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

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

OBJECTIVES: We prospectively evaluated the effects of W-Mn, from fetal life to school age, on children's cognitive abilities and behavior.

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

RESULTS: The 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 y. In multivariable-adjusted linear regression analyses, restricted to children with low arsenic (As) exposure, none of the W-Mn exposures was associated with the children's cognitive abilities. Stratifying by gender (p for interaction in general <0.081) showed that prenatal W-Mn ($<3\,\mathrm{mg/L}$) was positively associated with cognitive ability measures in girls but not in boys. W-Mn at all time points was associated with an increased risk of conduct problems, particularly 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 [odds ratio (OR)=0.39 (95% CI: 0.19, 0.82)] in 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, whereas boys appeared to be unaffected. Early life W-Mn exposure appeared to adversely affect children's behavior. https://doi.org/10.1289/EHP631

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/d for pregnant women and 0.5–2.0 mg/d for children (EFSA 2013). 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, particularly in children (Frisbie et al. 2012; Zoni and Lucchini 2013). Several cross-sectional studies have indicated that elevated Mn concentrations in drinking water (W-Mn) are associated with impaired cognitive abilities, adaptive behaviors, or both in 6- to 13-year-old children (Bouchard et al. 2011; Khan et al. 2011; Oulhote et al. 2014a; Wasserman et al. 2006). Additional studies indicate associations with blood Mn, often with an inverted U-shaped dose–effect relationship (Sanders et al. 2015). However, there appears to be no association between Mn in water and that in blood (Ljung et al. 2009; Rahman et al. 2015).

The timing of exposure may be critical for Mn neurotoxicity because the susceptibility of the brain to toxic insult is known to vary during different phases of development (Grandjean and Landrigan 2014). In addition, exposure may vary over time. Because Mn easily passes through the placenta (Erikson et al. 2007), elevated maternal exposure during pregnancy, combined with 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 dentin measured using microspatial analysis, which estimated 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 with neurodevelopmental outcomes was observed (Chan et al. 2015). Early childhood may be another critical period of Mn exposure because regulation of intestinal absorption and biliary excretion is not yet fully developed (Erikson et al. 2007). Although breast milk contains very little Mn (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 cognitive abilities and behavior in a large cohort of boys and girls at 10 y of age. Elevated Mn concentrations were found mainly in medium-deep wells, many of which were constructed to decrease exposure to arsenic (As), which was present in many 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 y of age. The study was initially nested in a randomized food and micronutrient supplementation trial (MINIMat) conducted in pregnant women living in Matlab, in rural Bangladesh (Persson et al. 2012), to evaluate the health effects of early life exposure to As, which occurs frequently in shallow wells (Vahter et al. 2006). The installation of deeper wells, often with the aim to

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decrease As exposure, has resulted in elevated W-Mn (Kippler et al. 2016; Ljung et al. 2009), which motivated us to evaluate the potential health consequences. 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 y of age, and 95% ($n = 1{,}530$) agreed to participate. The main reasons for loss to follow-up were parental refusal and outmigration.

This study was approved by the ethics review committee at International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) in Bangladesh and by the Regional Ethical Review Board in Stockholm, Sweden. Written consent was obtained from all mothers before enrollment in the initial study and before the test performed at 10 y of age.

Manganese Exposure

Drinking water was sampled during pregnancy and at 5 and 10 y of age. Area-wide screening of water As 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 y, we had maternal water samples for 1,265 children; of these children, 1,162 also had their water sampled at 5 y. At the follow-up investigations, the families were interviewed about all 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 lifelong 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 Mn in the mothers' drinking water samples (collected in 2002–2003) were performed during 2008–2009, and the measurements of the children's drinking water samples (5 y: 2007–2008; 10 y: 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). The analytical performance was validated by analysis of a certified reference material (Table S1). No samples were below the limit of detection (LOD) of Mn (see Table S1). For As, 30 samples were below the LOD of 0.01 μg/L at 5 y, and one sample was below the LOD at 10 y; these concentrations were set to LOD divided by the square root of 2.

