NeuroToxicology 32 (2011) 450–457

Contents lists available at ScienceDirect

NeuroToxicology

Arsenic and manganese exposure and children's intellectual function

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ARTICLE INFO

Article history:
Received 5 January 2011
Accepted 21 March 2011
Available online 29 March 2011

Keywords:
Arsenic
Manganese
Children
Water
Bangladesh

ABSTRACT

Recently, epidemiologic studies of developmental neurotoxicology have been challenged to increase focus on co-exposure to multiple toxicants. Earlier reports, including our own work in Bangladesh, have demonstrated independent associations between neurobehavioral function and exposure to both arsenic (As) and manganese (Mn) in school-aged children. Our earlier studies, however, were not designed to examine possible interactive effects of exposure to both As and Mn. To allow investigation of possible synergistic impact of simultaneous exposures, we recruited a new sample of 299 8–11 year old children, stratified by design on As (above and below 10 μg/L) and Mn (above and below 500 μg/L) concentrations of household wells. When adjusted only for each other, both As and Mn in whole blood (BAs; BMn) were significantly negatively related to most WISC-IV subscale scores. With further adjustment for socio-demographic features and ferritin, BMn remained significantly associated with reduced Perceptual Reasoning and Working Memory scores; associations for BAs, and for other subscales, were expectably negative, significantly for Verbal Comprehension. Urinary As (per gram creatinine) was significantly negatively associated with Verbal Comprehension scores, even with adjustment for BMn and other contributors. Mn by As interactions were not significant in adjusted or unadjusted models (all p's > 0.25). Findings are consistent with other reports documenting adverse impact of both As and Mn exposure on child developmental outcomes, although associations appear muted at these relatively low exposure levels.

1. Introduction

Recent studies of school-aged children have reported associations between neurobehavioral function and exposure to arsenic (As) via drinking water or industrial sources (Calderon et al., 2001; Rosado et al., 2007; Siripitayakunit et al., 1999; Tsai et al., 2003; von Ehrenstein et al., 2007; Wang et al., 2007). Similarly, a small but growing body of evidence suggests adverse effects of exposure to excessive levels of manganese (Mn), an essential mineral, on neurobehavioral functioning. Levels of Mn in hair (Riojas-Rodríguez et al., 2011) and drinking water (Bouchard et al., 2011) have been related to children's lower intelligence test scores. Recently, epidemiologic studies of developmental neurotoxicology have been challenged to increase focus on the role of co-exposure to multiple toxins (Bellinger, 2009). As an example, among Korean school children, while current levels of both blood lead (BPb) and blood Mn (BMn) were independently associated with lower intelligence scores, evidence of effect modification was also noted, with the BPb associations stronger among those with higher levels of BMn (Kim et al., 2009). In a small study relating hair levels of various metals to children's intelligence (Wright et al., 2006), both Mn and As hair levels were associated with poorer verbal learning and memory scores, with those above the median for both exposures scoring significantly lower in verbal memory.

We previously documented the adverse impact of chronic exposure to elevated levels of well-water As on 6- and 10 year old children's intellectual function, in Araihazar, Bangladesh (Wasserman et al., 2006a, 2004). In the same region, we also documented decrements in intellectual functioning in 10-year-

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doi:10.1016/j.neuro.2011.03.009

olds exposed to elevated levels of well-water Mn in a region where water As levels were extremely low (Wasserman et al., 2006b). That work, however, was not designed to examine possible interactive effects of simultaneous exposure to both As and Mn. Accordingly, in order to allow investigation of the possible synergistic impact of both exposures, we recruited a new sample of children from a neighboring region, stratified by design on As and Mn concentrations of household wells.

2. Materials and methods

2.1. Overview

Work reported here is a component of a large ongoing multi-disciplinary study by health, earth, and social scientists working collaboratively in Araihazar, Bangladesh. We have previously described the region and the larger cohort study of adults (Health Effects of Arsenic Longitudinal Study: HEALS), in which 12,000 adults were recruited in 2000–2002 and their household wells assessed for As and Mn concentrations (Ahsan et al., 2006). Subsets of children of those participants were the focus of earlier investigations (Wasserman et al., 2004, 2006a,b). From 07/06, to 08/08, 8287 additional adults residing in Araihazar were recruited into the HEALS cohort, and 6092 additional wells were assessed for As and Mn levels.

Reporting the results from well testing for As to households of our study area has resulted in a significant drop in As exposure (Chen et al., 2007). Further reductions have been achieved by installation of 50 deeper community wells that, in most cases, meet the WHO guideline for As (and in all cases the Bangladesh standard for As in drinking water of 50 μg/L) but not always the WHO guideline for Mn of 0.4 mg/L. The Mn content of these deeper wells, on the other hand, is typically much lower than the Mn content of shallow wells from which most of the population continues drinking. The status of deeper wells in Araihazar with respect to As and Mn is not unlike that of ~100,000 such wells installed throughout the country by the Bangladesh government and helped motivate design aspects of this study (van Geen et al., 2007).

