at these locations. Nurses and doctors receive the most advanced training in birthing techniques, followed by a TBA, and then followed by relatives who may or may not have any specialized training in delivering newborn infants. Most births that were delivered in a hospital were attended to by a nurse or doctor (97.9%) while the births delivered at home were attended to by a TBA (69.6%) or relative (27.1%). While there are large differences between birth-location and the type of attendant used, there does not seem to be any statistically significant differences between the birth-location and outcomes, such as birth-weight ($p=0.3987$). These are typical limitations of population-based pregnancy studies in developing countries where women do not receive extensive medical care. However, for all these health outcomes, there is no reason to expect that misclassification would vary with arsenic-exposure status. Such non-differential misclassification would decrease any observed statistical relationship between arsenic exposure and adverse health outcomes. This effect could have obscured a weak relationship if one truly existed. However, analysis looking at the subset of data where the CNC records and the responses of the participants completely agreed, did not indicate any differences in associations between the arsenic exposure and adverse health outcome, indicating that non-differential misclassification was not an issue during this analysis.

Rates of adverse outcomes in this study were compared with rates from three recent independent sources in an effort to determine whether misclassification could be affecting the results. These sources in-

cluded two national population-based surveys (29,30) and the National Nutrition Programme (NNP; an outgrowth of BINP) within the study areas (Mahmud F. Personal communication, 2004). These comparisons, as detailed in Table 5, show that the study population experienced low birth-weight (looking at full-term births only), childhood stunting, and childhood underweight at rates comparable to the national population. The comparison for stillbirth was equivocal. Comparable data for the birth-defect outcomes addressed in

have meant the prevalence of this outcome would be 21%, an implausible figure in view of the prevalence rates seen elsewhere.

Potential misclassification of the outcome could push the estimate towards or away from the null value. By studying the impact of the additional (or reduced) number of cases to achieve a hypothetical OR, the sensitivity analysis provides a theoretical bound to determine whether or not these values are probable.

Table 5. Birth outcomes in Faridpur Sadar, Matlab and Shahrasti upazilas in study population compared to the National Nutrition Programme, 2003-2004; Bangladesh Maternal Health Services and Maternal Mortality Survey, 2001; and the Bangladesh Demographic and Health Survey, 1999-2000

Variable	Study population, 2002			National Nutrition Programme, November 2003–August 2004				Bangladesh Maternal Health Services and Maternal Mortality Survey, 2001	Demographic and Health Survey, 1999-2000
	Faridpur (%)	Matlab (%)	Shahrasti (%)	Faridpur (%)	Matlab South (%)	Matlab North (%)	Shahrasti (%)	National (%)	National (%)
Livebirths*	94.6	98.0	93.4	90.2	90.2	83.1	82.2	90.2	
Stillbirth	2.5	0.8	4.2	3.8	4.0	12.1	13.2	2.5	
Low birth-weight [†]	6.3	14.6	9.9	9.6	13.8	9.9	10.2		
Stunting	46.7	48.9	69.5						44.7
Under-weight	40.7	44.9	58.3						47.7

*All births, excluding spontaneous abortions and stillbirths. It is possible to be in more than 1 category (e.g. livebirth and low birth-weight)

this study were not available. Nevertheless, the comparable rates of these adverse health outcomes within the study population indicated that the study population did not differ appreciably from the nationwide surveys.

To assess the probability that missing or misclassified outcomes could affect the study results, a sensitivity analysis was undertaken for the outcomes that were not directly measured by the field staff (Table 6). The analysis examined the potential number of arsenic-exposed women with poor outcomes who were missed if the true population OR for each outcome was 0.5, 2.0, or 3.0. For example, for low birth-weight, the study would have estimated an OR of 2.0 if the study participants included 24 more women with low-birth-weight newborns who had an arsenic exposure above 300 ppb, or 176 more women with low-birth-weight newborns who had an arsenic exposure above 50 ppb. Note that the addition of these individuals would increase the sample size such that by adding 24 women, all would have low-birth-weight infants, and the overall sample size would increase. However, adding even 24 women with low-birth-weight newborns would

