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NeuroToxicology 27 (2006) 210–216
NeuroToxicology

Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site

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Received 7 July 2005; accepted 12 October 2005
Available online 28 November 2005

Abstract

A pilot study was conducted to explore the potential associations between hair metal levels and the neuropsychological function and behavior of school-aged children. Thirty-two children, 11–13 years old, were administered a battery of tests that assessed general intelligence, visual-motor skills, receptive language, verbal memory, nonverbal problem-solving, and behavior problems. Parents and teachers rated the children's attention, executive functions, and behavior problems. The concentrations of manganese (Mn), arsenic (As), and cadmium (Cd) were measured in hair samples provided by 31 of the children. The mean hair metal levels were: Mn, 471.5 parts per billion (ppb); As, 17.8 ppb; Cd, 57.7 ppb. Children's general intelligence scores, particularly verbal IQ scores, were significantly related, inversely, to hair Mn and As levels, as were scores on tests of memory for stories and a word list. In some cases, a significant Mn-by-As interaction was found. It appeared that it was the low scores of children for whom both Mn and As levels were above the median values in the sample that were responsible for the main effects observed for each metal. No other significant relationships were found. These results suggest the need to study further the neuropsychological correlates of developmental exposure to Mn and As, particularly as a mixture.

Keywords: Children; Neuropsychology; Behavior; Manganese; Arsenic; Cadmium; Metal mixtures

1. Introduction

Many studies indicate that children exposed, at low levels, to metals such as lead (Pb) and mercury present signs of neurotoxicity, including reduced performance on neuropsychological tests and altered behavior (Bellinger and Adams, 2001). The extent to which low-level exposures to other metals, such as arsenic (As), manganese (Mn), and cadmium (Cd), are also neurotoxic is considerably less certain.

As has generally been considered to be a peripheral neurotoxicant, producing a clinical picture of severe polyneuropathy after acute poisoning. Recent mechanistic and neurobehavioral studies in animal models (Rao and Avani, 2004; Chaudhuri et al., 1999) and in humans (Calderon et al., 2001; Tsai et al., 2003; Wasserman et al., in press) suggest that this neurotoxicity includes the central nervous system as well. Mn, a transition metal, is both a nutrient and toxicant. Occupational exposures to Mn produce a clinical syndrome of memory loss, behavioral/mood changes and, in its final stages, a Parkinsonian-like motor dysfunction. Some studies suggest that Mn is a neurodevelopmental toxicant at environmental levels of exposure (Takser et al., 2003; He et al., 1994; Crinella et al., 1998). The central nervous system has generally not been considered to be an important target organ for Cd, although neurotoxicity has been reported in both adults and children (Bellinger et al., 2003).

In this cross-sectional pilot study, we evaluated the associations between the hair levels of As, Mn, and Cd and

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0161-813X/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2005.10.001

neurospsychological function and behavior in school-aged children. The study was conducted among children residing in Ottawa County, an area of northeast Oklahoma with a 100-year history of Pb and zinc (Zn) mining. Part of this region, called the Tar Creek Superfund site, was first listed on the U.S. Environmental Protection Agency's National Priorities List in 1983 and remains on the NPL (EPA site ID 0601269 (http://www.epa.gov/superfund/sites/npl/ok/htm). More than 75 million tonnes of mining waste (referred to as ''chat'') was left on the surface in piles reaching as much as 200 ft in height. Chat contains metals other than Pb and Zn, including Cd and Mn. Mine tailings also contaminate more than 800 acres of flotation ponds in the Tar Creek region. Although As is not found in chat at levels above background environmental concentrations, Oklahoma is known to have high background levels of As. Due to community concerns about As, we included this metal in our study.

2. Methods

2.1. Study sample

In order to minimize a volunteer bias that might have resulted from community-wide requests for participants, the study sample was recruited from two science classes in the Miami, OK, school system. A letter describing the study was sent home with each child, along with a postcard for parents to return indicating interest in being contacted about participating. Positive responses were received from the parents of 32 of 80 children (40%), and appointments with the examiner (DCB) were scheduled. The examiner made five visits to Miami in 2004 to conduct the evaluations. Each child was given $50 for participating. This study was approved by the Committee on Clinical Investigation of Children's Hospital Boston. Each child provided assent, and a parent or guardian provided consent. In addition, a Certificate of Confidentiality was obtained from the National Institutes of Health.

Table 1 presents the demographic characteristics of the study sample. Children ranged in age from 11 years 7 months to 13 years 8 months. Almost one-third of the parents self-identified themselves as American Indian. Fewer than half of the children were living with both biological parents, although 85% were living with at least one biological parent. Approximately 56% of the women and 40% of the men serving in a guardianship role had attended or graduated from college.

