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Manganese in Drinking Water and Cognitive Abilities and Behavior at 10 Years of Age: A Prospective Cohort Study

Syed Moshfiqur Rahman[1,2,#], Maria Kippler[1,#,†], Fahmida Tofail[2], Sven Bölte[3,4], Jena Derakhshani Hamadani[2], and Marie Vahter[1]

[1]Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

[2]International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh.

[3]Center of Neurodevelopmental Disorders (KIND), Pediatric Neuropsychiatry Unit, Department of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden;

[4]Center for Psychiatry Research, Stockholm County Council, Stockholm, Sweden

#Authors contributed equally

[†]Corresponding author: Maria Kippler, Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-171 77, Stockholm, Sweden. Phone +46 8 524 87407, Fax +468336981, Email: maria.kippler@ki.se.

Running title: Water manganese and IQ and behavior in children

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Abstract

Background: Cross-sectional studies have indicated impaired neurodevelopment by elevated drinking water manganese concentrations (W-Mn), but potential susceptible exposure windows are unknown.

Objective: To prospectively evaluate effects of W-Mn, from fetal life to school-age, on children's cognitive abilities and behavior.

Methods: We assessed cognitive abilities using Wechsler Intelligence Scale for Children (WISC-IV) and behavior using Strengths and Difficulties Questionnaire (SDQ) in 1,265 ten-year-old children in rural Bangladesh. Manganese in drinking water, used during pregnancy and by the children at 5 and 10 years, was measured using inductively coupled plasma mass spectrometry.

Results: Median W-Mn was 0.20 mg/L (range 0.001-6.6) during pregnancy and 0.34 mg/L (<0.001-8.7) at 10 years. In multivariable-adjusted linear regression analyses, restricted to children with low arsenic exposure, none of the W-Mn exposures were associated with the children's cognitive abilities. Stratifying by gender (*p* for interaction in general <0.081) showed that prenatal W-Mn (<3 mg/L) was positively associated with cognitive ability measures in girls, but not in boys. W-Mn at all time-points was associated with increased risk of conduct problems, especially in boys (range 24-43% per mg/L). At the same time, the prenatal W-Mn was associated with a decreased risk of emotional problems (OR: 0.39, 95% CI 0.19, 0.82) in the boys. In girls, W-Mn was mainly associated with low prosocial scores (prenatal W-Mn: OR 1.48, 95% CI 1.06, 1.88).

Conclusions: Elevated prenatal W-Mn exposure was positively associated with cognitive function in girls, while boys appeared unaffected. However, early-life W-Mn exposure appeared to adversely affect children's behavior.

Introduction

Manganese (Mn) is an essential element that functions as a cofactor in a number of enzymes and in certain antioxidants, which makes it important during early life development (Mistry and Williams 2011). The primary source of Mn is the diet, which usually provides the required 3.0 mg/day for pregnant women and 0.5-2.0 mg/day for children (EFSA 2013). However, excess exposure through drinking water is common worldwide (Frisbie et al. 2012; Ljung and Vahter 2007), and there is increasing concern that such exposure may adversely affect the central nervous system, especially in children (Frisbie et al. 2012; Zoni and Lucchini 2013). Several cross-sectional studies have indicated that elevated drinking water Mn concentrations (W-Mn) are associated with impaired cognitive abilities and/or adaptive behaviors in 6-13 years old children (Bouchard et al. 2011; Khan et al. 2011; Oulhote et al. 2014a; Wasserman et al. 2006). Even more studies indicate associations with blood Mn, often with an inverted U-shaped dose-effect relationship (Sanders et al. 2015). However, there appear to be no association between Mn in water and blood (Ljung et al. 2009; Rahman et al. 2015).

The timing of exposure may be critical for manganese neurotoxicity, as the susceptibility of the brain to toxic insult is known to vary during the different phases of the development (Grandjean and Landrigan 2014). Also the exposure may vary over time. Because Mn easily passes through the placenta (Erikson et al. 2007), an elevated maternal exposure during pregnancy, in combination with an increased gastrointestinal absorption (Takser et al. 2004), may lead to excess fetal exposure. Indeed, a few studies have indicated inverse associations between Mn concentrations in umbilical cord blood and child neurodevelopment (Takser et al. 2003; Yu et al. 2014). Similarly, Mn in tooth dentine measured using microspatial analysis, estimating prenatal and early postnatal exposure, was associated with adverse neurodevelopmental outcomes (Gunier et al. 2015; Mora et al. 2015). In another study that

measured Mn levels in pulverized whole teeth, no association of Mn and neurodevelopmental outcomes was observed (Chan et al. 2015). Early childhood may be another critical period of manganese exposure as the regulation of the intestinal absorption and the biliary excretion is not yet fully developed (Erikson et al. 2007). While breast-milk contains very little manganese (Ljung et al. 2009), introduction of food and drinking water may lead to high-level exposure. The present study aimed to prospectively evaluate potential adverse effects of elevated W-Mn, from fetal life to school-age, on the cognitive abilities and behavior in a large cohort of boys and girls at 10 years of age. Elevated Mn concentrations were found mainly in medium deep wells, many some of which were constructed to decrease the exposure to arsenic, present in many of the shallow wells (Kippler et al. 2016; Ljung et al. 2009).

