Published by Oxford University Press on behalf of the International Epidemiological Association
International Journal of Epidemiology 2011;**40**:1593–1604
doi:10.1093/ije/dyr176

Critical windows of exposure for arsenic-associated impairment of cognitive function in pre-school girls and boys: a population-based cohort study

JD Hamadani,[1]* F Tofail,[1,2] B Nermell,[2] R Gardner,[2] S Shiraji,[1] M Bottai,[2] SE Arifeen,[1] SN Huda[3] and M Vahter[2]

[1]International Center for Diarrhoeal Disease Research, Dhaka, Bangladesh, [2]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden and [3]Institute of Nutrition and Food Science, Dhaka University, Dhaka, Bangladesh

*Corresponding author. Head, Child Development Unit, Clinical Sciences Division, ICDDR,B: International Center for Diarrhoeal Disease Research, Bangladesh, 68 Shaheed Tajuddin Ahmed Sharani, Mohakhali, Dhaka 1212, Bangladesh.
E-mail: jena@icddrb.org

Accepted 6 October 2011

Background Exposure to arsenic through drinking water has been associated with impaired cognitive function in school-aged children in a few cross-sectional studies; however, there is little information on critical windows of exposure.

Methods We conducted a population-based longitudinal study in rural Bangladesh. We assessed the association of arsenic exposure, based on urinary arsenic (U-As; twice during pregnancy and twice in childhood), with the development of about 1700 children at 5 years of age using Wechsler Pre-school and Primary Scale of Intelligence [intelligence quotient (IQ)].

Results Median maternal U-As in pregnancy was 80 μg/l (10–90 percentiles: 25–400 μg/l). Children's urine contained 35 (12–155) μg/l and 51 (20–238) μg/l at 1.5 and 5 years, respectively. Using multivariable-adjusted regression analyses, controlling for all potential confounders and loss to follow-up, we found that verbal IQ (VIQ) and full scale IQ (FSIQ) were negatively associated with (log) U-As in girls. The associations were consistent, but somewhat stronger with concurrent arsenic exposure [VIQ: $B = -2.4$, 95% confidence interval (CI) $= -3.8$ to -1.1; FSIQ: $B = -1.4$, 95% CI $= -2.7$ to -0.1, $n = 817$), compared with that at 1.5 years (VIQ: $B = -0.85$, 95% CI $= -2.1$ to 0.4; FSIQ: $B = -0.74$, 95% CI $= -1.9$ to 0.4, $n = 902$), late gestation (VIQ: $B = -1.52$, 95% CI $= -2.6$ to -0.4; FSIQ: $B = -1.35$, 95% CI $= -2.4$ to -0.3, $n = 874$) and early gestation (VIQ: $B = -1.23$, 95% CI $= -2.4$ to -0.06; FSIQ: $B = -0.92$, 95% CI $= -2.0$ to -0.2, $n = 833$). In boys, U-As showed consistently low and non-significant associations with all IQ measures. An effect size calculation indicated that 100 μg/l U-As was associated with a decrement of 1–3 points in both VIQ and FSIQ in girls.

Conclusion We found adverse effects of arsenic exposure on IQ in girls, but not boys, at 5 years of age.

Keywords Pregnancy, maternal urinary arsenic, child urinary arsenic, cognitive function, intelligence quotient, arsenic exposure, gender differences

Introduction

Inorganic arsenic (iAs) is a known carcinogenic toxicant that is prevalent in drinking water in many areas of the world.[1–3] The vast majority of published literature focuses on health effects in exposed adults. A few cross-sectional studies have reported impairment of cognitive and neurobehavioural function in school-aged children with increasing arsenic exposure through drinking water.[4–9] However, we found no significant association between pre- or post-natal arsenic (maternal and child urinary arsenic) and child development at 7 or 18 months of age in our ongoing population-based, longitudinal study in rural Bangladesh.[10,11] However, we observed significant association of maternal arsenic exposure with reduced size at birth and increased infant morbidity and mortality in the same population.[12–16]

It is conceivable that arsenic affects higher cognitive functions which develop with increasing age, or that the effects can be detected first in school-aged children even if induced much earlier. Although many chemicals induce neurotoxicity *in utero* or early infancy while basic brain structures are forming and neurons are proliferating and migrating, arsenic may affect the development of synaptic connections, receptors and transmitter systems, which continues for years after birth.[17] Children in this population may be particularly vulnerable to arsenic neurotoxicity later in childhood because the prevalence of undernutrition in Bangladeshi children increases after their first year in life.[18] Particularly, a low intake of antioxidants may aggravate arsenic-induced oxidative stress.[19] Also, a low intake of nutrients essential for arsenic metabolism may result in poor arsenic detoxification.[20] Arsenic is methylated via one-carbon metabolism to methylarsonic acid (MMA) and dimethylarsinic acid (DMA). A high fraction of the toxic metabolite MMA in urine is an established risk factor for adverse effects in adults.[21]

The aim of this study was to assess cognitive and language development at 5 years of age in relation to arsenic exposure at multiple time points throughout gestation and during childhood. Also, we wanted to elucidate if malnutrition and poor arsenic metabolism could influence such associations. Though sex differences were not specifically hypothesized a priori, based on the knowledge about toxicological mechanisms of arsenic and our previous experience of gender-differences in arsenic toxicity,[21,22] we also evaluated whether arsenic would affect development differently in girls and boys.

Materials and Methods

Study area and population

Details of the study area and population are given in our previous publications.[10,11,23] In short, the study was conducted in Matlab, a rural area 53 km south-east of the capital Dhaka (for a map of the study area, see Sohel *et al*. 2010)[15] where International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b) has been collecting vital health and demographic information for the past 60 years.[24] A parallel water-screening project found that 70% of the tube wells, used by >95% of the population, had arsenic concentrations >10 μg/l.[25] The arsenic concentration of the water used by the women during pregnancy had a median of 66 μg/l (90th percentile: 410 μg/l).[26]

The study was part of a population-based randomized maternal and infant nutrition intervention in Matlab (MINIMat) evaluating the effects of food and micronutrient supplementation in pregnancy.[23] Infants born between May 2002 and December 2003 ($n = 2853$) were included in the child development component of the study. Children were tested at 7 months ($n = 2116$) and at 1.5 ($n = 2112$) and 5 ($n = 2260$) years. Only infants of mothers with low body mass index (BMI) benefited from supplementation during pregnancy at 7 months,[23] whereas there was no effect of supplementation at 1.5 years (Hamadani, unpublished data).