As in most of rural Bangladesh, people in Araihazar live in houses with floors made from mud or cement, with roofs and walls constructed from concrete, tin or straw. Members of extended families live in clusters of individual houses (a 'bari'), surrounded by farmland. Each bari has one or more tube wells, usually owned by a senior family member. This region is not particularly poor by Bangladeshi standards. Following a survey of the well characteristics of HEALS cohort villages within commuting distance of our field clinic, we designated household wells into one of four groups: High As High Mn (HAs–HMn: As > 10 μg/L and Mn > 500 μg/L), High As Low Mn (HAs–LMn), Low As High Mn (LAs–HMn) and Low As Low Mn (LAs–LMn). We identified all children estimated to be between 8 and 11 years old and continued recruitment at random until approximately 75 were included in each well category group.

Prior to conducting this study, we secured approval from Institutional Review Boards at Columbia and in Bangladesh, and obtained written informed consent from parents, as well as child assent.

2.2. Participants

Between January and December 2008, field staff visited each household with a potentially eligible child, when we verified current address, the As and Mn status of the home's well water, and checked study eligibility. Household visits continued until approximately 75 children in each of four groups had been enrolled, resulting in visits to 772 households. Inclusion criteria restricted enrollment to children aged 8–11 years who attended school in an age-appropriate grade, who had no known physical disability or chronic illness, and who were not twins. We further restricted enrollment to children who did not share a home well with other child participants. Of 772 children whose families were visited for eligibility review, we were unable to locate 46 because the family had moved or no one was home at the time of the visit. Some children were ineligible because of chronic illness (e.g., tuberculosis or asthma), disability or multiple births ($n = 14$), or because their families had switched wells ($n = 30$) since the initial visit, or because we restricted recruitment to one child per well ($n = 51$). Forty children who attended school only irregularly were excluded. For 34 children, parents refused to participate in the study mainly because of the distance and time to visit the study field clinic. Some children were ineligible because their age could not be determined ($n = 21$), or because they were either younger ($n = 123$) or older ($n = 102$) than eligible ages. Altogether, 310 children (and their families) agreed to participate, 304 of whom (from 37 villages, and relying on 304 unique home wells) received the WISC-IV at our clinic. Of the 304 participants, one child was excluded from analyses because inspection of his hematological data indicated that he was suffering from a hemoglobinopathy. Urinary and well measures were available on all; blood measurements of BAs and BMn were obtained on 299 children, whose data contributed to analyses predicting children's intelligence.

2.3. Procedure

During home visits, once parental consent and child assent were obtained, the field team collected sociodemographic information, and appointments were made for mother and child to visit the field clinic. Field staff also recorded information on the home characteristics for seven items related to the child's rearing environment that we previously found to be related to children's intellectual function in Bangladesh (Wasserman et al., 2004). At the field clinic, a physical exam and a neurodevelopmental assessment were done; urine and blood samples were collected. Children received a small age-appropriate gift in appreciation for participation.

2.4. Measures

2.4.1. Markers of exposure

Well water: Information about mother's well use was collected when families were originally recruited into the HEALS cohort expansion (2006–2008). During recruitment for the current study, only families who had not switched wells were recruited. At recruitment, almost all ($n = 297$, 97.7%) mothers of children in the current sample reported drinking exclusively from the index well, and 78% had drunk from that well for 5 or more years.

Field sample collection and laboratory analysis procedures are described elsewhere in detail (Cheng et al., 2004; van Geen et al., 2005). To evaluate household wells, water samples were collected in 20-mL polyethylene scintillation vials. Following acidification in the laboratory with high-purity HCl for at least 24 h (van Geen et al., 2007), water samples were analysed by high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS). The analytical detection limit of the method is 0.1 μg/L; the standard deviation of a single measurement is estimated at 4 μg/L for concentrations $\leq$150 μg/L (van Geen et al., 2005). Mn concentrations were also determined by HR ICP-MS. The detection limit of the method for Mn is also 0.1 μg/L and its precision 2% (Cheng et al., 2004).

Urinary measurements: UAs concentrations were assayed by graphite furnace atomic absorption spectrophotometry (GFAA), using a Perkin–Elmer Analyst 600 system as described (Nixon et al., 1991). Our laboratory participates in a quality control program coordinated by the Quebec Toxicology Center (QTC), Quebec, Canada. During the course of this study, intraclass correlation coefficients between our laboratory's values and samples calibrated at the QTC laboratory were 0.99. UAs levels were also adjusted for UCr concentrations, which were analysed by a colorimetric method based on Jaffe's reaction (Heinegard and Tiderstrom, 1973).