Materials and methods

Study area and population

The study involves a large mother-child cohort, covering early pregnancy to 10 years of age. It was initially nested in a randomized food and micronutrient supplementation trial (MINIMat) conducted in pregnant women living in Matlab, rural Bangladesh (Persson et al. 2012) to evaluate the health effects of early life exposure to arsenic, frequently occurring in the shallow wells (Vahter et al. 2006). The installation of deeper wells, often with the aim to decrease people's exposure to arsenic, has resulted in elevated W-Mn (Kippler et al. 2016; Ljung et al. 2009), which motivated us to evaluate the potential health consequences of that. The 1,607 singleton children born within the MINIMat trial between October 2002 and December 2003 were invited for follow-up of child growth and development at 10 years of age and 95% (n=1,530) agreed to participate. Main reasons for loss to follow-up were parents' refusal and outmigration.

The study was approved by the ethical review committee at icddr,b, Bangladesh and the Regional Ethical Review Board in Stockholm, Sweden. Written consent was obtained from all mothers prior to enrollment in the initial study and before the testing at 10 years of age.

Manganese exposure

Drinking water was sampled during pregnancy and at 5 and 10 years of age. Area-wide screening of water arsenic concentrations (W-As) was performed in 2002-2003 (Rahman et al. 2006), and from this screening we identified the wells used during pregnancy as previously described (Rahman et al. 2013). Out of the 1,530 children with neurodevelopmental assessment and water samples at 10 years, we had maternal water samples for 1,265 children, and out of these children 1,162 also had their water sampled at 5 years of age. At the follow-up investigations, the families were interviewed about all the water sources used since the children were born and for how long each source had been used. Thereafter, we collected drinking water samples from available indicated wells, enabling construction of the children's life-long W-Mn exposure after measuring Mn in the water samples.

Water collection and analysis

Water samples were collected in 20 mL trace element-free polyethylene containers containing 50 μL of nitric acid (69% HNO_3, Suprapur, MERCK, Germany) to minimize precipitation of metals. The measurements of manganese in the mothers' drinking water samples (collected: 2002 to 2003) were performed during 2008 to 2009 and the measurements of the children's drinking water samples (5 years: 2007-2008 and 10 years: 2012-2013) were performed during 2010 and 2014, respectively. Element concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS; Agilent

7700x, Agilent Technologies, Tokyo, Japan), as previously described (Rahman et al. 2013). Analytical performance was validated by analysis of a certified reference material (Supplemental Table S1). No samples were below the limit of detection of manganese (See Supplemental Material, Table S1). For arsenic, 30 samples were below the LOD of 0.01 μg/L at 5 years and one sample at 10 years, and these concentrations were set to LOD/√2.

Outcome assessments

The Wechsler Intelligence Scale for Children, 4th Edition (WISC-IV) (Wechsler 2003) was used to assess children's general cognitive abilities (IQ) and more specific cognitive functions through its subtests. The WISC-IV is the most widely used intelligence battery in clinical practice. In the present study we applied WISC-IV raw scores in order to exclude bias owing to comparison with foreign culture norms. Ten subtests generate four composite scores: *i)* Verbal Comprehension (vocabulary, information, and comprehension), *ii)* Perceptual Reasoning (block design, picture concepts, and matrix reasoning), *iii)* Working Memory (digit span and arithmetic), and *iv)* Processing Speed (coding and symbol search). The full scale IQ, which represents a child's general intellectual ability, was derived from the raw scores of the 10 subtests. All test materials and questionnaires were translated to Bengali. A pilot study with 52 children from the study area was conducted to culturally adapt the tests, without changing the underlying constructs. For example, pictures unfamiliar to the rural Bangladeshi children were replaced by similar local pictures, and too difficult questions were changed; e.g." what is fossil" was changed to "what is skeleton", and "who was Columbus" was changed to "who was Rabindranath Tagore". To assess children's cognitive abilities, we recruited four female psychologists with a Master's degree in psychology and they were trained for 6 weeks on the above tests. During the training, inter-rater reliabilities were measured between each tester and the trainer until the testers achieved >90% agreement with

the trainer. To minimize testers' bias during data collection, they were also observed by a supervisor (senior female psychologist), who visited the testing from time to time and observed about 5% of all tests by scoring them independently. An adequate inter-rater reliability (intra-class r>0.85) was achieved between each of the testers and the supervisor. Behavior problems were assessed using the parent-report of Strengths and Difficulties Questionnaire (SDQ) for 4-17 years old children (Goodman 2001). The SDQ is a screening test for childhood psychopathology adapted for many different cultures and languages and has demonstrated excellent psychometric properties (http://www.sdqinfo.com/). It consists of 25 items composing five scales concerning both negative and positive behaviors of children to be addressed by the caregivers on a 0-2 point scale; conduct problems, hyperactivity/inattention, emotional symptoms, and peer relationship problems with max score of 40, and on prosocial behavior with max score of 10. Female interviewers with a Bachelor's degree, who had completed two weeks of training, visited the families' homes and conducted the SDQ interviews with the mothers or other female care-givers in absence of mothers. The testers and interviewers were blinded to Mn concentrations in the children's drinking water, as all water samples were analyzed after completion of data collection.

Covariates

Information about the mother's background characteristics [age, early pregnancy weight, height, date of delivery, education, and hemoglobin concentrations (Hb)] and children's anthropometry at birth and exclusive breast feeding at four months (yes/no) were available from the MINIMat trial (Persson et al. 2012). Maternal education was defined as the number of years at school and categorized as zero, 1-5 and ≥6 years. During the follow-up of the children, we collected information on the number of siblings, years of education (formal schooling categorized as <3, 3, and >3 years), and type of schools [public primary school,

Madrasa (Islamic) school, NGO (nonprofit private) school, and English medium (private) school], and the families socio-economic status. SES was estimated via a wealth index constructed from information on family ownership of a number of consumer items, housing structure, and dwelling characteristics (Gwatkin et al. 2000). The index were divided into quintiles, where the lowest quintile represented the poorest and the highest quintile the richest families. The children's weight and height were measured by the testers at the health clinics after the developmental assessment. The measures were converted into age- and sex-standardized Z-scores [weight for age (WAZ) and height for age (HAZ)] using the AnthroPlus software (WHO 2009). The Hb concentrations (g/L) were measured in peripheral blood (finger prick) by a HemoCue photometer (HemoCue AB, Ängelholm, Sweden).