Exposure assessment

Pre-natal arsenic exposure was measured by arsenic concentrations in mothers' urine collected in early (gestational week 8) and late (gestational week 30) pregnancy. At 1.5 and 5 years, children's urine was collected during home interviews to measure childhood arsenic exposure.[27,28] Urine samples were transported frozen to Karolinska Institute for arsenic analysis. For mothers' urine, we assessed the sum of metabolites of inorganic arsenic using hydride-generation atomic-absorption spectroscopy.[26] For children's urine, the concentrations of the different arsenic metabolites (MMA, DMA and remaining un-methylated inorganic arsenic) were measured using high-pressure liquid chromatography on line with hydride generation and inductively coupled mass spectrometry (HPLC–ICPMS).[26–28] The measurements showed excellent agreement between the two methods ($r = 0.98$; $n = 319$) and accuracy was demonstrated using reference materials. In both cases, the concentration of urinary arsenic (U-As) is defined as

the sum of iAs, MMA and DMA measured in the urine. We adjusted for variation in urine dilution by specific gravity (average 1.012 g/ml for urine of mothers and 5-year olds, and 1.009 g/ml for the 1.5-year olds). Adjustment for specific gravity is known to be less dependent on age, nutritional status and arsenic exposure than is adjustment for creatinine excretion.[29]

Socio-economic status

A detailed socio-economic status (SES) history was collected. It included questions on parental education, number of family members, assets owned by the family, imbalance between income and expenditure in the previous month and the quality of the roof, floor and walls of the house.[23] An asset score was developed based on the factor analyses of all the assets owned in the household. A housing index was produced according to the materials used for the roof, floor and walls of the house.

Anthropometry

At enrolment during early pregnancy, mothers' body weight and height were measured and the BMI (kg/m^2) was calculated. Birthweight, length and head circumference were measured using standard methods.[23] Children's weight, length and head circumference at 1.5[11] and 5 years were measured according to standard procedures.[30] Heights and weights were converted to standard scores using the World Health Organization (WHO) growth references.[31]

Cognitive function

Children were tested using the third edition of Wechsler Pre-school and Primary Scale of Intelligence (WPPSI)[32] at the nearest health clinic. WPPSI was not previously used in this population by the present research group and was therefore culturally adapted and modified for use in Bangladeshi children. Initially, five testers were trained to test the children, and test–retest and inter-observer reliability with the trainer were assessed. Unfortunately, for unavoidable reasons, three testers left at different times in the study and new testers were trained. Testers were regularly supervised. Supervisors rated 10% of all tests, and adequate inter-observer reliabilities and test–retest reliability were achieved.

Seven subtests of the WPPSI were used. 'Block Design' (20 items), 'Matrix Reasoning' (29 items) and 'Picture Completion' (32 items) subtests were summed to calculate the performance intelligence quotient (PIQ). 'Information' (34 questions), 'Vocabulary' (25 questions) and 'Comprehension' (20 questions) subtests provided the verbal IQ (VIQ). 'Coding' measured the speed of processing of the children. The three scaled scores were summed to calculate the full scale IQ (FSIQ).

Home stimulation

Quality of home stimulation was assessed using a modified version of home observation for measurement of environment (HOME) for early childhood.[33] Four research assistants were trained to interview mothers at home. Ten per cent of the interviews were supervised and good inter-observer agreement was achieved.

Maternal IQ

When mothers brought their children to the health clinic, they were asked to take Raven's coloured and progressive matrices test in order to measure their IQ.[34] The sum of the correct answers was used as the mother's IQ.

Statistical methods

Data were analysed using SPSS for Windows version 17 (SPSS Inc., Chicago). Age of the child at the time of testing was negatively correlated to FSIQ ($r = -0.07$, $P = 0.002$) and PIQ ($r = -0.13$, $P < 0.001$) and we therefore controlled for age in any analyses relating to FSIQ and PIQ. Plots of the IQ scores against U-As indicated a non-linear relationship. Therefore, U-As in mothers (gestational weeks 8 and 30) and children (1.5 and 5 years of age) were first categorized into quartiles for evaluation of differences in developmental scores using one-way analysis of variance (ANOVA). The distributions of the mothers' and children's U-As were strongly right-skewed. For linear regression analyses, U-As values were log-transformed to meet the requirements of normal distribution of residuals. Correlations of log-transformed U-As with developmental scores and potential confounders, i.e. socio-demographic variables, were calculated for boys and girls separately. Variables correlating with both exposure (at any time point) and outcomes were considered as possible confounders and were used in multivariable-adjusted regression analyses. Mothers' education was highly correlated with mothers' IQ, fathers' education and assets, and was therefore not used in the regression models. We evaluated separate multivariable-adjusted regression models examining the effects of mothers' and children's U-As, controlling for potential confounders. Although the testers were continuously supervised and showed good agreement in terms of the supervisor evaluation, ratings between testers still differed. Therefore, to control for testers' differences, we used dummy variables for testers in the multivariable-adjusted regression analyses.

In the first step of the analysis, we entered U-As (mothers' or children's), sex and age (except for VIQ) in the model. In the second step, we additionally entered HOME, and in the third step we entered all of the potential confounders. HOME was entered separately in the second step as it was highly related to the developmental outcomes and most of the SES variables. For models evaluating the effect of children's

U-As, we included fathers' education, mothers' BMI and IQ, assets, housing, number of children in the household, gestational age at birth, birth length, height-for-age *z*-score (HAZ) and dummy variables representing testers. For models using mothers' U-As, we controlled for fathers' education, mothers' BMI and IQ, assets, housing, number of children in the household, gestational age at birth, birth length, head circumference at 5 years and testers. The sex of the children predicted IQ and we therefore calculated interaction terms for sex and U-As measures, and tested these in models fully adjusted as described above. Next, we omitted gestational age and anthropometric measures from the regression analyses to check if the effect of arsenic was mediated through those measures. Additionally, we assessed the probability of being tested in the study using a logistic regression of the covariates that had missing values (SES, housing, assets, income–expenditure deficits, maternal BMI at enrolment, maternal age, parity, parents' education, gestational age, birthweight, length and head circumference) and used the inverse of the predicted probability as weights in the multiple regression analyses to determine if the loss to follow-up affected the estimates for the effect of arsenic. Finally, we evaluated potential effects of (i) poverty and (ii) poor arsenic metabolism by stratifying for (i) SES quintiles and (ii) low (below median) and high (above median) percentage of MMA in children's urine at 5 years. We checked if the food and micronutrient supplementation during pregnancy had any effect on children's IQ, but none of the supplementation groups differed from others at 5 years of age (data not shown).