Outcome Assessments

The Wechsler Intelligence Scale for Children, Fourth Edition (WISC-IV) (Wechsler 2003) was used to assess the children's general cognitive abilities (IQ), and more specific cognitive functions were assessed through its subtests. The WISC-IV is the most widely used intelligence battery in clinical practice. In the present study, we used WISC-IV raw scores to exclude bias owing to comparison with foreign culture norms. Ten subtests generate four composite scores: *a*) Verbal Comprehension (vocabulary, information, and comprehension), *b*) Perceptual Reasoning (block design, picture concepts, and matrix reasoning), *c*) Working Memory (digit span and arithmetic), and *d*) 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 with similar local pictures, and overly difficult questions were changed: For example, "What is a fossil" was changed to "What is a skeleton," and "Who was Columbus" was changed to "Who was Rabindranath Tagore." To assess the children's cognitive abilities, we recruited four female psychologists with Master's degrees in psychology; they were trained for 6 wk on the above-mentioned 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 ~5% of all tests by scoring them independently. 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-reported Strengths and Difficulties Questionnaire (SDQ) for 4–17 y old children (Goodman 2001). The SDQ is a screening test for childhood psychopathology adapted for many different cultures and languages, and it has demonstrated excellent psychometric properties (http://www.sdqinfo.com/). The test 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 a maximum score of 40; and prosocial behavior, with a maximum score of 10. Female interviewers with Bachelor's degrees who had completed two weeks of training visited the families' homes and conducted the SDQ interviews with the mothers or other female caregivers in the absence of mothers. The testers and interviewers were blinded to Mn concentrations in the children's drinking water because all water samples were analyzed after the data collection was completed.

Covariates

Information about the mother's background characteristics [age, early pregnancy weight, height, date of delivery, education, and hemoglobin concentrations (Hb)] and the children's anthropometry at birth and exclusive breastfeeding at four months (yes/no) was available from the MINIMat trial (Persson et al. 2012). Maternal education was defined as the number of years at school and was categorized as zero, 1–5, and ≥ 6 y. 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 y) and type of school [public primary school, Madrasa (Islamic) school, NGO (nonprofit private) school, and English medium (private) school], and the families' socioeconomic status (SES). 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 was divided into quintiles wherein the lowest quintile represented the poorest families, and the highest quintile represented the richest families. The children's weight and height were measured by the testers at health clinics after the developmental assessment was performed. The measures were converted into age- and sex-standardized *z*-scores [weight for age (WAZ) and height for age (HAZ)] using AnthroPlus software [World Health Organization (WHO), Geneva, Switzerland]. The Hb concentrations (g/L) were measured in peripheral blood (finger prick) using a HemoCue photometer (HemoCue AB, Ängelholm, Sweden).

A modification of the middle childhood version (6–10 y) of the Home Observation For Measurement of Environment (HOME) was used to assess the quality and quantity of stimulation and support for the children at home (Caldwell and Bradley 2003). The HOME consists of 58 questions addressing *a*) responsibility, *b*) encouragement of maturity, *c*) emotional climate, *d*) learning materials and opportunities, *e*) enrichment, *f*) family companionship, *g*) family integration, and *h*) physical environment. The questionnaire was tested in a pilot study with 20 children in the study area, and seven questions were dropped because the mothers could not understand them. Raven's Coloured Progressive Matrices™ test (Raven 2003) was used to measure the mothers' nonverbal IQ in terms of abstract logical reasoning (Hamadani et al. 2011). The sum of correct answers (from a total of 60 questions) was used as a proxy of the mothers' IQ. Results from Raven's matrices have demonstrated good convergence with IQ estimates on the Wechsler scales in normative and clinical samples (Bölte et al. 2009) and were sensibly correlated with education and SES.