A modification of the middle childhood version (6-10 years) of the home observation for measurement of environment (HOME) was used to assess the quality and quantity of stimulation and support to the children at home (Caldwell and Bradley 2003). The HOME consists of 58 questions addressing *i*) responsibility, *ii*) encouragement of maturity, *iii*) emotional climate, *iv*) learning materials and opportunities, *v*) enrichment, *vi*) family companionship, *vii*) family integration, and *viii*) physical environment. The questionnaire was tested in a pilot study with 20 children in the study area and seven questions were dropped because the mother couldn't understand them. The Raven's colored and Progressive Matrices test (Raven 1992) was used to measure the mothers' non-verbal IQ in terms of abstract logical reasoning (Hamadani et al. 2011). The sum of correct answers (in total 60 questions) was used as a proxy of the mothers' IQ. Results on Raven matrices have demonstrated good convergence with IQ estimates on the Wechsler scales in normative and clinical samples (Bolte et al. 2009) and were sensibly correlated with their education and SES.

Because exposure to arsenic, cadmium and lead through drinking water and food may affect children's development (Hamadani et al. 2011; Hu et al. 2006; Kippler et al. 2012), we

tested whether the urinary concentrations of arsenic metabolites (marker of exposure to inorganic arsenic), cadmium and lead at gestational week (GW) 8 and at 5 and 10 years of age influenced the associations of manganese with the children's cognitive development and behavior.

Statistical analysis

Bivariate associations between exposures, covariates and outcomes were assessed using Spearman's rank correlation coefficient (r_s), Kruskal–Wallis test, Mann-Whitney U-test, or χ^2 tests, depending on the type of data. Scatter plots with Lowess smoothing function did not indicate non-linear associations between W-Mn and the different outcomes. Associations of W-Mn with cognitive abilities (WISC-IV) were explored using linear regression analyses, whereas the associations with the problem behavior subtest scores were explored using logistic regression, using the following cut-offs: Conduct problems (normal <3 and slightly raised ≥ 3 scores), Hyperactivity problems (normal <6 and slightly raised ≥6 scores), Peer problems (normal <3 and slightly raised ≥ 3 scores), Emotional problems (normal <4 and slightly raised ≥4scores), and Prosocial behavior (normal >5 and slightly raised ≤5) (Network 2005).

The models were adjusted for children's age at assessment and gender (*a priori*), variables known to affect child development or that were significantly associated with the exposure and outcome (p<0.05), and variables that changed the estimates of W-Mn by at least 10% (maternal IQ and SES, number of siblings, child HAZ at 10 years, education in years, type of school attended, Hb concentrations, total HOME score, the testers of cognitive abilities, and the natural log transformed urinary arsenic concentrations at each exposure window. In case of strongly associated variables (r_s>0.60; i.e. WAZ and HAZ at 10 years of age), we included the variable which had the largest influence on the effect estimates. We

excluded one boy with very high W-Mn concentration (6.5 mg/L) at 5 years, which had a disproportionately large influence on the associations. Because W-Mn concentrations were skewed at all sampling occasions we also repeated the analyses with log_2-tranfomed W-Mn. As this gave essentially the same results (significance levels), we chose to use the untransformed concentrations, which also simplify the interpretation. The association between W-Mn and W-As showed a complex pattern (Ljung et al. 2009), with elevated W-Mn concentrations mainly at fairly low W-As concentrations (<20 µg/L) and *vice versa* (See Supplemental Material, Figure S1). We controlled for this by adjusting for urinary arsenic (natural log transformed), a biomarker of ongoing arsenic exposure (Vahter 2002) in our analyses. In an additional step, we restricted the models to children with water arsenic concentrations <20 µg/L (at each respective exposure time-point), where most of the variation in W-Mn occurred (See Supplemental Material, Figure S1). In sensitivity analysis, we additionally adjusted the models for either food (two groups) or micronutrient supplementation (three groups), or the combination of both (six groups), which was taken by the mothers during pregnancy.

As gender differences have previously been indicated in cross-sectional studies exploring associations of Mn exposure and children's cognitive function (Bouchard et al. 2011; Riojas-Rodriguez et al. 2010), we tested for a multiplicative interaction between W-Mn and child gender (significant at $p<0.10$) and we also repeated the above mentioned analyses stratified by child gender. In girls, scatter plots indicated non-linear associations of maternal W-Mn with cognitive abilities; therefore, we evaluated these associations using linear spline regression analyses with a knot at the turning point of the curve (3 mg/L, according to the scatter plot).

Statistical significance was considered as a 95% CI that did not include zero or $p<0.05$ (two-sided). The statistical analyses were conducted using SPSS (version 22.0, IBM Corporation, USA) and STATA (version 11; STATA Corp, College Station, TX, USA).