We calculated effect sizes by evaluating U-As in a fully adjusted model using non-transformed U-As concentrations $<100\,\mu g/l$, where residuals were normally distributed, the relationship between U-As and IQ was approximately linear and the association was not unduly influenced by a few individuals with very high U-As concentrations. We compared the estimated effect with the standard deviation of the sample.

Ethics

Informed written consent was obtained from mothers at enrolment and again before the testing of 5-year olds. The institutional review boards of the Karolinska Institute and icddr,b approved the project. In a parallel project, information about the water arsenic concentrations was provided to the well owners, and the pumps were painted red if water exceeded the national drinking water standard of $50\,\mu g/l$.[25] Pregnant women and people showing symptoms of arsenic intoxication were given priority for mitigation efforts.

Results

Singleton children born between May 2002 and December 2003 ($n=2853$) comprised the sample for the child development component. At 5 years of age, we tested 2260 (79%) of those children, whereas 593 (21%) children were lost due to (i) refusal to come for the test ($n=44$, 7% of all lost to follow-up), (ii) migration to other areas ($n=63$, 11%), (iii) death ($n=96$, 16%), (iv) unavailability on several visits ($n=358$, 60%), (v) disability ($n=4$, 1%) and (vi) illness at the time of testing ($n=28$, 5%). The children who were not tested came from higher SES families and had higher parental education ($P<0.001$), mother's BMI ($P=0.006$) and asset score ($P=0.007$), as well as better housing ($P=0.001$) (Table 1).

The mean age of the mothers was 26 years at enrolment and one-third were undernourished with BMI $<18.5\,kg/m^2$ (Table 1). The children were on average 63 months old [Standard Deviation (SD) 1.9 months] with an average body weight of 14.9 kg (SD 1.9 kg) and average body height of 103 cm (SD 5 cm) at the time of the 5-year developmental testing. Many children in this population were underweight [41% had weight-for-age *z*-scores (WAZ) less than −2] and stunted (33% had HAZ less than −2) at 5 years of age.

Among the children who were tested for their IQ or whose mothers were interviewed at home for the quality of home stimulation at 5 years, we had U-As measures for 2010 children at 5 years and 2101 children at 1.5 years. In addition, we had U-As data for 1945 of the mothers in early pregnancy and 2019 mothers in late pregnancy. The median U-As was similar in early ($81\,\mu g/l$, $n=1945$) and late ($84\,\mu g/l$, $n=2019$) gestation (Table 1). Children's median U-As at 1.5 years was $34\,\mu g/l$ and at 5 years was $51\,\mu g/l$ (Table 1). The distribution of U-As measured at all time points was right skewed. For example, the arithmetic mean concentration at 5 years was $100\,\mu g/l$.

Mean FSIQ, PIQ and VIQ by quartiles of mothers' and children's U-As are presented in Table 2. There were group differences and linear trend across the arsenic quartiles for all the developmental variables. U-As was negatively related to most socio-economic and anthropometric variables (Table 3). FSIQ, PIQ and VIQ were related to all socio-economic and anthropometric variables (data not shown).

Table 4 shows the associations between VIQ and U-As at all four time points of exposure assessment using multivariable-adjusted regression analysis. Controlling for HOME in the models caused substantial changes in the estimates of the effect of arsenic, but the associations remained after this adjustment and also after controlling for additional potential confounders.

For VIQ, there were effects of sex at all four time points ($P<0.05$ for all) and there were interactions of sex with U-As during late gestation [$B=-1.5$, 95% confidence interval (CI) $=-3.0$ to 0.004, $P=0.051$]

Table 1 Characteristics of the study cohort compared with those children not tested

Variable	Tested	Not tested	*P*-value
Family characteristics	$n = 1945–2200$	$n = 584–592$ (U-As: $n = 320$)	
Poor housing (%)	21	18	0.001
Income–expenditure deficit (%)	20	18	0.423
Assets[a]	0.3 (−1.8, 1.8)	0.7 (−1.5, 2.1)	0.008
Fathers' education (years)[a]	5.0 (0, 12)	6.5 (0, 12)	<0.001
Mothers' education (years)[a]	5.0 (0, 10)	6.0 (0, 10)	<0.001
Mothers' BMI (kg/m^2)[b]	20.1 (2.6)	20.4 (2.8)	0.006
Arsenic in mothers' urine in gestational week 8 (μg/l)[b]	81 (24, 380)	67 (19, 396)	0.998
Arsenic in mothers' urine in gestational week 30 (μg/l) [b]	84 (26, 415)	67 (23, 408)	0.173
Children's characteristics	$n = 2101–2260$	$n = 269–585$	
Males (%)	52	52	0.507
Birthweight (g)[a]	2688.4 ± 393.7	2668.9 (469.5)	0.342
Birth length (cm)[a]	47.8 (2.2)	47.7 (2.7)	0.177
Birth head circumference (cm)[a]	32.5 (1.7)	32.4 (2.0)	0.248
Gestational age (weeks)[a]	39.2 (1.7)	39.0 (2.3)	0.050
Concurrent anthropometry			
HAZ	−1.6 (0.9)	Not measured	
WAZ	−1.8 (0.9)	Not measured	
U-As at 1.5 years (μg/l)[b]	34 (12, 155)	35 (12, 225)	0.050
U-As at 5 years (μg/l)[b]	51 (20, 238)	49 (15, 284) ($n = 77$)	0.610

[a]Mean (SD).
[b]Median (10th and 90th percentiles).

and at 5 years of age ($B = -2.9$, 95% CI = −4.6 to −1.1, $P = 0.001$) (Table 4). When boys and girls were evaluated separately, the effects were only present in girls, and somewhat stronger with concurrent arsenic exposure ($B = -2.4$, 95% CI = −3.8 to −1.1, $P < 0.001$), compared with that at 1.5 years, and early and late gestation (Table 4).