Because exposure to As, 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 urinary concentrations of As metabolites (a marker of exposure to inorganic As), cadmium, and lead at gestational week (GW) 8 and at 5 and 10 y of age influenced the associations of Mn 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), the Kruskal-Wallis test, the Mann-Whitney *U*-test, or chi-square tests, depending on the type of data. Scatter plots with Lowess smoothing did not indicate nonlinear associations between W-Mn and the different outcomes. Associations of W-Mn with cognitive abilities (WISC-IV) were explored using linear regression analyses, whereas associations with the problem behavior subtest scores were explored using logistic regression with 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 ≥ 4 scores), and prosocial behavior (normal >5 and slightly raised ≤ 5 scores) (AMHOCN 2005).

The models were adjusted for the children's age at assessment and gender (*a priori*), for variables known to affect child development or that were significantly associated with the exposure and outcome ($p < 0.05$), and for variables that changed the estimates of W-Mn by ≥10% (maternal IQ and SES, number of siblings, child HAZ at 10 y, education in years, type of school attended, Hb concentrations, total HOME score, the testers of cognitive abilities, and the natural log–transformed urinary As concentrations at each exposure window). In case of strongly associated variables ($r_s > 0.60$; i.e., WAZ and HAZ at 10 y of age), we included the variable that had the largest influence on the effect estimates. We excluded one boy with a very high W-Mn concentration (6.5 mg/L) at 5 y, 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 $\log_2$-transfomed W-Mn. Because this analysis gave essentially the same results (significance levels), we chose to use the untransformed concentrations, which also simplified 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 ($<20\ \mu g/L$) and vice versa (see Figure S1). We controlled for this pattern by adjusting for urinary As (natural log–transformed), a biomarker of ongoing As exposure (Vahter 2002) in our analyses. In an additional step, we restricted the models to children with water As concentrations $<20\ \mu g/L$ (at each exposure time point), where most of the variation in W-Mn occurred (see Figure S1). In sensitivity analyses, we additionally adjusted the models for either food (two groups) or micronutrient supplementation (three groups) or for the combination of both (six groups), which was taken by the mothers during pregnancy.

Because 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-Rodríguez 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 abovementioned analyses stratified by child gender. In girls, scatter plots indicated nonlinear 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% confidence interval (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; STATACorp LLC, 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 y. 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 they 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 y of age in early pregnancy. Approximately 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 y, these values were quite similar (median, 228 μg/L; range, 0.1–6,550 μg/L), and at 10 y, the median was 339 μg/L (range, 0.1–8,680 μg/L). In total, 810 and 733 of the children had the same water source at 5 and 10 y of age, respectively, as their mother had used during pregnancy (W-Mn prenatally and at 5 y $r_s = 0.76, p < 0.001$; W-Mn prenatally and 10 y $r_s = 0.58, p < 0.001$), and 656 of the children had used the same water source from birth to 10 y of age (W-Mn at 5 and 10 y $r_s = 0.74, p < 0.001$). 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 y; $p < 0.05$), but not with child anthropometry measures (WAZ and HAZ at 5 and 10 y; $p > 0.10$).

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 y of age (Table 2). However, adjustment for other covariates, in particular SES, markedly decreased the associations, which were no longer significant. Restricting the analyses

Table 1. Background characteristics of the studied children, and their cognitive and behavioral scores at 10 y 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 y)	28/39/33	33/39/29	22/40/38	<0.001
Number of siblings (%, 1–2/3/>3)	31/42/27	34/41/25	29/44/28	0.12
WAZ	−1.8 ± 1.0	−1.7 ± 1.0	−1.8 ± 1.0	0.081
HAZ	−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
Hb (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
WAZ	−1.8 ± 1.8	−1.8 ± 0.9	−1.9 ± 0.8	0.007
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: As, arsenic; BMI, body mass index; GW, gestational week; HAZ, height for age; Hb, hemoglobin concentration; HOME, Home Observation for Measurement of Environment; Mn, manganese; SD, standard deviation; SDQ, Strengths and Difficulties Questionnaire; WAZ, weight for age; WISC-IV, Wechsler Intelligence Scale for Children, 4th Edition.