Results

Background characteristics

The main characteristics of the studied 1,265 children are summarized in Table 1. The age range at testing was narrow, 8.8-10.1 years. The girls were more underweight (46% with WAZ<-2 z-scores) and stunted (30% with HAZ<-2 z-scores) than the boys (41% and 25%, respectively). In general, boys obtained higher scores for verbal comprehension, perceptional reasoning and working memory, and showed more conduct problems, hyperactivity, and peer problems, whereas girls had higher scores for processing speed and prosocial behavior, but more emotional problems (Table 1). The mothers of the tested children were 14-45 years of age in early pregnancy. About 30% had a BMI <18.5 kg/m^2 (range 12.8-35.3 kg/m^2) and 32% were anemic as indicated by Hb<110 g/L in early gestation (WHO 2011). None of the women had smoked or used alcohol during pregnancy.

The median concentration of prenatal W-Mn was 204 µg/L with a wide range (1.3-6,550 µg/L). At 5 years it was quite similar (median 228 µg/L; range 0.1-6,550 µg/L), while at 10 years it was 339 µg/L (0.1-8,680 µg/L). In total, 810 and 733 of the children had the same water source at 5 and 10 years of age, respectively, as their mother had used during pregnancy (W-Mn prenatally and at 5 years $r_s=0.76$, $p<0.001$ and W-Mn prenatally and 10 years $r_s=0.58$, $p<0.001$), and 656 of the children had used the same water source since birth to 10 years of age (W-Mn at 5 and 10 years $r_s=0.74$, $p<0.001$). W-Mn correlated weakly with SES ($r_s=0.16$, 0.10, and 0.14, respectively, for water collected prenatally, and at 5 and 10

years; $p<0.05$), but not with child anthropometry measures (WAZ and HAZ at 5 and 10 years; $p>0.10$).

Manganese and children's cognitive abilities

In the age- and gender-adjusted analyses, both prenatal and child W-Mn were positively associated with all cognitive ability measures at 10 years of age (Table 2). However, adjustment for other covariates, in particular SES, markedly decreased the associations, which were no longer significant. Restricting the analyses to children of women with W-As <20 μg/L further reduced the associations (Table 2). Therefore, children with low W-As were selected for the subsequent analyses.

Gender was strongly influential in the models of prenatal W-Mn with the different cognitive ability measures and the interaction between gender and W-Mn was significant for full scale IQ, verbal comprehension, working memory, and processing speed ($p<0.10$; Table 2). When boys were evaluated separately (n=288 with W-As<20 μg/L), the multivariable-adjusted associations of prenatal or childhood W-Mn concentrations with the different cognitive ability measures were generally inverse, although non-significantly (with the exception of working memory; Table 3). For girls, the linear spline regression analysis with prenatal W-Mn showed positive associations below the knot at 3 mg/L for all cognitive ability measures (Table 3). For full scale IQ and verbal comprehension they differed significantly from the inverse associations above the knot (Wald test $p<0.05$), although there were only 27 mothers with W-Mn concentrations above 3 mg/L. The positive associations of W-Mn concentrations at 5 and 10 years of age were much weaker than those with the prenatal W-Mn and no longer statistically significant (Table 3). Additionally adjusting the association of W-Mn at 5 or 10 years with full scale IQ for the mothers' urinary arsenic concentrations during pregnancy had marginal impact on the estimates (data not shown).

Manganese and children's behavior

The multivariable-adjusted logistic regression analyses showed that W-Mn at all time-points was significantly associated with increased risk of conduct problems (Table 4; Model 1). Restricting the analyses to the children with low W-As (Model 2) slightly strengthened the risk estimates for conduct problems and low prosocial behavior, which were statistically significant at all exposure time points. Thus, the following analyses concern children using water with <20 µg/L of arsenic.

We found a significant interaction between gender and prenatal W-Mn for hyperactivity, and between gender and W-Mn at 10 years of age for peer problems (Table 4). Stratifying the models by gender (Table 5) indicated slightly stronger associations of prenatal W-Mn with conduct problems in boys (statistically significant) than in girls (not significant). For boys, the obtained odds ratios for conduct problems in relation to W-Mn at 5 and 10 years were slightly lower than those for the prenatal exposure. On the other hand, the prenatal W-Mn was associated with decreased risk of emotional problems in the boys (only 27 cases out of which 20 also had conduct problems), but with W-Mn and 5 and 10 years of age (37 and 40 cases, respectively), this inverse association was weaker and no longer significant. In girls, W-Mn was mainly associated with increased risk of low prosocial scores.

Discussion

This large prospective cohort study indicates that elevated water Mn concentrations prenatally and at 5 and 10 years of age were associated with a similar increased risk (~18-29%) of parents-reported conduct problems, especially in boys. Simultaneously, W-Mn was associated with a lower risk of emotional problems in the boys, probably because emotional problems are less frequent in children with conduct problems (often temper tantrums or hot tempers, disobedient, fighting with other children, etc.) or less appraised by the parents of such children. In the girls,

elevated W-Mn was mainly associated with low scores for prosocial behavior. We found no clear evidence for impairment of cognitive abilities by W-Mn. There was a tendency of inverse associations in the boys, especially with the early childhood exposure, however the effect on full scale IQ corresponded to less than 0.1 SD only. On the contrary, the prenatal W-Mn was positively associated with all cognitive test scores in the girls up to about 3 mg/L, above which the positive influence disappeared. The total range of W-Mn at 10 years was 0.001-8.7 mg/L, with 52% exceeding the U.S. lifetime health advisory value of 0.3 mg/L (EPA 2012). The U.S. Institute of Medicine has indicated an Tolerable Upper Intake Level of 6 mg Mn/day for children aged 9-13 years (Institute of Medicine 2001).