Similarly for FSIQ, there were interactions of sex with U-As in late gestation ($B = -1.4$, 95% CI = −2.8 to 0.07, $P = 0.063$) and at 5 years of age ($B = -2.0$, 95% CI = −3.7 to −0.3, $P = 0.021$), and FSIQ showed slightly weaker associations with arsenic exposure in the girls (Table 5). For FSIQ, there was again a tendency in girls for stronger association with concurrent arsenic exposure ($B = -1.40$, 95% CI = −2.7 to −0.1, $P = 0.029$) compared with exposure at 1.5 years and early and late gestation (Table 5).

In boys, all exposures showed consistently low and non-significant associations with all IQ measures (Tables 4 and 5).

In all children, PIQ was negatively associated only with maternal U-As in late gestation (fully adjusted model: $B = -1.31$, $P = 0.028$), not with exposure earlier or later ($P = 0.32–0.80$; data not shown).

We also excluded gestational age at birth, birth anthropometry measures and concurrent anthropometry as covariates in the models, as arsenic has been found to affect size at birth[12] and could potentially mediate the effects on child cognition. However, exclusion of these covariates did not change the estimates of the effect of arsenic. Moreover, weighting the regressions by the inverse probability of being tested did not change the result.

Stratified analysis by SES (quintiles) or percentage of MMA in child urine at 5 years did not indicate any influence of SES or arsenic methylation efficiency on the associations between arsenic exposure and IQ measures, in either girls or boys.

It was not possible to measure the cumulative exposure to arsenic (μg/l × years) as we did not have information on time points for possible changes of water sources or on length and completeness of breastfeeding, which would markedly affect the cumulative exposure. In order to test for the effect of high exposures throughout life, we selected children with above median pre-natal U-As. Among these children, we compared children with above median U-As in childhood to children with U-As below median in

Table 2 Means (SD) of developmental outcomes by quartiles of mothers' and children's U-As concentrations

U-As (μg/l)	*n*	FSIQ[a]	PIQ[a]	VIQ
Mothers' U-As at GW 8				
0–36	479	76.6 (99)	77.6 (9.5)	81.2 (10.3)
37–79	481	75.9 (8.8)	76.1 (9.5)	79.5 (8.5)
80–206	490	74.2 (8.5)	75.2 (8.9)	79.1 (8.8)
>206	495	74.2 (9.4)	76.0 (10.3)	78.6 (8.7)
Total	1945	74.9 (9.2)	76.2 (9.6)	79.6 (9.1)
Group difference, *P*-value		<0.001	0.001	<0.001
Linear trend, *P*-value		<0.001	0.003	<0.001
Mothers' U-As at GW 30				
0–40	482	76.7 (10.4)	77.3 (10.2)	81.5 (10.2)
41–82	512	75.6 (9.0)	76.6 (9.0)	80.2 (9.4)
83–228	513	74.4 (8.6)	76.0 (9.4)	79.0 (8.5)
>228	512	73.9 (9.0)	75.2 (10.0)	78.8 (8.5)
Total	2019	75.1 (9.3)	76.3 (9.7)	79.8 (9.2)
Group difference, *P*-value		<0.001	0.003	<0.001
Linear trend, *P*-value		<0.001	<0.001	<0.001
Children's U-As at 1.5 years				
0–17	522	77.1 (9.7)	77.6 (9.4)	78.0 (9.8)
18–35	542	74.9 (9.1)	75.9 (9.7)	76.2 (9.4)
36–80	522	74.1 (9.0)	75.8 (9.0)	75.5 (9.4)
>80	515	74.3 (8.6)	75.6 (10.2)	75.7 (9.6)
Total	2101	75.1 (9.2)	76.2 (9.6)	76.3 (9.6)
Group difference, *P*-value		<0.001	0.005	<0.001
Linear trend, *P*-value		<0.001	0.003	<0.001
Children's U-As at 5 years				
0–29	505	76.6 (10.2)	77.2 (9.8)	81.6 (10.6)
30–50	502	75.6 (9.8)	76.6 (10.0)	80.1 (9.3)
51–120	502	74.1 (8.3)	75.6 (9.0)	78.8 (8.3)
>120	501	74.3 (9.1)	76.0 (10.1)	78.8 (8.6)
Total	2010	75.1 (9.4)	76.3 (9.8)	79.8 (9.3)
Group difference, *P*-value		<0.001	0.021	<0.001
Linear trend, *P*-value		<0.001	0.008	<0.001

[a]Controlling for age.
GW: gestational week.

childhood. However, the differences in VIQ, PIQ or FSIQ at 5 years between these groups were negligible. Alternatively, we selected children with above median post-natal exposure and within that group we compared those with above and below median pre-natal exposure. There were no significant difference on any of the measures; however, the children with above median exposure in the early pre-natal measure tended to have lower VIQ compared with those with below median exposure on unadjusted (mean difference = 1.6 and 95% CI = −0.08 to 3.2; $P = 0.06$) and adjusted ($B = -1.1$, 95% CI = −2.6 to 0.4; $P = 0.15$) analysis.

Effect size calculations using non-transformed U-As values <100 μg/l indicated that an arsenic concentration in urine of 100 μg/l U-As was associated with a decrease in VIQ and FSIQ of 1–3 points, which equals 0.1–0.3 SD (Tables 4 and 5).

Discussion

This is the first longitudinal study on the effects of early-life exposure to inorganic arsenic on child IQ at pre-school age. We found that arsenic exposure, measured by individual biomarker at four different points in time, was negatively associated with children's verbal and FSIQ, even after carefully controlling for the most relevant potential confounders, most notably the quality of home stimulation. An important new finding is the differential effect of arsenic on boys and girls, with the negative effects of arsenic on IQ observed predominantly in girls. In boys, there was no evidence of adverse effects of arsenic exposure on IQ. In the girls, concurrent exposure at 5 years seemed to be somewhat more influential than pre-natal and early childhood exposure, in spite of the fact that U-As concentrations were lower in 5-year-old children compared with their mothers' during gestation. Although the effect size of arsenic in the present study was moderate and may be compensated for by other factors of importance for development in the individual child, such a negative effect of arsenic may be important on a population basis, considering the prevalence of arsenic in drinking water in Bangladesh and elsewhere in the world. We believe that the present results are also relevant for other populations, as the effects of arsenic on children's IQ were independent of SES.