ICP-MS blood measurements: Venous whole blood samples were analysed for BPb, BMn, BSe and BAs concentrations using a Perkin–Elmer Elan DRC II ICP-MS equipped with an AS 93+ auto sampler. ICP-MS-DRC methods for metals in whole blood were developed according to published procedures (Pruszkowski et al., 1998; Stroh, 1988), with modifications for blood sample preparation as suggested by the Laboratory for ICP-MS Comparison Program, Institut National de Sante Publique du Quebec. A 3 mL EDTA vacutainer of whole blood was thawed, thoroughly mixed, and then diluted 50 times with the following diluent: 1% HNO_3 + 0.2% Triton-X-100 + 0.5% NH_4OH + 1% Methanol. The sample was then centrifuged for 10 min at 3500 rpm, and the supernatant used for analysis. One multi-element standard solution was used for instrument calibration. The metal concentrations of that solution were chosen to cover the expected ranges of analyte concentrations in the blood samples: 5, 25 and 125 μg/L (for Pb, Mn, and As) and 10, 50, 250 μg/L for Se. Special attention was given to correction for matrix-induced interferences. Matrix suppression is compensated very well by the selection of suitable internal standards (IS), which are matched to masses and, if possible, to ionization properties of the analytes. For As we used iridium (Ir); for Se we used rhodium (Rh); for Pb and Mn we used lutetium (Lu) and gallium (Ga), respectively. A stock IS spiking solution containing 25 ng of each IS was delivered to each tube. Polyatomic interferences were suppressed with the instrument's Dynamic Reaction Cell (DRC) technology feature, utilizing oxygen as a second gas for As and Se. After calibrating the instrument, and after every ten samples, we ran quality control samples, i.e., blood samples with known analyte concentrations obtained from the Laboratory for ICP-MS Comparison Program in Quebec (see above).

Quality-control blood samples were purchased to cover the range of concentrations of analytes of interest and were run during the course of each day. During the time period of these analyses, the intraprecision coefficients of variation for BPb, BMn, BSe and BAs were 1.9, 3.1, 2.6, and 3.3%, respectively, and the interprecision coefficients were 3.3%, 6.3%, 5.3% and 7.7%, respectively. We also participate in the Quebec Multi-Elements External Quality Assessment Scheme (QMEQAS) run by the aforementioned Quebec laboratory. Three times yearly, that lab sends blood urine and serum samples with known concentrations of 23 elements. Reported concentrations for BPb, BMn, BSe and BAs were well within the expected target ranges. Venous blood samples were used for measuring hemoglobin (Hgb) and serum ferritin (SF: (Miles et al., 1974).

2.4.2. Outcomes

The Wechsler Intelligence Scale for Children-Fourth Edition (WISC-IV: (Wechsler, 2003)) is an individually administered assessment of intellectual function, suitable for children 6 to 16 years old. This revised version of the WISC-III (Wechsler, 1991) has excellent psychometrics, and provides measures of general intellectual ability (Full Scale IQ) and specific cognitive domains (Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed Indices). As discussed in our earlier work (Wasserman et al., 2004, 2006a,b), neither the WISC nor any other recently well-standardized child IQ test has been adapted or standardized for use in Bangladesh, and not all subtests are appropriate for children there. We derived a battery of the following subtests (listed with their respective Composites): Similarities, Comprehension, and Information (Verbal Comprehension); Block Design, Matrix Reasoning, and Picture Completion (Perceptual Reasoning); Digit Span and Letter-Number Sequencing (Working Memory); Coding and Symbol Search (Processing Speed).

As in our earlier work, WISC-IV materials were translated into Bengali and back-translated into English, with the incorporation of culturally appropriate adaptations. As examples, in the Similarities subtest, we substituted "Bamboo and bricks" for "Lumber and bricks"; in Picture Concepts, we did not administer items presenting objects, such as a wading pool or an ice cube tray, that would be unfamiliar to Bangladeshi children; we did not administer Comprehension items referring to seatbelts or libraries, as villagers rarely ride in automobiles, and there are no local public libraries; in Information, we did not ask about Christopher Columbus, and asked children to identify the *six* seasons of the year; in Picture Completion, we did not administer the bathtub item, and substituted a goat for the picture of a pig. Scores for Verbal Comprehension, Perceptual Reasoning, Working Memory and Processing Speed were added to generate a measure of Full Scale intelligence. Assessments were administered by two college-graduate assessors, who had also performed this role in our previous studies [12–14] and who were blind to families' exposure characteristics.