[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 test.

[c] Available for n = 1,162.

[d] Sampled at gestational week 14.

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 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 nonsignificantly (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, these associations differed significantly from the inverse associations above the knot (Wald test $p < 0.05$), although only 27 mothers had W-Mn concentrations >3 mg/L. The positive associations of W-Mn concentrations at 5 and 10 y of age were much weaker than those with the prenatal W-Mn and were no longer statistically significant (Table 3). Further adjusting the association of W-Mn at 5 or 10 y with Full-Scale IQ for the mothers' urinary As 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 at all time points, W-Mn was significantly associated with an increased risk of conduct problems (Table 4; Model 1). Restricting the analyses to 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 As.

We found a significant interaction between gender and prenatal W-Mn for hyperactivity and between gender and W-Mn at 10 y 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 y were slightly lower than those for prenatal exposure. In contrast, prenatal W-Mn was associated with a decreased risk of

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

	Age- and gender-adjusted model		Multivariable-adjusted model 1[a]		Multivariable-adjusted model 2[b]		
Exposure windows	β (95% CI)	*p*-Value	β (95% CI)	*p*-Value	β (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 y	(*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 y	(*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: 95% CI, 95% confidence interval; W-Mn, water manganese concentration.

[a] Adjusted for mother's IQ, socioeconomic status (SES) (quintiles), child age at intelligence testing (years), gender, education (<3, 3, and >3 y), height for age (HAZ) at 10 y, hemoglobin concentration (Hb) (g/dL), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], Home Observation for Measurement of Environment (HOME), tester, number of siblings, and urinary arsenic (As) 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 W-Mn and gender.

emotional problems in boys (only 27 cases, out of which 20 also had conduct problems), but for W-Mn at 5 and 10 y of age (37 and 40 cases, respectively), this inverse association was weaker and no longer significant. In girls, W-Mn was mainly associated with an increased risk of low prosocial scores.

Discussion

This large prospective cohort study indicates that exposure to elevated W-Mn prenatally and at 5 and 10 y of age was associated with a similar increased risk (~18–29%) of parent-reported conduct problems, particularly in boys. Simultaneously, W-Mn was associated with a lower risk of emotional problems in the boys, most likely because emotional problems occur less frequently in children with conduct problems (frequent temper tantrums or hot tempers, disobedience, fighting with other children, etc.) or are noted less frequently by the parents of such children. In girls, exposure to 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 toward inverse associations in boys, particularly in those

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

	Boys		Girls	
Exposure windows	β (95% CI)	*p*-Value	β (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 y[a,c]	(*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 y[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: 95% CI, 95% confidence interval; Hb, hemoglobin concentration; W-As, water arsenic concentration; W-Mn, water manganese concentration.

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

[b] Spline regression analyses with a knot at 3 mg/L; β 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 y in relation to W-Mn (mg/L) during pregnancy and childhood.

	Age- and gender-adjusted model		Multivariable-adjusted model 1[a]		Multivariable-adjusted model 2[b]		
Exposure windows	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 y	(*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 y	(*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: 95% CI, 95% confidence interval; OR, odds ratio; SDQ, Strengths and Difficulties Questionnaire; W-Mn, water manganese concentration.

[a] Adjusted for mother's IQ, socioeconomic status (SES) (quintiles), child age at intelligence testing (years), gender, education (<3, 3, and >3 years), height for age (HAZ) at 10 years, hemoglobin concentration (Hb) (g/dL), school type [public primary school, Madrasa (Islamic), NGO (nonprofit private), and English medium (private) school], Home Observation for Measurement of Environment (HOME), number of siblings, and urinary arsenic (As) concentration 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.