The main strengths of this cohort study include the large sample size (more than 1700 children) and the longitudinal design with repeated measures of individual arsenic exposure (twice pre-natally and twice during childhood) and repeated measures of child development, both at 0.6,[10] 1.5[11] and 5 years of age (this study). We also carefully controlled for potential confounders including home stimulation and other socio-economic variables. The developmental tests were culturally adapted and showed good test–retest reliability. In the present study, the scores correlated with parental education, maternal IQ, SES and nutritional status in theoretically expected ways. The testers were rotated around the study area throughout the study period and we statistically controlled for testers in the analyses. Hence, they are unlikely to affect the results. The home environment had a particularly strong impact on the IQ of the children, and adjusting for the home environment decreased the association between

Table 3 Univariate associations (Pearson's correlation) of maternal and child characteristics with mean maternal and child arsenic exposure

Variable	Mother's U-As early gestation (log)	Mother's U-As late gestation (log)	Child's U-As 1.5 years (log)	Child's U-As 5 years (log)
Family characteristics				
Father's education	−0.11**	−0.09**	−0.08**	−0.11**
Mother's education	−0.11**	−0.11**	−0.08**	−0.12**
Mother's age	−0.03	−0.03	−0.02	−0.008
Mother's BMI	−0.08**	−0.08**	−0.05*	−0.07*
Mother's IQ	−0.06*	−0.06*	−0.03	−0.09**
Parity	0.02	0.01	0.02	0.06*
Assets	−0.14**	−0.08**	−0.07**	−0.09**
Housing	−0.19**	−0.16**	−0.12**	−0.12**
Wealth index	−0.13**	−0.08**	−0.08**	−0.10**
Income–expenditure deficit	−0.03	0.001	0.03	−0.04
Number of children <6 years of age in the family	0.10**	0.13**	0.13**	0.09**
Child characteristics at birth				
Gestational age	−0.03	−0.05*	−0.05*	−0.04
Birth order	0.009	0.01	0.003	0.04
Weight	−0.01	−0.04	−0.04	−0.04
Length	−0.03	−0.06*	−0.06*	−0.06*
Head circumference	−0.05*	−0.06*	−0.11**	−0.04
Child characteristics at 5 years of age				
WAZ 64 months	−0.04	−0.02	−0.03	−0.07*
HAZ 64 months	−0.04	−0.05*	−0.03	−0.05*
BMIZ 64 months	−0.02	0.02	−0.002	−0.05*
Head circumference 64 months	−0.05*	−0.04	−0.03	−0.01
HOME at 64 months	−0.09**	−0.11**	−0.11**	−0.14**
FSIQ[a]	−0.10**	−0.11**	−0.11**	−0.10**
PIQ[a]	−0.07*	−0.08**	−0.08**	−0.05*
VIQ	−0.11**	−0.10**	−0.11**	−0.12**

[a]Controlling for age at tests.
*$P < 0.05$, **$P < 0.01$.
MDI: mental developmental index; BMIZ: body mass index *z*-score.

arsenic and the children's test scores substantially. Given the marked attenuation of association after adjustments, residual confounding cannot be totally excluded.

We based the exposure assessment on the concentration of arsenic metabolites in urine, as it reflects the actual individual exposure to inorganic arsenic from both water and food. Thus it provides a more reliable exposure estimate than water arsenic concentrations, which have been used in most of the previous large-scale epidemiological studies on arsenic-related health effects. Recent studies demonstrated elevated arsenic concentrations in foods of plant origin, mainly rice,[35] seen also in the present study population.[26,28] Moreover, we identified fairly low exposure at 1.5 years due to partial breastfeeding.[27] Measuring arsenic metabolites in urine also provides the possibility to evaluate the influence of arsenic metabolism, which is largely genetically determined.[36] However, children with poor methylation of arsenic did not differ in performance compared with children with efficient arsenic methylation.

Although there was considerable loss to follow-up, we found only small differences in arsenic exposure, anthropometry and SES between lost and tested children. The lost children came from higher SES

Table 4 Regression coefficients and 95% CIs for the effect of maternal and child U-As (log μg/l) on VIQ scores at 5 years of age adjusting for HOME and other potential confounders

VIQ	GW 8		GW 30		1.5 years		5 years	
	B (95% CI)	*P* (*n*)	B (95% CI)	*P* (*n*)	B (95% CI)	*P* (*n*)	B (95% CI)	*P* (*n*)
Model 1[a]								
All the children controlling for sex								
Step 1	−1.8 (−2.7 to −0.9)	<0.001	−1.9 (−2.8 to −1.0)	<0.001	−2.0 (−3.0 to −1.1)	<0.001	−2.3 (−3.3 to −1.2)	<0.001
Step 2	−1.2 (−2.0 to −0.4)	0.004	−1.1 (−1.9 to −0.3)	0.007	−1.2 (−2.0 to −0.3)	0.009	−1.0 (−2.0 to −0.1)	0.030
Step 3	−0.9 (−1.7 to −0.13)	0.022	−0.7 (−1.4 to 0.1)	0.090	−0.9 (−1.7 to −0.1)	0.026	−0.9 (−1.8 to 0.03)	0.058
Interaction of sex with U-As	−0.6 (−2.1 to 0.9)	0.443 (1731)	−1.5 (−3.0 to 0.004)	0.051 (1805)	0.06 (−1.6 to 1.7)	0.946 (1884)	−2.9 (−4.6 to −1.1)	0.001 (1726)
R^2	0.28		0.30		0.29		0.30	
Model 2[b]								
Girls								
Step 1	−2.0 (−3.3 to −0.7)	0.004	−2.6 (−3.8 to −1.3)	<0.001	−2.2 (−3.6 to −0.8)	0.002	−3.4 (−4.9 to −1.8)	<0.001
Step 2	−1.5 (−2.7 to −0.3)	0.017	−1.7 (−2.9 to −0.5)	0.004	−1.2 (−2.5 to 0.03)	0.055	−2.4 (−3.8 to −1.0)	0.001
Step 3	−1.2 (−2.4 to −0.06)	0.039 (833)	−1.5 (−2.6 to −0.4)	0.007 (874)	−0.9 (−2.1 to 0.4)	0.164 (902)	−2.4 (−3.8 to −1.1)	<0.001 (817)
R^2	0.276		0.303		0.290		0.302	
Boys								
Step 1	−1.6 (−2.9 to −0.4)	0.008	−1.3 (−2.5 to −0.07)	0.038	−1.9 (−3.2 to −0.6)	0.004	−1.2 (−2.6 to 0.2)	0.083
Step 2	−1.0 (−2.1 to 0.1)	0.088	−0.5 (−1.6 to 0.6)	0.340	−1.1 (−2.2 to 0.1)	0.074	0.2 (−1.1 to 1.4)	0.770
Step 3	−0.6 (−1.7 to 0.5)	0.258 (898)	0.06 (−1.0 to 1.1)	0.907 (931)	−1.0 (−2.1 to 0.16)	0.092 (982)	0.5 (−0.7 to 1.7)	0.394 (909)
R^2	0.283		0.282		0.288		0.288	
Points per 100 μg/l in girls	−2.0		−1.1		−1.8		−2.6	

[a]Model 1: Step 1: sex, U-As and interaction of sex with U-As; Step 2: HOME; Step 3: father's education, mother's BMI and IQ, assets, housing, number of children in the household, gestational age, birth length, concurrent HAZ and dummy variables representing testers. For models using mother's U-As, concurrent head circumference was used instead of HAZ.