2.4.3. Socio-demographic measures

Maternal intelligence was measured on the Wechsler Abbreviated Scale of Intelligence (WASI: (The Psychological Corporation, 1999)), a short and reliable measure of intelligence, across the age span. It consists of two performance subtests (Block Design and Matrix Reasoning) and two Verbal (Vocabulary and Similarities) subtests. As is the case for the WISC-IV, not all subtests are appropriate in the Bangladesh context. Pilot testing had revealed that many mothers without education were unable to master the abstract nature of the Similarities and Block Design subscales. Our battery included Vocabulary and Matrix Reasoning subtests; scores for these subtests were combined by adding them together, generating a measure that reflected both Verbal and Performance skills. Some slight changes were made to the Vocabulary subscale to reflect local conditions: a vocabulary item for "flashlight" was changed to "hurricane [lamp]"; another vocabulary item ("cart") was eliminated. Maternal education and IQ scores were substantially correlated ($r = 0.63$), providing support for validity. As was described for the WISC-IV, above, the lack of standardized norms for Bangladesh required that we employ raw scores (sum of items passed) as a marker of intelligence, rather than standardized scores or "IQ".

Additional sociodemographic characteristics were assessed during a structured interview with a parent during the home visit. The interview inquires about maternal and paternal age, ethnicity, education and occupation, maternal and paternal lifestyle variables (smoking, alcohol use) both currently and while the mother was pregnant with the child, and characteristics and outcomes of that pregnancy, i.e. complications (e.g. hypertension, diabetes), birthweight, gestational length, complications of delivery, and health status of the child at delivery (e.g. congenital defects). School attendance was estimated by asking mothers for how many months the child had attended school. Height, weight and head circumference were measured at the clinic visit after the WISC-IV.

Home environment: Characteristics of the home environment commonly contribute to child intelligence, often measure via the HOME observation (Caldwell and Bradley, 1984, 2001). Because many items in this interview/observation, including availability of a library card, are very rare in the Bangladeshi setting, we administered a limited set of similar-type items that asked about materials and opportunities available to the child at home. We developed a scale comprised of the sum of 6 items (with a Cronbach's alpha of 0.61), including the presence of a clock, displayed artwork, availability of a watch in the family, availability of any age-appropriate toy, a wall calendar, and trips away from the region within the past six months.

2.5. Translation and training

As noted, all tests and interviews were translated (and back-translated) between Bangla (Bengali) and English. Materials were piloted to ensure maternal and child comprehension. Subsequently, three testers were trained (by GW) and continued with supervised practice sessions for two weeks. All written test responses were rechecked when data were sent to Columbia for entry and analysis.

2.6. Statistical analyses

We first examined differences in outcomes, exposure and potential covariates across the four groups of well-water exposures, and used Chi-square and Kruskal–Wallis tests to detect group differences in categorical and quantitative variables. Analyses next examined associations between blood measures of exposure to arsenic (BAs) and manganese (BMn) and their interaction and each of the five measures of intellectual function (Verbal Comprehension, Working Memory, Performance, Processing Speed, Perceptual Reasoning and Full Scale raw scores). These analyses first adjusted only for the other element of interest, and then adjusted further for sociodemographic and maternal factors, using linear regression. From the pool of potential covariates we selected those associated with WISC outcomes ($p < 0.10$); linear models were further confined to those remaining significant ($p < 0.05$) in regression analysis for one or another WISC-IV outcome. Because iron and manganese are chemically similar and share many aspects of their metabolism and transport (Fitsanakis et al., 2010), we also controlled for plasma ferritin, a marker of iron status, using two indicator variables, one for higher ferritin (>median of 32.5) and one to denote missing values. Final models were adjusted for head circumference, maternal age and intelligence and school attendance, as well as for ferritin; all contributors, except for ferritin, were considered as continuous measures. Next, analyses were repeated, with measures of WAs and WMn replacing the blood measures, and then substituting urinary As (UAs) for BAs, with and without further adjustment for creatinine. In order to make distributions more symmetrical and to reduce any potential impact of extreme values in regression analyses, As and Mn measures were log-transformed. To test for interactions between exposure variables, regression analyses included a term reflecting the product of the corresponding As and Mn variables.

3. Results

3.1. Sample characteristics

The 310 participating families did not differ significantly from non-participants (eligible but refused) in mother's age, her years of schooling, or the number of her living children (data not shown). Nor did they differ with regard to land or television ownership, the number of years they had relied on their designated family well, or the home well As concentrations. However, families who refused participation had home wells with significantly lower Mn concentrations [530.3 μg/L vs. 719.8 μg/L, $t_{(96)} = 2.46$, $p < 0.001$].

WAs and WMn were somewhat correlated ($r = 0.13$, $p < 0.03$, $n = 303$): only two children drank from wells with very high levels of both exposures (WAs > 150 μg/L and WMn >1000 (data not shown). Table 1 presents data on exposures and potential covariates across the four exposure groups. There were no differences across groups in demographic, anthropometric or social characteristics, except that mothers of children in the two Low Mn groups had more years of education than those in other groups [Kruskal–Wallis $\chi^2_{(3)} = 8.12$, $p < 0.05$]; hemoglobin was significantly higher in children in the Low As/Low Mn group [Kruskal–Wallis $\chi^2_{(3)} = 8.17$, $p < 0.0001$]. As expected, measures of water As and Mn tracked group membership [for WAs and WMn,

Table 1
Sample, exposure and outcome characteristics.