For the stillbirth outcome, including sufficient women to bring the OR to 2.0 would again have raised the prevalence rate to values that seem unlikely in view of national survey results (4.7% or more), although within the range of the highest NNP values. Misclassification would have a greater impact in the higher exposure categories due to the small number of cases. Indeed, if misclassification did occur in the higher exposure categories due to the small numbers of cases needed, the prevalence of stillbirth and low birth-weight would be in line with national estimates. This only holds true in the higher exposure categories and not in the lower exposure categories. It is unlikely that differential misclassification only occurred in the higher exposure areas and not in the lower exposure areas as well. Furthermore, it is unlikely that the effect of arsenic is protective (OR <1) since the prevalence of health outcome would become too low. Thus, while the possibility cannot be ruled out that missing or systematically misclassified data may have affected the results, a large bias in the results appears improbable.

Earlier studies on other populations in Bangladesh reported associations between arsenic exposure

Table 6. Results of sensitivity analysis to determine impact of missing data on pregnancy outcomes at various arsenic-exposure levels

Arsenic concentration and hypothetical odds ratio	Pregnancy outcome			
	Stillbirth		Low birth-weight*	
	Number of additional cases needed to achieve hypothetical odds ratio	Prevalence of health outcome after adding additional cases (%)	Number of additional cases needed to achieve hypothetical odds ratio	Prevalence of health outcome after adding additional cases (%)
300 ppb				
OR=0.5	1	1.70	-15	6.02
OR=2	11	5.71	24	20.70
OR=3	18	8.33	49	28.01
200 ppb				
OR=0.5	-10	1.41	-55	5.60
OR=2	18	5.16	55	19.39
OR=3	37	7.55	128	26.51
100 ppb				
OR=0.5	-19	1.27	-73	5.93
OR=2	26	4.88	126	20.12
OR=3	57	7.14	257	27.48
50 ppb				
OR=0.5	-23	1.22	-75	6.36
OR=2	28	4.70	176	21.31
OR=3	62	6.88	343	28.87

*Calculated only for full-term pregnancies; ppb=Parts per billion; OR=Odds ratio

and spontaneous abortion, stillbirth, preterm birth rates, and neonatal mortality (9,10). Ecological studies in other countries, including Chile, Hungary, and Taiwan, have also found associations between exposure and poor pregnancy outcomes (8,11,12). In this study population, however, apart for a small association with all birth-defects combined, no association was found between exposure to arsenic-contaminated drinking-water and the selected pregnancy outcomes.

Several associations were found between the health outcomes of interest and selected covariates. These findings were consistent with what has been reported in the literature, lending support to negative findings that arsenic exposure is not spurious. For instance, maternal under-nutrition, as reflected by low BMI, is a risk factor for low-birth-weight infants, preterm birth, and other delivery complications for mothers (11,31). Improved maternal nutrition, as reflected by weight gained during pregnancy, reduces the risk of adverse health outcomes, such as low-birth-weight infants, stillbirth, and premature birth (31-32). The findings of this study concurred with earlier findings in that elevated BMI and increased weight gain during pregnancy reduced the odds of having a low-birth-weight infant. Similarly, a protective effect was observed between weight gained during pregnancy and

reduced odds of stillbirth and low birth-weight. Girls were more likely to be of low birth-weight, and it has been found previously that the mean birth-weight in girls is typically lower than in boys (33). The finding that rates of stillbirth are higher when women deliver in a hospital versus in their homes can perhaps be explained by most women seeking advanced medical care only when having complications with their pregnancies. Thus, hospitals would have more adverse outcomes than the home or location of local health center simply because the individuals who deliver at the hospitals tend to have more complications with their pregnancy.

As stated above, there was a small but statistically significant association found between concentrations of arsenic and all birth-defects combined. However, with only 11 birth-defects reported, it is possible that the positive association found may reflect a statistical artifact rather than a true association since this study does not have the sufficient sample size to study such a rare disease outcome in-depth. If there were a real relationship between arsenic exposure and birth-defects, one would expect to see that arsenic is particularly associated with a specific defect or defects reflecting a consistent biological mechanism of effect. In this study, the small number of all reported birth-defects precluded analysis by type of defect.