[b]Model 2: as above, except sex and interaction of sex with U-As were not included.

GW: gestational week.

Table 5 Regression coefficients and 95% CIs for the effect of maternal and child U-As (log μg/l) on FSIQ scores at 5 years of age, adjusting for HOME and other potential confounders

FSIQ	GW 8		GW 30		1.5 years		5 years	
	B (95% CI)	*P* (*n*)	*B* (95% CI)	*P* (*n*)	*B* (95% CI)	*P* (*n*)	*B* (95% CI)	*P* (*n*)
Model 1[a]								
All the children controlling for sex								
Step 1	−1.6 (−2.5 to −0.7)	<0.001	−2.0 (−2.9 to −1.1)	<0.001	−1.9 (−2.8 to −0.9)	<0.001	−2.0 (−3.0 to −0.9)	<0.001
Step 2	−0.9 (−1.7 to −0.1)	0.024	−1.1 (−1.8 to −0.3)	0.006	−0.8 (−1.7 to −0.009)	0.048	−0.6 (−1.5 to 0.3)	0.215
Step 3	−0.5 (−1.3 to −0.2)	0.167	−0.6 (−1.3 to 0.1)	0.114	−0.6 (−1.4 to −0.15)	0.113	−0.4 (−1.2 to 0.5)	0.415
Interaction of sex with U-As	−0.6 (−2.1 to 0.8)	0.394 (1731)	−1.4 (−2.8 to 0.07)	0.063 (1805)	−0.2 (−1.7 to 1.3)	0.792 (1884)	−2.0 (−3.7 to −0.3)	0.021 (1726)
R^2	0.35		0.36		0.36		0.36	
Model 2[b]								
Girls								
Step 1	−1.9 (−3.2 to −0.6)	0.005	−2.6 (−3.9 to −1.3)	<0.001	−2.2 (−3.6 to −0.9)	0.002	−2.6 (−4.2 to −1.1)	0.001
Step 2	−1.3 (−2.5 to −0.2)	0.027	−1.6 (−2.7 to −0.5)	0.004	−1.1 (−2.3 to 0.1)	0.072	−1.5 (−2.9 to −0.2)	0.029
Step 3	−0.9 (−2.0 to −0.2)	0.104 (833)	−1.3 (−2.4 to −0.3)	0.012 (874)	−0.7 (−1.9 to 0.4)	0.203 (902)	−1.4 (−2.7 to −0.1)	0.029 (817)
R^2	0.35		0.37		0.36		0.37	
Boys								
Step 1	−1.4 (−2.6 to −0.1)	0.033	−1.4 (−2.6 to −0.2)	0.026	−1.5 (−2.8 to −0.2)	0.022	−1.4 (−2.8 to 0.05)	0.059
Step 2	−0.6 (−1.6 to 0.5)	0.311	−0.5 (−1.6 to 0.6)	0.330	−0.6 (−1.7 to 0.6)	0.315	0.3 (−1.0 to 1.5)	0.689
Step 3	−0.2 (−1.2 to 0.9)	0.739 (898)	0.1 (−0.9 to 1.1)	0.845 (931)	−0.5 (−1.6 to 0.6)	0.364 (982)	0.7 (−0.5 to 1.8)	0.271 (909)
R^2	0.34		0.35		0.35		0.35	
Points per 100 μg/l in girls	−3.0		−1.0		−0.9		−0.9	

[a]Model 1: Step 1: age, sex, U-As and interaction of sex with U-As; Step 2: HOME; Step 3: father's education, mother's BMI and IQ, assets, housing, number of children in the household, gestational age, birth length, concurrent HAZ and dummy variables representing testers. For models using mother's U-As, concurrent head circumference was used instead of HAZ.

[b]Model 2: as above, except sex and interaction of sex with U-As were not included.

GW: gestational week.

families, and the effect of SES was controlled in the analyses. In addition, when we adjusted for the characteristics of the lost children through inverse probability weighting, the results were changed minimally. In a subsample of the cohort of mothers, we previously found that water concentrations of manganese and arsenic were inversely related.[37] Thus, it is possible that manganese exposure might have reduced the association between arsenic and child development, and we have initiated studies to examine that question in a larger group of mothers and children. Manganese exposure has been shown to influence children's behaviour in recent cross-sectional studies in Bangladesh.[38]

There are several possible mechanisms by which arsenic may affect the developing brain. A direct effect on brain cells is possible as arsenic is a pro-oxidant and may cause oxidative stress in the sensitive developing brain.[39–41] We found associations between arsenic exposure and oxidative stress markers both in the mothers' urine in early pregnancy[19] and in their placentas at delivery.[13] Another possible mode of action of arsenic is hormonal interactions, especially with oestrogen and thyroid hormones,[42] both of which are essential for brain development.[43] A hormonal interaction may be supported by the observed sex differences in our study. Interestingly, recent experimental studies showed that female rats were more susceptible to arsenic-induced neurotoxicity than male rats, particularly to effects on the dopaminergic system.[44] In addition, girls were recently found to be more susceptible to manganese-induced impairment of child development than boys.[45] Unfortunately, none of the previously reported epidemiological studies on arsenic and child development (all cross-sectional) evaluated the associations separately in boys and girls.