In support of the present findings, a previous cross-sectional study indicated more classroom behavioral problems at 8-11 years of age (n=211) with increasing W-Mn concentrations (Khan et al. 2011), but the time of initiation of the behavioral effects and potential gender differences were not evaluated. Interestingly, higher perinatal Mn exposure, assessed by concentrations in deciduous tooth dentine, was recently associated with poorer behavioral outcomes in 248 school-aged U.S. children (Mora et al. 2015). Unlike the present findings, a few cross-sectional studies have shown inverse associations between W-Mn and cognitive abilities in 6-13 years old children (Bouchard et al. 2011; Khan et al. 2012; Wasserman et al. 2006) (n=362, 840 and 142, respectively). The reason for this discrepancy is not known and further prospective studies are warranted. To note, excess Mn exposure is believed to affect dopamine and other neurotransmitters in the brain (Vorhees et al. 2014), and experimental studies indicate an increased susceptibility to Mn very early in life (Beaudin et al. 2013).

The mode(s) of action behind the observed gender differences in the effects of Mn are unknown. Possible, the underlying mode of action is the same for the observed behavioral effects. The parents' general scoring of more difficult problems in boys and better prosocial

behavior in girls supports the influence of gender-specific norms. Still, previous studies have indicated gender-related differences in the kinetics of Mn, as shown by higher blood Mn concentrations in girls than in boys before 2-3 years of age (Berglund et al. 2011; Oulhote et al. 2014b) and markedly higher urinary excretion of Mn in girls than in boys at 6-12 years of age (Berglund et al. 2011). In experimental studies, postnatal exposure to Mn has been shown to alter the levels of monoamines and corticosterone in a sex-dependent manner (Vorhees et al. 2014) and cause more morphological changes in striatal medium spiny neurons in male than in female mice (Madison et al. 2011).

The indicated positive associations of the prenatal W-Mn with the girls' cognitive abilities, while no such association was observed in boys, suggest a sex-difference already during fetal development. Therefore, another hypothesis is that the prenatal Mn exposure affects epigenetic and/or hormonal factors. The early fetal epigenome is sensitive to environmental influences, especially to poor nutrition (Barker et al. 2013), and the sensitivity has been shown to differ by sex (Tarrade et al. 2015). As it was mainly the prenatal exposure that appeared beneficial for the girls 'cognitive development, residual confounding seems unlikely. Gender differences were also apparent with the W-Mn at 5 years of age; there was no association of W-Mn with the girls' cognitive abilities, whereas boys showed mainly inverse associations. Unrelated to W-Mn, girls generally scored lower than the boys on the cognitive tests, which is in contrast to the results at 5 years of age (Hamadani et al. 2011). Possibly, this is related to the discrimination against female gender, resulting in poorer nutrition, less stimulation and more violence (Adams et al. 2013; VanderEnde et al. 2014).

The strengths of this study include the large sample size and the population-based prospective design with Mn measured in all water sources used by the children, as well as that used by their mothers during pregnancy. Based on the life time water histories of the children, we did an attempt to evaluate also the effects of life-time cumulative exposure. However,

because the correlations of W-Mn between years were generally very high (0.7-1.0), the results were essentially the same as with the childhood exposure at 5 and 10 years of age. The cognitive assessment was expressed as raw scores as the WISC-IV has not been standardized for Bangladeshi children, giving rise to low scoring according to the international standard. A limitation of the study is the lack of water samples from the children's schools, which might have caused non-differential misclassification of children's exposure, especially at 10 years of age. At 5 years, only 30% of the children attended school, and then only for a short time each day. However, we observed a strong correlation (r_s=0.66; *P*=<0.001) between the concentrations of arsenic in the children's water and urine, a biomarker of ongoing arsenic exposure, indicating that the included W-Mn concentrations were indeed representative of the children's exposure. Also, we only had information on exclusive breast feeding at four months (yes/no), showing that only 55% of the infants were still exclusively breast fed at this age. In another study from the same area, infants that were not exclusively breast-fed were mainly given cow milk (about 25% of the infants at 4 months), plain water, fruit juice or semisolid food (all less than 20% at 4 months) (Saha et al. 2008). Thus, the age at which drinking water was first introduced likely varied among the infants. Another limitation is the lack of an exposure biomarker, especially blood Mn concentrations, which have repeatedly been used in previous studies on child neurodevelopment (Sanders et al. 2015). However, in a sub-cohort of the mothers we found no association whatsoever between the concentrations of Mn in water and blood (Ljung et al. 2009), and as the aim of the present study was to evaluate potential adverse effects of the high W-Mn concentrations, we prioritized analyzing all the collected water samples. An additional limitation is that iron status of mothers and their children was only assessed via hemoglobin, while more precise markers of iron deficiency such as serum ferritin were only present for a sub-sample of the mothers.

Conclusions

In conclusion, elevated prenatal and early childhood exposure to W-Mn (mainly from medium deep wells) appeared to increase the risk of children´s behavior problems at 10 years of age. However, we also found positive associations of prenatal W-Mn and cognitive abilities in girls. Thus, compared to the severe health risks of the elevated concentrations of arsenic, a potent carcinogen and general toxicant, present in many of the shallow wells; the presently observed effects of elevated Mn concentrations in water from the increasingly installed deeper wells seem modest. Potential temporal variations in W-Mn in the different types of water sources need to be evaluated.

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Table 1 Background characteristics of the studied children, and their cognitive and behavioral scores at 10 years of age.