Table 1
Characteristics of study participants

Age in months (mean, S.D., range)	151.5, 6.8, 139–164
Sex	47% male
Ethnicity	31% American Indian 69% White, non-Hispanic
Family structure	44% two parent household: both biological parents 25% two parent household: one biological and one step/adoptive parent 16% one parent household: biological parent
Maternal education	22% less than high school 22% high school graduate 19% some college 37% college graduate or graduate degree
Paternal education	10% less than high school 50% high school graduate 23% some college 17% college graduate or graduate degree
Number of children <18 years in the home (mean, S.D.)	1.9, 1.3
Hair metals (ppb)	
Arsenic	17.8, 14.1, 1.4–55.4
Manganese	471.5, 455.5, 89.1–2145.3
Cadmium	57.7, 57.7, 16.4–293.1
Lead	1680, 5178, 137–29,343

2.2. Data collection

2.2.1. Neuropsychological evaluation

Each child was administered a 2-h battery of neuropsychological tests in a private room at the public library or in a small conference room at the local hospital. Because of limited knowledge about potential neuropsychological correlates of children's exposures to the metals of interest, the battery was broad-based *and included assessments of most major domains*. The battery included the following instruments: Wechsler Abbreviated Intelligence Scale (WASI) (The Psychological Corporation, 1999), Wide Range Assessment of Visual Motor Ability (WRAVMA) (Adams and Sheslow, 1995), the 3 receptive scales of the Clinical Evaluation of Language Fundamentals-Third Edition (CELF-3) (Semel et al., 1995), the Children's Category Test-Level II (CCT) (Boll, 1993), the California Verbal Learning Test-Children (CVLT-c) (Delis et al., 1994), and the story memory subtest of the Wide Range Assessment of Memory and Learning (WRAML) (Sheslow and Adams, 1990). Children also completed two self-report instruments, the Children's Depression Inventory (CDI) (Kovacs, 2001), and the Behavior Assessment System for Children (BASC) (Reynolds and Kamphaus, 1992). Both parents and the teacher completed the appropriate versions of the CADS-IV (Connors, 1997, 1999), a questionnaire that assesses attention, and the Behavior Rating Inventory of Executive Functions (BRIEF) (Gioia et al., 2000). The children's teacher also completed the BASC.

Parents also completed a brief questionnaire that elicited information about ethnicity, primary language spoken in the home, family structure, maternal and paternal education, the child's medical history (conditions, hospitalizations, medications), and the child's educational history (grade retention, receipt of remedial services).

2.2.2. Hair sampling and analysis

Before the neuropsychological evaluation, a hair sample was collected from each child. Approximately 30–40 strands were taken from the occiput. If necessary to obtain a sufficient

sample, additional strands were taken from other areas of the scalp. A hair sample was not collected if a child had gel in his or her hair, and arrangements were made to collect the hair sample at a later date when the gel had been removed by shampooing. All of the children denied recent use of hair dye. Because the hair sample obtained from one child was insufficient for analysis, the sample size of children with both hair metal levels and neuropsychological outcome data is 31.

Hair samples were cleaned by sonicating them for 15 min in ~10 ml of 1% Triton X-100 solution in a 50-ml beaker, rinsing them several times with distilled deionized water, and drying them in a drying oven at 70 °C for 24 h. Samples were placed in a 15-ml plastic tube and digested with 1 ml of concentrated HNO_3 acid for 24 h and then diluted to 10 ml with deionized water.

Acid digested samples were analyzed by inductively coupled plasma-mass spectrometry (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwark, CT). Analyses of Cd, Mn, and As were performed using an external calibration method, using indium as the internal standard for Cd and Mn, and tellurium as the internal standard for As. In addition, Pb was also measured, using thalium (Tl) as the internal standard.

Quality control measures included analysis of initial calibration verification standard, National Institute of Standards and Technology Standard Reference Material (NISTSRM) 1643e (trace elements in water), a solution of 5 ng ml mixed element standard solution of Pb, Cd, Mn, and As solution (NIST traceable), continuous calibration verification standards, procedural blank, Certified Reference Material (CRM) GBW 09101 (Human Hair, Shanghai Institute of Nuclear Research, Academia Sinica, China). Results were given as the average of five replicate measurements. Recovery of the analysis of QC standard by this procedure is 90–110% and <5% precision.

3. Results

3.1. Hair metal levels

Table 1 shows the hair metals levels of the 31 children. The correlations among the three metals measured were substantial (As and Mn: 0.65, $p = 0.0001$; As and Cd: 0.47; $p = 0.008$; Mn and Cd: 0.64; $p = 0.0001$). Arsenic levels were significantly lower in girls than boys (mean (S.D.) = 25.9 (4.0) parts per billion versus 11.0 (2.3) parts per billion, respectively; $p = 0.002$). Neither Mn nor Cd hair levels were related significantly to sex. Hair metals levels of American Indian and non-American Indian children did not differ significantly.