	Overall $N = 303$	Low As–Low Mn $n = 77$	Low As–High Mn $n = 74$	High As–Low Mn $n = 73$	High As–High Mn $n = 79$
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Child age (years)	9.64 (0.77)	9.52 (0.80)	9.67 (0.73)	9.80 (0.75)	9.56 (0.80)
Male [% (n)]	49.83 (151)	61.04 (47)	48.65 (36)	43.84 (32)	45.57 (36)
Months attending school	42.03 (16.27)	43.36 (18.52)	42.40 (14.87)	42.58 (14.05)	39.87 (17.18)
BMI	14.06 (1.32)	13.94 (1.03)	14.16 (1.42)	13.96 (1.17)	14.17 (1.59)
Head circumference (cm)	49.37 (1.42)	49.32 (1.27)	49.38 (1.45)	49.08 (1.32)	49.71 (1.75)
Mother's age (years)	35.30 (6.02)	34.55 (5.48)	35.95 (5.96)	35.08 (6.32)	35.63 (6.30)
Mother's education (years)	3.74 (3.69)	4.17 (3.69)	3.05 (3.44)	4.33 (3.69)	3.39 (3.80)
Mother's WASI Raw Score	31.00 (10.60)	32.05 (9.73)	30.27 (10.66)	31.51 (10.83)	30.20 (11.20)
Home stimulation	3.48 (1.67)	3.75 (1.77)	3.39 (1.72)	3.16 (1.57)	3.61 (1.56)
Water As (μg/L)	43.32 (73.65)	2.39 (2.32)	3.33 (2.71)	97.33 (108.89)	70.74 (58.16)
Water Mn (μg/L)	725.54 (730.04)	202.14 (145.49)	1111.19 (686.16)	184.06 (146.19)	1367.13(692.61)
Urine As (μg/L)	78.09 (72.16)	46.70 (35.81)	52.30 (38.78)	106.01 (93.01)	107.05 (79.61)
Urine Creatinine (mg/dL)	35.00 (24.16)	35.01 (25.06)	33.79 (24.40)	36.81 (24.95)	34.46 (22.61)
Urine As (mg/g creatinine)	246.54 (183.93)	149.59 (74.40)	173.37 (111.75)	330.51 (247.41)	332.0 (170.42)
Blood As (μg/L)	4.81 (3.22)	3.20 (1.73)	3.42 (1.75)	6.27 (4.21)	6.34 (3.04)
Blood Mn (μg/L)	14.78 (3.72)	14.58 (3.86)	15.49 (4.15)	14.38 (3.52)	14.69 (3.28)
Blood Pb (μg/L)	114.56 (37.22)	123.93 (38.43)	113.94 (42.49)	100.97 (30.59)	118.55 (33.12)
Blood Se (μg/L)	104.97 (17.23)	106.46 (16.29)	103.64 (15.58)	106.77 (18.06)	103.09 (18.76)
Hemoglobin (g/dL)	12.46 (0.99)	12.79 (1.15)	12.22 (0.83)	12.33 (1.01)	12.47 (0.88)
Serum ferritin (ng/mL)	33.43 (17.89)	31.59 (16.88)	32.19 (17.50)	36.93 (18.15)	33.15 (18.83)
WISC-4 Raw Scores					
Verbal Comprehension	23.80 (8.77)	24.56 (8.32)	22.93 (7.91)	23.82 (8.41)	23.85 (10.26)
Perceptual Reasoning	31.24 (11.24)	30.21 (10.61)	30.69 (10.16)	32.86 (10.53)	31.25 (13.30)
Working Memory	20.25 (6.06)	20.05 (5.79)	20.28 (6.01)	20.42 (6.87)	20.24 (5.66)
Processing Speed	33.21 (12.48)	32.57 (12.12)	33.82 (11.35)	33.63 (13.46)	32.87 (13.07)
Full Scale	108.50 (31.32)	107.39 (30.90)	107.73 (27.34)	110.74 (32.51)	108.22 (34.45)

respectively, $\chi^2_{(3)} = 229.51$ and 229.06, p's < 0.0001], as did measures of UAs and UAs/g creatinine [$\chi^2_{(3)} = 60.83$ and 86.01, p's < 0.0001, respectively]. Measures of BAs also tracked group membership [$\chi^2_{(3)} = 80.23$ and 122.40, p's < 0.0001]. In contrast, BMn did not vary predictably across the Low and High WMn groups, suggesting that it may not be a good biomarker of WMn exposure. Median values (and ranges) for WAs and WMn, respectively, were 10.37 (0.1–464 μg/L) and 527.249 μg/L (40–3442.45 μg/L). For reasons that are unclear, BPb was higher in the LAs/LMn group and lower in the HAs/LMn group [$\chi^2_{(3)} = 16.46$, $p < 0.001$]. Table 1 also presents group differences in children's intelligence scores. Before covariate adjustment, we observed no significant differences in mean scores for any subtest across exposure groups.