Only for clubfoot (n=3) and neural tube defect (n=2) were multiple instances of a single defect seen. The neural tube defect finding is consistent with reports from animal and human studies that have found associations with elevated levels of arsenic exposure (in the form of pesticide exposure) and neural tube defects (34). Nevertheless, given that birth-defects are rare events and the multitude of causative factors that could contribute to birth-defects are unknown with respect to arsenic exposure through drinking-water, future investigations should be designed to study this relationship more closely.

One aspect that should be noted is that the CNCs and mothers do not have the diagnostic tools available to detect birth-defects that may not be readily obvious, such as metabolic problems and malformations of internal organs. Thus, the birth-defects reported are likely to be skewed towards gross physical abnormalities. Future studies should explore birth-defects, especially neural tube defects, and their association with arsenic exposure in greater detail. A different study design, such as case-control, would be appropriate for studying very rare outcomes, such as individual birth-defects. Such a study should be performed. In addition, this topic should also be studied in different populations as effects may vary with differences in genes, diet, or other environmental factors. Finally, studies could be undertaken in areas where populations are exposed to very high arsenic concentrations to determine if higher doses are harmful to human fetuses.

Ideally, future population-based studies could incorporate prospective data collection and personal dose measurement to obtain robust measures of outcomes and exposures to arsenic-contaminated drinking-water. Regardless of whether arsenic is ultimately found to be a special hazard during pregnancy, given that known serious health effects can result from chronic arsenic exposure, efforts to find safe alternatives of drinking-water for the population must continue.

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REFERENCES

1. Bhumbla D, Keefer R. Arsenic mobilization and bioavailability in soils. *In: Nriagu J, editor. Arsenic in the environment. Pt. I: Cycling and characterization.* New York: Wiley, 1994;51-82. (Advances in environmental science and technology, v. 26).
2. Arsenic Rule Benefits Review Panel. Arsenic rule benefits analysis: an SAB review. A review by the Arsenic Rule Benefits Review Panel (ARBRP) of the US EPA Science Advisory Board, EPA-SAB-EC-01-008. Washington, DC: U.S. Environmental Protection Agency, 2001.
3. National Research Council. Arsenic in drinking-water. 2001 update. Washington, DC: National Academy Press, 2001. 225 p.
4. Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH, Axelson O. Hypertension and arsenic exposure in Bangladesh. *Hypertension* 1999;33:74-8.
5. Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM *et al.* Increased prevalence of hypertension and long-term arsenic exposure. *Hypertension* 1995;25:53-60.
6. Mitra SR, Mazumder DN, Basu A, Block G, Haque R, Samanta S *et al.* Nutritional factors and susceptibility to arsenic-caused skin lesions in West Bengal, India. *Environ Health Perspect* 2004;112:1104-9.