Variables	All children[a] (n=1,265)	Boys[a] (n= 656)	Girls[a] (n= 609)	*p* Value[b]
Children at 10 years				
Age at testing (years)	9.5±0.1	9.5±0.1	9.5±0.1	0.61
Education (%, <3/ 3/or >3 years)	28/39/33	33/39/29	22/40/38	<0.001
No. of siblings (%, 1-2/3/>3)	31/42/27	34/41/25	29/44/28	0.12
Weight for age (WAZ)	-1.8±1.0	-1.7±1.0	-1.8±1.1	0.081
Height for age (WAZ)	-1.4±0.9	-1.4±0.9	-1.5±1.0	0.23
HOME	27.0±5.0	26.9±5.1	27.2±4.8	0.18
Hemoglobin (g/L)	119+11.1	119+11.1	118+11.3	0.15
Drinking water Mn (µg/L)	339 (4.0-3,203)	334 (3.1-2,877)	348 (4.9-3,435)	0.13
Drinking water As (µg/L)	2.2 (0.1-311)	3.6 (0.1-305)	1.3 (0.1-319)	0.015
WISC-IV				
Full scale IQ	131±34	133±34	130±33	0.22
Verbal comprehension	36±11	37±11	36±10	0.043
Perceptual reasoning	31±12	33±12	30±11	<0.001
Working memory	30±6.1	30±6.2	29±6.0	<0.001
Processing speed	34±12	32±11	36±12	<0.001
SDQ				
Conduct problems	2.9± 1.7	3.2± 1.7	2.6± 1.5	<0.001
Hyperactivity/inattention	4.5±1.7	4.7±1.7	4.2±1.6	<0.001
Peer relationship problems	2.1±1.3	2.2±1.3	2.0±1.3	0.0046
Emotional symptoms	1.6±1.4	1.5±1.4	1.8±1.4	<0.001
Prosocial behavior	6.6±1.8	6.3±1.8	6.9±1.7	<0.001
Children at 5 years[c]				
Water Mn (µg/L)	228 (8.2-2,605)	226 (7.9-2,494)	231 (8.6-2,748)	0.53
Water As (µg/L)	3.6 (0.1-266)	4.5 (0.1-281)	2.9 (0.1-264)	0.16
Weight for age (WAZ)	-1.8±1.8	-1.8±0.9	-1.9±0.8	0.007
Height HAZ)	-1.6±1.6	-1.6±1.0	-1.6±0.9	0.75
Children at birth				
Gestational age (weeks)	39.1±2.1	39.0±2.2	39.3±2.1	0.004
Weight (g)	2,688±378	2,730±396	2,643±656	<0.001
Length (cm)	47.7±2.1	48.0v2.2	47.4±1.9	<0.001
Mothers				
Age (years)	26.8±5.8	26.9±5.8	26.6±6.2	0.26
BMI at GW9 (kg/m^2)	20.0±2.6	20.0±2.6	20.0±2.6	0.84
Education in years	4.5±4.0	4.5±4.0	4.5±4.0	0.83
Maternal IQ	24.7±11.6	24.7±11.8	24.6±11.8	0.87
Hb (g/L)[d]	116±13.1	116±12.6	115±13.6	0.16
Drinking water Mn (µg/L)	204 (23-2,494)	218 (22-2,251)	200 (25-2,715)	0.53
Drinking water As (µg/L)	33 (0.1- 412)	29 (0.1- 411)	35 (0.1- 425)	0.90

Abbreviations: SD, standard deviation; GW, gestational week; Hb, hemoglobin; WISC-IV, Wechsler Intelligence Scale for Children, 4th Edition; SDQ, Strength and Difficulties Questionnaire

[a]Data shown as the arithmetic mean±SD, or percentage or median (5th and 95th percentile) for As and Mn concentrations

[b]Derived by using either Mann-Whitney U-test or χ^2
[c]Available for n= 1,162
[d]Sampled at gestational week 14c

Table 2 Associations of W-Mn (mg/L) during pregnancy and at 5 and 10 years of age with measures of cognitive abilities at 10 years of age.

Exposure windows	**Age- and gender-adjusted model**		**Multivariable-adjusted model 1**[a]		**Multivariable-adjusted model 2**[b]		
	B (95% CI)	*p* value	B (95% CI)	*p* value	B (95% CI)	*p* value	*p* interaction[c]
W-Mn in pregnancy	n=1,265		n=1,201		n=554		
Full scale IQ	4.5 (2.5, 6.5)	<0.001	1.0 (-0.69, 2.7)	0.25	0.42 (-1.6, 2.5)	0.69	0.029
Verbal comprehension	1.5 (0.87, 2.1)	<0.001	0.37 (-0.17, 0.92)	0.18	0.070 (-0.62, 0.76)	0.84	0.050
Perceptual reasoning	1.2 (0.50, 1.9)	0.001	0.26 (-0.40, 0.92)	0.45	0.16 (-0.65, 0.96)	0.70	0.20
Working memory	0.61 (0.24, 0.98)	0.001	0.17 (-0.17, 0.51)	0.32	0.072 (-0.33, 0.47)	0.73	0.081
Processing speed	1.2 (0.47, 1.9)	0.001	0.20 (-0.47, 0.86)	0.56	0.12 (-0.64, 0.88)	0.76	0.059
W-Mn at 5 years	n=1,162		n=1,124		n=705		
Full scale IQ	2.6 (0.51, 4.6)	0.015	0.23 (-1.5, 2.0)	0.79	-0.37 (-2.3, 1.5)	0.70	0.097
Verbal comprehension	0.88 (0.22, 1.5)	0.009	0.096 (-0.46, 0.66)	0.74	-0.10 (-0.73, 0.52)	0.74	0.11
Perceptual reasoning	0.87 (0.16, 1.6)	0.017	0.31 (-0.36, 0.99)	0.36	0.10 (-0.64, 0.84)	0.79	0.12
Working memory	0.30 (-0.084, 0.68)	0.13	-0.030 (-0.37, 0.31)	0.86	-0.043 (-0.43, 0.34)	0.83	0.013
Processing speed	0.53 (-0.20, 1.3)	0.15	-0.15 (-0.83, 0.53)	0.66	-0.32 (-1.0, 0.40)	0.38	0.96
W-Mn at 10 years	n=1,265		n=1,232		n=801		
Full scale IQ	2.3 (0.70, 3.9)	0.005	0.71 (-0.64, 2.1)	0.30	0.18 (-1.3, 1.6)	0.81	0.44
Verbal comprehension	0.57 (0.072, 1.08)	0.025	-0.12 (-0.56, 0.32)	0.59	-0.33 (-0.81, 0.15)	0.18	0.42
Perceptual reasoning	0.56 (0.012, 1.1)	0.045	0.12 (-0.41, 0.66)	0.65	-0.050 (-0.63, 0.53)	0.87	0.46
Working memory	0.37 (0.081, 0.66)	0.012	0.19 (-0.084, 0.46)	0.17	0.12 (-0.17, 0.40)	0.42	0.43
Processing speed	0.78 (0.22 1.3)	0.014	0.52 (-0.016, 1.06)	0.057	0.44 (-0.13, 1.0)	0.13	0.88