[d] OR for low prosocial scores.

with early childhood exposure; however, the effect on Full-Scale IQ corresponded to <0.1 standard deviation (SD) only. On the contrary, prenatal W-Mn was positively associated with all cognitive test scores in girls up to approximately 3 mg/L, above which the positive influence disappeared. The total range of W-Mn at 10 y was 0.001–8.7 mg/L, with 52% exceeding the U.S. lifetime health advisory value of 0.3 mg/L [U.S. Environmental Protection Agency (EPA) 2012]. The U.S. Institute of Medicine has indicated a Tolerable Upper Intake Level of 6 mg Mn/day for children ages 9–13 y (Institute of Medicine 2001).

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

	Boys		Girls	
Exposure windows	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 y[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 y[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

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio; W-As, water arsenic concentration; W-Mn, water magnesium concentration.

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

[b] OR of low prosocial scores.

In support of the present findings, a previous cross-sectional study indicated more classroom behavioral problems at 8–11 y of age ($n = 211$) with increasing W-Mn concentration (Khan et al. 2011), but the time of initiation of the behavioral effects and the potential gender differences were not evaluated. Interestingly, higher perinatal Mn exposure, assessed by concentrations in deciduous tooth dentin, was recently associated with poorer behavioral outcomes in 248 school-age 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 y 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. Notably, 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. It is possible that 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. Nevertheless, 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 y of age (Berglund et al. 2011; Oulhote et al. 2014b) and by markedly higher urinary excretion of Mn in girls than in boys at 6–12 y 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 to cause more morphological changes in striatal medium spiny neurons in male mice than in female mice (Madison et al. 2011).

The indicated positive associations of prenatal W-Mn with girls' cognitive abilities, with no such associations observed in boys, suggest a sex difference during fetal development. Therefore, another hypothesis is that prenatal Mn exposure affects epigenetic and/or hormonal factors. The early fetal epigenome is sensitive to environmental influences, particularly to poor nutrition (Barker et al. 2013), and this sensitivity has been shown to differ by sex (Tarrade et al. 2015). Because it was mainly the prenatal exposure that appeared to be beneficial for the girls' cognitive development, residual confounding seems unlikely. Gender differences were also apparent with W-Mn at 5 y 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 boys on the cognitive tests, which is in contrast to the results at 5 y of age (Hamadani et al. 2011). This difference may be related to discrimination against the 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 and in all those used by their mothers during pregnancy. Based on the lifetime water histories of the children, we also attempted to evaluate the effects of lifetime 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 those obtained for childhood exposure at 5 and 10 y of age. The cognitive assessment was expressed as raw scores because 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 nondifferential misclassification of the children's exposure, particularly at 10 y. At 5 y, only 30% of the children attended school, and then only for a short time each day. However, we observed a strong correlation ($r_s = 0.66$; $p \leq 0.001$) between the concentration of As in the children's water and that in their urine, a biomarker of ongoing As exposure, indicating that the included W-Mn concentrations were indeed representative of the children's exposure. In addition, we only had information on exclusive breastfeeding at four months (yes/no), which showed that only 55% of the infants were still exclusively breastfed at this age. In another study from the same area, infants that were not exclusively breastfed were mainly given cow's milk (~25% of the infants at 4 mo), plain water, fruit juice, or semisolid food (all <20% at 4 mo) (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, in particular blood Mn concentration, which has been used repeatedly in previous studies on child neurodevelopment (Sanders et al. 2015). However, in a subcohort of the mothers, we found no association whatsoever between the concentration of Mn in water and that in blood (Ljung et al. 2009), and because the aim of the present study was to evaluate potential adverse effects of high W-Mn concentrations, we prioritized analyzing all the collected water samples. An additional limitation is that the iron status of the mothers and their children was only assessed via hemoglobin, and more precise markers of iron deficiency such as serum ferritin were only present for a subsample 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 y of age. However, we also found positive associations of prenatal W-Mn and cognitive abilities in girls. Thus, compared with the severe health risks of exposure to elevated concentrations of As, a potent carcinogen and general toxicant that is present in many shallow wells, the effects observed here of elevated Mn concentrations in water from the increasingly common deeper wells seem modest. Potential temporal variations in W-Mn in different types of water sources need to be evaluated.

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