7. Guha Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakraborty D *et al.* Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. *Int J Epidemiol* 1998;27:871-7.
8. Borzsonyi M, Bereczky A, Rudnai P, Csanady M, Horvath A. Epidemiological studies on human subjects exposed to arsenic in drinking water in southeast Hungary. *Arch Toxicol* 1992;66:77-8.
9. Milton AH, Smith W, Rahman B, Hasan Z, Kulsom U, Dear K *et al.* Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. *Epidemiology* 2005;16:82-6.
10. Ahmad SA, Sayed MH, Barua S, Khan MH, Faruquee MH, Jalil A *et al.* Arsenic in drinking water and pregnancy outcomes. *Environ Health Perspect* 2001;109:629-31.
11. Hopenhayn-Rich C, Browning SR, Hertz-Picciotto I, Ferreccio C, Peralta C, Gibb H. Chronic arsenic exposure and risk of infant mortality in two areas of Chile. *Environ Health Perspect* 2000;108:667-73.
12. Yang CY, Chang CC, Tsai SS, Chuang HY, Ho CK, Wu TN. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. *Environ Res* 2003;91:29-34.
13. United States Environmental Protection Agency. Method 1632: inorganic arsenic in water by hydride generation quartz furnace atomic absorption. Washington, DC: Office of Water, Engineering and Analysis Division, U.S. Environmental Protection Agency, 1996. (EPA/821/R-96-013).
14. World Health Organization. A growth chart for international use in maternal and child health care: guidelines for primary health care personnel. Geneva: World Health Organization, 1978. 36 p.
15. Cunningham FG, Gant NF, Leveno KJ, Gilstrap LC, Hauth JC, Wenstrom KD. Williams Obstetrics. 21 ed. New York: McGraw-Hill, 2001. 1,668 p.
16. Filmer D, Pritchett L. Estimating wealth effects without expenditure data-or tears: an application to educational enrollments in States of India. *Demography* 2001;38:115-32.
17. Harvey CF, Swartz CH, Badruzzaman AB, Keonblute N, Yu W, Ali MA, *et al.* Arsenic mobility and groundwater extraction in Bangladesh. *Science* 2002;298:1602-6.
18. Tareq SM, Safiullah S, Anawar HM, Rahman MM, Ishizuka T. Arsenic pollution in groundwater: a self-organizing complex geochemical process in the deltaic sedimentary environment, Bangladesh. *Sci Total Environ* 2003;313:213-26.
19. Kendall MG. Rank correlation methods. 4th ed. London: Charles Griffin, 1975. 202 p.
20. Rahman M, Tondel M, Ahmad S, Axelson O. Diabetes mellitus associated with arsenic exposure in Bangladesh. *Am J Epidemiol* 1998;148:198-203.
21. Yoshida T, Yamauchi H, Fan Sun G. Chronic health effects in people exposed to arsenic via the drinking water: dose-response relationships in review. *Toxicol Appl Pharmacol* 2004;198:243-52.
22. Garite TJ, Clark RH, Elliott JP, Thorp JA. Twins and triplets: the effect of plurality and growth on neonatal outcome compared with singleton infants. *Am J Obstet Gynecol* 2004;191:700-7.
23. Kabir MI, Rahman M, Flora MS, Azad AK. Arsenicosis and body mass index (BMI) in a selected area of Bangladesh. *J Preven Soc Med* 2001;29:6-12.
24. Cooney CA. Dietary selenium and arsenic affect DNA methylation. *J Nutr* 2001;131:1871-2.
25. Spallholz JE, Mallory Boylan L, Rhaman MM. Environmental hypothesis: is poor dietary selenium intake an underlying factor for arsenicosis and cancer in Bangladesh and West Bengal, India. *Sci Total Environ* 2004;323:21-32.
26. Miyazaki K, Watanabe C, Mori K, Yoshida K, Ohtsuka R. The effects of gestational arsenic exposure and dietary selenium deficiency on selenium and selenoenzymes in maternal and fetal tissues in mice. *Toxicology* 2005;208:357-65.
27. Van Geen A, Cheng Z, Seddique AA, Hoque MA, Gelman A, Graziano JH *et al.* Reliability of a commercial kit to test groundwater for arsenic in Bangladesh. *Environ Sci Technol* 2005;39:299-303.
28. Ahsan H, Perrin M, Rahman A, Parvez F, Stute M, Zheng Y *et al.* Associations between drinking-water and urinary arsenic levels and skin lesions in Bangladesh. *J Occup Environ Med* 2000;42:1195-201.

29. National Institute of Population Research and Training. Bangladesh demographic and health survey 1999-2000. Dhaka: National Institute of Population Research and Training, 2001. 280 p.
30. National Institute of Population Research and Training. Bangladesh maternal health services and maternal mortality survey 2001. Dhaka: National Institute of Population Research and Training, 2003. 234 p.
31. Ehrenberg HM, Dierker L, Milluzzi C, Mercer BM. Low maternal weight, failure to thrive in pregnancy, and adverse pregnancy outcomes. *Am J Obstet Gynecol* 2003;189:1726-30.
32. Ortolano SE, Mahmud Z, Iqbal Kabir AF, Levinson FJ. Effect of targeted food supplementation and services in the Bangladesh Integrated Nutrition Project on women and their pregnancy outcomes. *J Health Popul Nutr* 2003;21:83-9.
33. Osmani S, Sen A. The hidden penalties of gender inequality: fetal origins of ill-health. *Econ Hum Biol* 2003;1:105-21.
34. Shalat SL, Walker DB, Finnell RH. Role of arsenic as a reproductive toxin with particular attention to neural tube defects. *J Toxicol Environ Health* 1996;48:253-72.