3.2. Associations between markers of exposure and intellectual function

Table 2 presents results of analyses predicting children's intellectual function from BAs and BMn before and after adjustment for sociodemographic measures; in each case, analyses adjust for one exposure while examining the other. The distributions of some outcomes were skewed; the removal of extreme values resulted in reduced skew so that the previously marginal BMn/Full Scale association achieved significance with removal of two outliers (data not shown). In each instance, unlike sociodemographic contributors, exposure made relatively small contributions to children's scores. When adjusted only for each other, both BAs and BMn were negatively related to all measures of children's intellectual function, significantly so for Full Scale and Working Memory scores; BAs was significantly related to Verbal Comprehension scores, and BMn to Perceptual Reasoning scores. Together BAs and BMn explained less than 5% of the variance in children's scores.

When sociodemographic and other factors were added to the model, they made consistent contributions to intellectual function (Table 2). As expected, children who had attended more months of school, and those whose mothers scored higher on a test of intelligence, achieved significantly higher scores in all domains (all p's < 0.05). Maternal Age was significantly and positively related to children's Verbal Comprehension. Further, children with larger head circumferences achieved higher Full Scale, Working Memory, and Perceptual Reasoning scores. Children whose plasma ferritin levels were above 32.5 ng/mL received significantly higher Full Scale, Perceptual Reasoning and Processing Speed scores.

With adjustment, the explained variances for all measures increased expectably. Altogether, these features explained 48%, 31%, 36%, 31% and 36% of the respective variances in Full Scale, Verbal Comprehension, Working Memory, Perceptual Reasoning and Processing Speed scores. The strength of the associations with BAs and BMn were cut approximately in half, so that significance in many instances was reduced. Negative associations between BMn and both Working Memory and Perceptual Reasoning remained significant even with full adjustment. To offer some estimation of the magnitude of these associations, with estimated regression coefficient for exposure in the models adjusting for covariates, we derived the decrement of test scores for exposure increasing from lowest to higher quartiles, first based on BAs and then on BMn. Comparing the lowest and highest BAs quartiles, Full Scale IQ (adjusted for other contributors) decreased by 6.19 raw points, while Verbal Comprehension decreased by 2.39 points and Working Memory scores decreased by 1.46 points. Comparing the lowest and highest BMn quartiles, Full Scale IQ (adjusted for other contributors) decreased by 9.71 raw points, while Perceptual Reasoning scores decreased by 4.89 points and Working Memory scores decreased by 2.57 points.

In the models with Mn by As interaction, the estimated regression coefficients for main effects of Mn and As were negative, while mostly positive for interaction. The Mn by As interactions were not statistically significant in either covariate-adjusted or unadjusted models; all p's were > 0.26. BPb at these low levels made no significant contribution to child outcomes in any model examined.

When we substituted UAs for BAs, UAs was unrelated to test scores. In a model that also included BMn and urinary creatinine, UAs was significantly and negatively associated with Full Scale, Verbal Comprehension and Working Memory, but with further control for other covariates, the effect of UAs was attenuated. Neither WAs nor WMn made significant contributions to children's scores, before or after adjustment for other covariates.

We considered the possibility that adjustment for mothers' intelligence might represent statistical overcontrol, since mothers utilized the same wells as their children. However, neither WAs nor WMn made significant contributions to mothers' WASI scores, indicating that associations between maternal and child intelligence were independent of exposure.

Table 2
Associations between WISC-4 raw scores and measures of arsenic and manganese exposure, before and after adjustment ($N = 299$).

	Full Scale b (se)	Verbal Comprehension b (se)	Working Memory b (se)	Perceptual Reasoning b (se)	Processing Speed b (se)
Adjusted only for the other metal					
Blood As	−6.11 (2.93)*	−1.98 (0.82)*	−1.35 (0.57)*	−1.60 (1.05)	−1.18 (1.20)
Blood Mn	−18.52 (7.44)*	−3.77 (2.10)+	−3.84 (1.44)**	−7.68 (2.67)**	−3.23 (3.04)
R^2	4.0%	3.4%	4.7%	3.9%	0.8%
Adjusted for other contributors					
Maternal intelligence	0.59 (0.14)****	0.17 (0.04)***	0.15 (0.03)****	0.15 (0.06)**	0.13 (0.06)*
Maternal age	0.07 (0.23)	0.16 (0.07)*	−0.02 (0.05)	0.04 (0.09)	−0.11 (0.10)
School months	1.01 (0.09)****	0.21 (0.03)****	0.14 (0.02)****	0.24 (0.04)****	0.42(0.04)****
Head circumference	3.28 (0.96)***	0.73 (0.31)*	0.64 (0.21)**	1.62 (0.40)****	0.31(0.43)
Plasma ferritin High (>32.5) vs. low	5.55 (2.76)*	1.39 (0.89)	0.38 (0.60)	2.44 (1.14)*	1.33 (1.24)*
Not available vs. low	6.05 (6.85)	1.02 (2.21)	0.66 (1.48)	−0.19 (2.84)	4.55 (3.07)
Blood As	−3.80 (2.20)+	−1.49 (0.71)*	−0.91(0.47)+	−1.13 (0.91)	−0.27 (0.99)
Blood Mn	−9.69 (5.65)+	−1.69 (1.82)	−2.56 (1.22)*	−4.88 (2.34)*	−0.56 (2.53)
R^2	47.9%	31.2%	35.8%	30.8%	35.5%