Our finding that children's concurrent arsenic exposure is more strongly associated with IQ scores, compared with exposure earlier in life, is supported by other studies. In particular, von Ehrenstein *et al*.[9] reported that concurrent exposure (measured by arsenic in urine) among 351 Indian children 5–15 years of age, but not the estimated long term or pre-natal exposure (based on water arsenic concentrations), was associated with small reductions in the adjusted scores of the vocabulary test, the object assembly test and the picture completion test. A weaker effect of pre-natal and early post-natal arsenic exposure might be related to the recently observed up-regulation of arsenic methylation efficiency very early in pregnancy,[46] which is likely to decrease toxicity, in combination with the poor transfer of arsenic to breast milk.[47] However, it is possible that the duration of exposure is important. We were not able to assess the exact cumulative exposure (μg/l × years), as we lacked detailed data on water source and breastfeeding practices. However, comparing children with differing urinary arsenic measures over time did not show any significant difference on VIQ, PIQ or FSIQ. Still, our results might suggest that arsenic exerts an effect on the brain in early gestation, and that this is sustained or somewhat aggravated by the continuing childhood exposure. An effect in early gestation is in line with the observed lower head circumference at birth for children with higher early pre-natal arsenic exposure,[12] as head circumference is associated with lower child IQ.[48] It is essential to follow the children to elucidate whether their IQ scores deteriorate with persistent exposure.

Conclusions

We demonstrate associations of early-life arsenic exposure and decreased VIQ and FSIQ in pre-school-aged children. The noteworthy finding of effects, particularly in girls, requires confirmation in future studies. We intend to continue following these children to determine whether effects become more apparent in later childhood and adolescence and whether the sex differences persist. At the same time, it is essential to improve the mitigation options particularly for water sources with high arsenic concentrations used by children.

Funding

EU project Public Health Impact of long term, low level Mixed element Exposure in susceptible population strata (PHIME), sponsored by the EC [FOOD-CT-2006-016253; the European Community is not liable for any use that may be made of the information contained therein], the Swedish Research Council, the Swedish International Development Cooperation Agency (Sida), Sida's Department for Research Cooperation (SAREC) and Karolinska Institutet. The MINIMat study was funded by United Nations Children's Fund (UNICEF), Sida, UK Medical Research Council, Swedish Research Council, Department for International Development (DFID), International Center for Diarrhoeal Disease Research, Bangladesh (icddr,b), Global Health Research Fund-Japan, Child Health and Nutrition Research Initiative (CHNRI), Uppsala University and United States Agency for International Development (USAID).

Acknowledgements

The authors gratefully acknowledge the participation of all pregnant women and families in Matlab. They also thank the members of the MINIMat team, supervisors (Fardina Mehrin, Afroza Hilaly, Shamima Shiraji, Fatema Khatun), testers (Zannatul Ferdousi, Nasrin Sultana, Sharmin Shiraji, Rafiqa

Banu, Parveen Sultana, Shirin Akhter, Shamima Yeasmin) and interviewers (Shiuli Rani Das, Roxana Khanum, Asma Khatun, Mina Begum, Fatema Begum); as well as the technical personnel analysing the urine samples (Margaretha Grandér, Brita Palm).

Conflict of interest: None declared.

KEY MESSAGES

- This is the first longitudinal study reporting association between pre-natal and childhood arsenic exposure and development of 5-year-old children.
- All four measures of early-life arsenic exposure were associated with lower VIQ and FSIQ in the girls, but not in boys, with more pronounced association of concurrent exposure compared with exposure earlier in life.
- The associations were not influenced by poor nutrition or poor arsenic metabolism.
- Given the persistent arsenic exposure beginning *in utero* in affected populations, and the documented impairment of child survival, health and development, it is an urgent task to improve access to safe drinking water.

References

[1] IARC. *Some Drinking-Water Disinfectants and Contaminants, Including Arsenic*. Lyon: International Agency for Research on Cancer, 2004.

[2] Smith AH, Steinmaus CM. Health effects of arsenic and chromium in drinking water: recent human findings. *Ann Rev Pub Health* 2009;**30:**107–22.

[3] Straif K, Benbrahim-Tallaa L, Baan R *et al*. A review of human carcinogens–part C: metals, arsenic, dusts, and fibres. *Lancet Oncol* 2009;**10:**453–54.

[4] Calderon J, Navarro ME, Jimenez-Capdeville ME *et al*. Exposure to arsenic and lead and neuropsychological development in Mexican children. *Environ Res* 2001;**85:** 69–76.

[5] Tsai SY, Chou HY. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. *Neurotoxicology* 2003;**24:**747–53.

[6] Wasserman GA, Liu X, Parvez F *et al*. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. *Environ Health Perspect* 2004;**112:**1329–33.

[7] Wang SX, Wang ZH, Cheng XT *et al*. Arsenic and fluoride exposure in drinking water: children's IQ and growth in Shanyin county, Shanxi province, China. *Environ Health Perspect* 2007;**115:**643–47.

[8] Wasserman GA, Liu X, Parvez F *et al*. Water arsenic exposure and intellectual function in 6-year-old children in Araihazar, Bangladesh. *Environ Health Perspect* 2007;**115:** 285–89.

[9] von Ehrenstein OS, Poddar S, Yuan Y *et al*. Children's intellectual function in relation to arsenic exposure. *Epidemiology* 2007;**18:**44–51.

[10] Tofail F, Vahter M, Hamadani JD *et al*. Effect of arsenic exposure during pregnancy on infant development at 7 months in rural Matlab, Bangladesh. *Environ Health Perspect* 2009;**117:**288–93.

[11] Hamadani JD, Grantham-McGregor SM *et al*. Pre-and postnatal arsenic exposure and child development at 18 months of age: a cohort study in rural Bangladesh. *Int J Epidemiol* 2010;**39:**1206–16.

[12] Rahman A, Vahter M, Smith AH *et al*. Arsenic exposure during pregnancy and size at birth: a prospective cohort study in Bangladesh. *Am J Epidemiol* 2009;**169:**304–12.

[13] Ahmed S, Khoda SM, Rekha RS *et al*. Arsenic-associated oxidative stress, inflammation, and immune disruption in human placenta and cord blood. *Environ Health Perspect* 2011;**119:**258–64.

[14] Rahman A, Persson LÅ, Nermell B *et al*. Arsenic exposure and risk of spontaneous abortion, stillbirth, and infant mortality. *Epidemiology* 2010;**21:**797–804.

[15] Nazmul S, Mohammad A, Mahfuzar R, Anisur R, Peter S, Pavlos K. Spatial patterns of fetal loss and infant death in an arsenic-affected area in Bangladesh. *Int J Health Geograp* 2010;**9:**53.

[16] Rahman A, Vahter M, Ekström EC, Persson LÅ. Arsenic exposure in pregnancy increases the risk of lower respiratory tract infection and diarrhea during infancy in Bangladesh. *Environ Health Perspect* 2011;**119:**719–24.