Abbreviations: CI, 95% confidence interval; W-Mn, water manganese concentration

[a]Adjusted for mother's IQ, SES (quintiles), child age at intelligence testing (years), gender, education (<3, 3, and >3 years), HAZ at 10 years, Hb (g/dL), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], HOME, tester, number of siblings, and urinary arsenic concentrations at each respective time point (natural log transformed).

[b]Adjusted as model 1, but restricted to children with water As <20 µg/L at each respective time point.

[c]In model 2 we also tested for a multiplicative interaction between water Mn and gender.

Table 3 Associations of W-Mn (mg/L) during pregnancy and childhood with cognitive abilities at 10 years when restricted to low W-As and stratified by gender.

Exposure windows	**Boys**		**Girls**	
	B (95% CI)	*p* value	B (95% CI)	*p* value
W-Mn in pregnancy[a]	n=288		n=266/n=27	
Full scale IQ	-1.8 (-5.3, 1.7)	0.31	5.2 (1.8, 8.6)/-5.4 (-13, 2.0)[b]	0.003/0.15
Verbal comprehension	-0.62 (-1.8, 0.53)	0.29	1.5 (0.31, 2.6)/-1.7 (-4.2, 0.81)[b]	0.013/0.18
Perceptual reasoning	-0.22 (-1.6, 1.2)	0.76	1.4 (0.033, 2.7)/-1.3 (-4.2, 1.5)[b]	0.045/0.37
Working memory	-0.32 (-1.0, 0.39)	0.38	0.72 (0.084, 1.4)/-0.51 (-1.9, 0.88)[b]	0.027/0.47
Processing speed	-0.64 (-1.9, 0.59)	0.31	1.6 (0.29, 3.0)/-1.9 (-4.8, 1.0)[b]	0.018/0.21
W-Mn at 5 years[a,b]	n=345		n=350	
Full scale IQ	-3.2 (-6.4, 0.065)	0.055	0.92 (-1.4, 3.3)	0.44
Verbal comprehension	-0.88 (-1.9, 0.17)	0.099	0.26 (-0.53, 1.0)	0.52
Perceptual reasoning	-1.1 (-2.3, 0.18)	0.093	0.65 (-0.27, 1.6)	0.17
Working memory	-0.70 (-1.4, -0.022)	0.043	0.38 (-0.074, 0.84)	0.10
Processing speed	-0.52 (-1.7, 0.67)	0.39	-0.37 (-1.3, 0.57)	0.44
W-Mn at 10 years[a]	n=406		n=395	
Full scale IQ	-0.56 (-3.0, 1.8)	0.64	0.70 (-1.2, 2.6)	0.46
Verbal comprehension	-0.59 (-1.4, 0.17)	0.13	-0.16 (-0.78, 0.46)	0.61
Perceptual reasoning	-0.35 (-1.3, 0.60)	0.47	0.17 (-0.55, 0.90)	0.64
Working memory	-0.058 (-0.53, 0.41)	0.81	0.23 (-0.13, 0.59)	0.21
Processing speed	0.44 (-0.44, 1.3)	0.33	0.45 (-0.31, 1.2)	0.24

Abbreviations: CI, 95% confidence interval; W-Mn, water manganese concentration; Hb, hemoglobin concentration

[a]Adjusted for mother's IQ, SES (quintiles), child age at intelligence testing (years, one decimal), gender, education (<3, 3, or >3 years), HAZ at 10 years, Hb (g/L), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], HOME, tester (4 psychologists), number of siblings , and urinary arsenic concentrations at each respective time point (natural log transformed).

[b]Spline regression analyses with a knot at 3 mg/L; B and 95% CI for <3mg/L/ ≥3 mg/L.

[c]Excluding one outlier with W-Mn 6.5 mg/L.

Table 4 Multivariable-adjusted odd ratios (95% confidence intervals) for raised SDQ difficult scores (low prosocial) at 10 years in relation to W-Mn (mg/L) during pregnancy and childhood.