Note: values for Blood As and Blood Mn have been log-transformed.
+ $0.05 < p < 0.09$
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.
**** $p < 0.0001$.

4. Discussion

In this cross-sectional study of children selected for their household well water As and Mn characteristics, we found significant decrements in a range of components of intellectual skill that were associated with exposure to both As and Mn. On the other hand, after adjustment for maternal age and intelligence, and for child head circumference, school attendance and plasma ferritin, most associations with exposure were attenuated. However, significant negative associations between BMn and both Perceptual Reasoning and Working Memory remained following adjustment. Associations between BAs and Verbal Comprehension remained significant (and for Working Memory, nearly significant), and in the expected direction, following adjustment. With or without correction for creatinine levels, and adjusting for other contributors, UAs remained negatively associated with all subtest scores (significantly so for Verbal Comprehension). While in the expected direction, associations between well-water As or Mn, and children's test scores were not statistically significant. While we found no indication of As by Mn interactive associations with child intellectual function, the fact that only two children drank from wells extremely high in both exposures may partially explain our inability to detect an interactive effect of As and Mn on child intelligence.

4.1. Arsenic exposure and children's intellectual function

In our earlier work (Wasserman et al., 2004), with similarly aged children (using the WISC-III), and with higher As exposure, we found significant associations between well-water As levels and children's scores, that persisted after adjustment for socio-demographic features, as well as for WMn. Children drinking from wells with WAs > 50 μg/L scored significantly worse than children whose home wells had WAs levels < 5 μg/L. Similar associations were noted for creatinine-adjusted UAs. In a second study of six-year-olds from the same area (Wasserman et al., 2006a), WAs was significantly related to WISC-III Performance and Processing Speed scores, even after adjustment for social features and WMn. In a neighboring region in West Bengal (von Ehrenstein et al., 2007), and in two studies of children residing near metallurgic complexes in Mexico (Calderon et al., 2001; Rosado et al., 2007), UAs concentration was negatively related to some subtests of earlier versions of the WISC. One of these found no association with WAs (von Ehrenstein et al., 2007). The range of UAs in the present investigation, while markedly lower than in our earlier work, was similar to that reported in these three other child studies.

Importantly, while each adjusted for some family social characteristics, none considered maternal intelligence. We document in the present study a contribution of maternal intelligence to children's intelligence that is more substantial than contributions of other social markers, such as measures of parental education or occupation. While our earlier work estimated maternal intelligence on the Raven's Progressive Matrices (Raven et al., 1983), here we made use of a more recently developed test that closely parallels the tests administered to children. Employing a more sound and updated measure of maternal intelligence may have allowed for more accurate characterization of impact of environmental exposures on children; across subtests, correlations between mothers' and children's intelligence scores ranged from 0.22 to 0.37.

Here, even with rigorous adjustment for other contributors, UAs/Cr remained significantly associated with Verbal Comprehension scores. None of the three other studies of children adjusted for creatinine. Among these recent investigations, only one (Calderon et al., 2001) adjusted for Pb exposure (though this would have been important in both Mexican sites) and the West Bengal study did not examine Mn, a likely co-exposure.

4.2. Manganese exposure and children's intellectual function

The present investigation found significant (or near significant) adverse impacts of a variety of markers of Mn exposure on perceptual reasoning and memory skills, consistent with studies of both occupational and animal exposures (Burton and Guilarte, 2009). Studies of inhalation exposure to manganese in occupational settings consistently report adverse impact on motor functioning (Agency for Toxic Substances and Disease Registry, 2000; Cook et al., 1974; Roels et al., 1999). Among heavily exposed adult workers, occupational manganese exposure has a progressive course, from non-specific symptoms such as weakness, somnolence and headaches, to (after prolonged elevations) memory loss and motor disturbances such as gait, postural and steadiness abnormalities (Cowan et al., 2009). Studies of Mn exposure (see (Burton and Guilarte, 2009) for a review of animal studies) point to its effects on dopaminergic systems within the prefrontal cortex and its connections (Guilarte et al., 2008) that impact on executive function and working memory (Lucchini et al., 1995).