[17] Rodier PM. Developing brain as a target of toxicity. *Environ Health Perspect* 1995;**103(Suppl 6):**73–76.

[18] Saha KK, Frongillo EA, Alam DS, Arifeen SE, Persson LÅ, Rasmussen KM. Household food security is associated with growth of infants and young children in rural Bangladesh. *Pub Health Nutr* 2009;**12:**1556–62.

[19] Engström KS, Vahter M, Johansson G *et al*. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7, 8-dihydro-2′-deoxyguanosine in urine. *Free Rad Biol Med* 2010;**48:**1211–17.

[20] Vahter M. Effects of arsenic on maternal and fetal health. *Ann Rev Nutr* 2009;**29:**381–99.

[21] Lindberg AL, Rahman M, Persson LÅ, Vahter M. The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. *Toxicol Appl Pharmacol* 2008;**230:** 9–16.

[22] Vahter M, Akesson A, Liden C, Ceccatelli S, Berglund M. Gender differences in the disposition and toxicity of metals. *Environ Res* 2007;**104:**85–95.

[23] Tofail F, Persson LÅ, El Arifeen S *et al*. Effects of prenatal food and micronutrient supplementation on infant development: a randomized trial from the Maternal and Infant Nutrition Interventions, Matlab (MINIMat) study. *Am J Clin Nutr* 2008;**87:**704–11.

[24] Icddr B. Health and Demographic Surveillance System, Matlab. *Registration of Health and Demographic Events 2007* 2009;**41.**

[25] Rahman M, Vahter M, Wahed MA *et al*. Prevalence of arsenic exposure and skin lesions. A population-based survey in Matlab, Bangladesh. *J Epidemiol Comm Health* 2006;**60:**242–48.
[26] Vahter ME, Li L, Nermell B *et al*. Arsenic exposure in pregnancy: a population-based study in Matlab, Bangladesh. *J Health, Populat Nutr* 2006;**24:**236–45.
[27] Fängström B, Hamadani J, Nermell B, Grandér M, Palm B, Vahter M. Impaired arsenic metabolism in children during weaning. *Toxicol App Pharmacol* 2009;**239:**208–14.
[28] Gardner R, Hamadani J, Grander M *et al*. Persistent exposure to arsenic via drinking water in rural Bangladesh despite major mitigation efforts. *Am J Pub Health* 2011; July 21: AJPH. 2010.300025 v1.
[29] Nermell B, Lindberg AL, Rahman M *et al*. Urinary arsenic concentration adjustment factors and malnutrition. *Environ Res* 2008;**106:**212–18.
[30] WHO. *Measuring Change in Nutritional Status: Guidelines for Assessing the Nutritional Impact of Supplementary Feeding Programmes for Vulnerable Groups*. Geneva: World Health Organization, 1983.
[31] WHO. *Global Database on Child Growth and Malnutrition*. Geneva: World Health Organization, 2006.
[32] Wechsler D (ed.). *Wechsler Preschool and Primary Scale of Intelligence – III*. San Antonio: The Psychological Corp, 2002.
[33] Caldwell BM. Descriptive evaluations of child development and of developmental settings. *Pediatrics* 1967;**40:** 46–54.
[34] Raven JC, John Hugh C, Raven JE. *Manual for Raven's Progressive Matrices and Vocabulary Scales (2)*. London, UK: H.k. Lenid and Co. Ltd, 1986.
[35] Norton GJ, Islam MR, Deacon CM *et al*. Identification of low inorganic and total grain arsenic rice cultivars from Bangladesh. *Environ Sci Technol* 2009;**43:**6070–75.
[36] Engstrom K, Vahter M, Mlakar SJ *et al*. Polymorphisms in arsenic(+III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism. *Environ Health Perspect* 2011;**119:**182–88.
[37] Ljung KS, Kippler MJ, Goessler W, Grander GM, Nermell BM, Vahter ME. Maternal and early life exposure to manganese in rural Bangladesh. *Environ Sci Technol* 2009;**43:**2595–601.
[38] Wasserman GA, Liu X, Parvez F *et al*. Arsenic and manganese exposure and children's intellectual function. *Neurotoxicology* 2011;**32:**450–57.
[39] Kalia M. Brain development: anatomy, connectivity, adaptive plasticity, and toxicity. *Metabolism* 2008;**57:** S2–S5.
[40] Rodríguez VM, Limón-Pacheco JH, Carrizales L, Mendoza-Trejo MS, Giordano M. Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in the expression of dopaminergic and antioxidant systems in the albino rat. *Neurotoxicol Teratol* 2010;**32:**640–47.
[41] Xi S, Guo L, Qi R, Sun W, Jin Y, Sun G. Prenatal and early life arsenic exposure induced oxidative damage and altered activities and mRNA expressions of neurotransmitter metabolic enzymes in offspring rat brain. *J Biochem Mol Toxicol* 2010;**24:**368–78.
[42] Davey JC, Nomikos AP, Wungjiranirun M *et al*. Arsenic as an endocrine disruptor: arsenic disrupts retinoic acid receptor–and thyroid hormone receptor–mediated gene regulation and thyroid hormone–mediated amphibian tail metamorphosis. *Environment Health Perspect* 2008; **116:**165–72.
[43] Masuo Y, Ishido M. Neurotoxicity of endocrine disruptors: possible involvement in brain development and neurodegeneration. *J Toxicol Environment Health, Part B* 2011; **14:**346–69.
[44] Bardullas U, Limón-Pacheco JH, Giordano M, Carrizales L, Mendoza-Trejo MS, Rodríguez VM. Chronic low-level arsenic exposure causes gender-specific alterations in locomotor activity, dopaminergic systems, and thioredoxin expression in mice. *Toxicol App Pharmacol* 2009;**239:**169–77.
[45] Bouchard MF, Sauvé S, Barbeau B *et al*. Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ Health Perspect* 2011;**119:** 138–43.
[46] Gardner RM, Nermell B, Kippler M *et al*. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. *Reprod Toxicol* 2011b;**31:**210–18.
[47] Fängström B, Moore S, Nermell B *et al*. Breast-feeding protects against arsenic exposure in Bangladeshi infants. *Environ Health Perspect* 2008;**116:**963–69.
[48] Gale CR, O'Callaghan FJ, Bredow M, Martyn CN. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. *Pediatrics* 2006;**118:**1486–92.