Exposure windows	**Age- and gender-adjusted model**		**Multivariable-adjusted model 1**[a]		**Multivariable-adjusted model 2**[b]		
	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	*p* interaction[c]
W-Mn in pregnancy	n=1,265		n=1,201		n=554		
Conduct problems	1.09 (0.96, 1.24)	0.18	1.20 (1.04, 1.39)	0.013	1.29 (1.08, 1.53)	0.005	0.22
Hyperactivity	1.11 (0.97, 1.27)	0.13	1.11 (0.95, 1.29)	0.20	1.03 (0.86, 1.23)	0.74	0.090
Peer problems	0.99 (0.87, 1.13)	0.88	0.99 (0.85, 1.14)	0.85	0.96 (0.81, 1.14)	0.66	0.16
Emotional problems	0.76 (0.60, 0.98)	0.031	0.81 (0.61, 1.07)	0.14	0.70 (0.51, 0.97)	0.034	0.18
Prosocial behavior[d]	1.10 (0.95, 1.28)	0.21	1.16 (0.97, 1.39)	0.093	1.27 (1.03, 1.57)	0.025	0.35
W-Mn at 5 years	n=1,162		n=1,124		n=705		
Conduct problems	1.15 (1.01, 1.31)	0.037	1.20 (1.03, 1.39)	0.016	1.26 (1.07, 1.48)	0.005	0.44
Hyperactivity	1.09 (0.95, 1.25)	0.23	1.05 (0.90, 1.23)	0.56	1.06 (0.89, 1.26)	0.49	0.25
Peer problems	0.99 (0.86, 1.13)	0.86	0.97 (0.84, 1.12)	0.66	0.98 (0.83, 1.15)	0.79	0.18
Emotional problems	0.74 (0.57, 0.97)	0.029	0.77 (0.57, 1.03)	0.077	0.72 (0.52, 0.99)	0.045	0.57
Prosocial behavior[d]	0.98 (0.84, 1.13)	0.77	1.04 (0.88, 1.24)	0.63	1.11 (0.92, 1.34)	0.28	0.60
W-Mn at 10 years	n=1,265		n=1,232		n=801		
Conduct problems	1.10 (1.00, 1.22)	0.052	1.17 (1.04, 1.31)	0.007	1.18 (1.05, 1.34)	0.007	0.31
Hyperactivity	0.99 (0.89, 1.11)	0.93	0.97 (0.86, 1.11)	0.69	0.96 (0.84, 1.11)	0.61	0.43
Peer problems	0.92 (0.83, 1.03)	0.14	0.91 (0.81, 1.02)	0.12	0.89 (0.78, 1.01)	0.073	0.074
Emotional problems	0.77 (0.63, 0.94)	0.010	0.79 (0.63, 0.99)	0.037	0.83 (0.66, 1.05)	0.12	0.71
Prosocial behavior[d]	1.09 (0.96, 1.22)	0.17	1.17 (1.01, 1.34)	0.031	1.23 (1.05, 1.43)	0.009	0.25

Abbreviations: CI, 95% confidence interval; W-Mn, water manganese concentration; Hb, hemoglobin

[a]Adjusted for mother's IQ, SES (quintiles), child age at intelligence testing (years), gender, education (<3, 3, and >3 years), HAZ at 10 years, Hb (g/dL), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], HOME, number of siblings, and urinary arsenic concentration at each respective time point (natural log transformed).

[b]Adjusted as model 1, but restricted to children with water arsenic <20 µg/L at each respective time point.

[c]In model 2 we also tested for a multiplicative interaction between water Mn and gender.

[d]OR for low prosocial scores.

Table 5 Multivariable-adjusted odd ratios (95% confidence intervals) of difficult behavior at 10 years in relation to W-Mn (mg/L) during pregnancy and childhood when restricted to low W-As and stratified by child gender.

Exposure windows	**Boys**		**Girls**	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
W-Mn in pregnancy[a]	n=288		n=266	
Conduct problems	1.43 (1.06, 1.91)	0.017	1.18 (0.95, 1.46)	0.13
Hyperactivity	1.14 (0.88, 1.48)	0.31	0.90 (0.69, 1.18)	0.45
Peer problems	1.11 (0.85, 1.44)	0.45	0.86 (0.68, 1.10)	0.23
Emotional problems	0.39 (0.19, 0.82)	0.013	0.80 (0.54, 1.18)	0.26
Prosocial behavior[b]	1.17 (0.87, 1.57)	0.29	1.48 (1.07, 2.06)	0.019
W-Mn at 5 years[a]	n=355		n=350	
Conduct problems	1.36 (1.04, 1.79)	0.026	1.21 (0.99, 1.49)	0.066
Hyperactivity	1.16 (0.90, 1.50)	0.25	0.99 (0.77, 1.27)	0.94
Peer problems	1.30 (0.89, 1.44)	0.31	0.90 (0.72, 1.12)	0.33
Emotional problems	0.61 (0.36, 1.03)	0.063	0.72 (0.47, 1.12)	0.15
Prosocial behavior[b]	1.10 (0.85, 1.42)	0.48	1.14 (0.85, 1.52)	0.39
W-Mn at 10 years[a]	n=406		n=395	
Conduct problems	1.24 (1.01, 1.53)	0.045	1.16 (0.99, 1.36)	0.062
Hyperactivity	1.01 (0.83, 1.23)	0.90	0.94 (0.77, 1.15)	0.54
Peer problems	1.00 (0.82, 1.20)	0.97	0.82 (0.68, 0.99)	0.039
Emotional problems	0.88 (0.62, 1.25)	0.47	0.77 (0.55, 1.08)	0.14
Prosocial behavior[b]	1.15 (0.93, 1.41)	0.20	1.34 (1.05, 1.73)	0.021

[a]Adjusted for mother's IQ, SES (quintiles), child age at intelligence testing (years), education (<3, 3, and >3 years), HAZ at 10 years, Hb (g/dL), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], HOME, number of siblings, and urinary arsenic concentration at each respective time point (natural log transformed).
[b]OR of low prosocial scores.