A small but growing body of evidence regarding children's exposure suggests adverse effects of Mn on neurobehavioral functioning. For example, among Parisian children followed from birth through their preschool years (Takser et al., 2003), after adjustment for sex and maternal education, cord BMn levels were associated with poorer functioning in attention, non-verbal memory, and hand skill. Among 11–13 year olds, a comparison (unadjusted for social features) of those from an area with high levels of Mn sewage irrigation with those from a control area revealed lower scores on tests of short-term memory, manual dexterity, and visuoperceptive speed in exposed children ((He et al., 1994), cited in Mergler (1999). In a small ($n = 92$) and unadjusted comparison of school-aged children in two Chinese villages with and without high levels of Mn in drinking water, those with higher exposure had significantly lower serum levels of dopamine (Zhang et al., 1995), and significantly lower scores in tests of memory and dexterity (Peng et al., 1994). In a recent study of children (Bouchard et al., 2011), WMn associations were generally stronger and more consistent for performance, rather than verbal, intelligence. Others have noted Mn associations with verbal, rather than performance, aspects of child intelligence (Kim et al., 2009; Menezes-Filho et al., 2011). In our earlier work with similarly aged Bangladeshi children (Wasserman et al., 2006b) where exposure to WAs was extremely low (by design), we observed decrements in both verbal and performance intelligence on an earlier version of the test used here (Wechsler, 1991) that were dose-related to WMn. While studies differ in the test (or version of a test) and biomarker(s) used, support appears most consistent for impact of Mn exposure on performance-related aspects of intelligence.

Some of the present findings stand in contrast to our earlier work. Most importantly, by design, half of the study participants utilized wells with As levels <10 μg/L, and half utilized wells with Mn < 500 μg/L. These cutoffs were selected to reflect the WHO guidelines (WHO, 2011), and to provide policy-relevant results. This resulted in substantially lower levels of exposure to both elements in the current sample (overall 43 μg/L and 725 μg/L for WAs and WMn, respectively, compared to earlier levels of 118 μg/L and 795 (Wasserman et al., 2004, 2006b). Beyond this design-driven aspect, well water As and Mn levels were somewhat correlated ($r = 0.13$, $p < 0.03$) here, so that As levels even in our High As–Low Mn group (97 μg/L) were lower than previously studied. The lower levels of exposure in the current study may have limited our capacity to detect significant interactions between Mn and As on children's test scores.

Mn-related associations with child intelligence have been noted in samples where WMn is considerably lower (e.g., (Bouchard et al., 2011), where mean WMn = 98 μg/L). Although WMn levels in the current sample were somewhat lower than in our earlier work, BMn levels here were slightly higher than before (14.8 μg/L compared to earlier levels of 12.8 μg/L). BMn levels in both Bangladesh samples are higher than those reported for children in mining communities (Riojas-Rodríguez et al., 2011; Menezes-Filho et al., 2011), where exposure presumably occurs via inhalation, perhaps offering a more direct route to brain regions than occurs with ingestion (Brenneman et al., 2000).

4.3. Biomarkers of Mn exposure

In our earlier work (Wasserman et al., 2006b), when levels of WAs were extremely low by design, WMn related to child intelligence while BMn did not. In the current sample, BMn, rather than WMn, is associated with child intelligence. A recent paper (Bouchard et al., 2011) reports associations between child IQ and Mn measured in hair and in water (with stronger associations for HMn); BMn was not assessed. Two other recent studies (Riojas-Rodríguez et al., 2011; Menezes-Filho et al., 2011) report associations with child intelligence for HMn (significant only for girls in (Riojas-Rodríguez et al., 2011)), but non-significant associations for BMn. Studies of adults, even community studies of environmental exposure (e.g., (Mergler et al., 1999)) have reported associations between BMn and a range of cognitive functions. BMn may not reliably stand in for the total body burden of Mn (Bouchard et al., 2011), since it seems to reflect recent exposure. Other factors such as duration, frequency, magnitude and latency of Mn exposure can also potentially impact BMn levels (Smith et al., 2007); exposure in our Bangladesh cohort is relatively constant. These discrepancies suggest that defining the optimal biomarker for Mn exposure remains an open question, although future studies should consider adding HMn as a marker.

5. Conclusions

In summary, we conducted a study of 8–11 year old children, half of whom consumed water with As concentrations below WHO health guideline levels, and close to half of whom relied on household wells below the WHO water guideline levels. When adjusted only for each other, BAs and BMn were significantly associated with poorer scores on most WISC-IV subscales. After adjustment for covariates, BMn remained significantly associated with lower Perceptual Reasoning and Working Memory scores. In fully adjusted models, BAs and UAs/gCr seemed to reduce WISC-IV scores, although most associations did not reach a statistical significance of 0.05. Overall, findings are consistent with other reports documenting adverse impact of both As and Mn exposure on child developmental outcomes.

Conflicts of interest

None.

Acknowledgements

This work was supported by National Institute of Environmental Health Sciences grants P42 ES 10349 and P30 ES 09089.

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