3.2. Intelligence

In the cohort as a whole, the mean Full-Scale IQ = was 94.5 (S.D. 12.6, range 74–119); mean Verbal IQ was 94.2 (S.D. 13.5, range 69–116); and mean Performance IQ was 96.0 (S.D. 13.4, range 77–131). In crude analyses, hair As was inversely associated with Full-Scale IQ (coefficient −0.37, p = 0.022) and Verbal IQ (coefficient −0.50, p = 0.003), but not to Performance IQ. Hair Mn was also inversely related to Full-Scale IQ (coefficient −0.01, p = 0.024), Verbal IQ (coefficient −0.02, p = 0.004), but not to Performance IQ. Hair Cd level was not significantly related to any IQ score. The associations between As and Mn and Full-Scale and Verbal IQ remained significant when adjustments were made for maternal education and sex (Table 2). The associations between Full-Scale IQ and both As and Mn were due largely to the associations between these metals and Verbal IQ. Scatterplots of the adjusted associations between As and Verbal IQ (Fig. 1) and between Mn and Verbal IQ (Fig. 2) suggest that the associations did not depart from linearity. Because of the demonstrated relationship between Pb and IQ deficits, we evaluated models of Full-Scale and Verbal IQ in which adjustments were made for hair Pb level as well as for maternal education and sex. The coefficients for hair As and hair Mn were essentially unchanged and both remained statistically significant predictors of both IQ scores.

Additional analyses were done to determine whether the IQ scores of children with higher exposures to both As and Mn were lower than the scores among children with high exposure to only one of the metals. Adjusting for maternal education and sex, an As-by-Mn interaction term was significant for Full-Scale IQ ($p = 0.02$) and Verbal IQ ($p = 0.016$), but not for Performance IQ ($p = 0.24$). The magnitudes of the significant interactions were explored by constructing 2 × 2 tables based on median splits of hair As and Mn levels. Boxplots of the adjusted Full-Scale IQ (Fig. 3A) and Verbal IQ (Fig. 3B) scores show that the scores of the children in the high As/high Mn cell were as many as 10 points lower than the scores of the children in the other three cells.

3.3. Neuropsychological test scores

Hair As and Mn levels were consistently associated, inversely, with children's scores on assessments of verbal learning and memory (Table 3). Children with higher As levels recalled fewer words over the five learning trials on the CVLT-C ($p = 0.03$) and tended to recall fewer story elements on the story memory subtest of the WRAML ($p = 0.064$). Children with higher Mn levels also recalled fewer words on the learning

Table 2
Associations between hair metals levels and IQ scores, adjusted for sex and maternal education

Metal	Full-Scale IQ			Verbal IQ			Performance IQ		
	Slope	S.E.	p	Slope	S.E.	p	Slope	S.E.	p
Arsenic	−0.44	0.17	0.01	−0.51	0.16	0.003	−0.27	−0.21	0.21
Manganese	−0.010	0.005	0.07	−0.12	0.005	0.02	−0.004	0.006	0.44
Cadmium	−0.03	0.04	0.5	−0.03	0.04	0.5	−0.03	0.05	0.6

Table 3
Associations between hair manganese and hair As levels and neuropsychological test scores, adjusted for sex and maternal education

Test score	Mn			As		
	Coefficient	S.E.	*p*	Coefficient	S.E.	*p*
WRAVMA						
Drawing	0.004	0.006	0.51	0.39	0.21	0.074
Matching	−0.001	0.005	0.86	−0.34	0.17	0.055
Pegboard	0.005	0.007	0.50	0.085	0.25	0.70
Visual motor composite	0.002	0.006	0.70	0.065	0.21	0.76
CELF-3						
Concepts and directions	0.0005	0.001	0.70	−0.025	0.046	0.60
Word classes	−0.001	0.001	0.39	−0.44	0.042	0.30
Semantic relationships	−0.07	0.04	0.11	−0.07	0.04	0.11
Receptive language	−0.005	0.007	0.48	−0.33	0.23	0.37
Children's category test						
Total	−0.004	0.00	0.33	−0.083	0.16	0.62
CVLT-C						
List A total trials 1–5	−0.007	0.003	0.03	−0.26	0.11	0.03
Short delay free recall	−0.0007	0.0003	0.03	−0.01	0.012	0.27
Long delay free recall	−0.0008	0.0003	0.005	0.016	0.11	0.14
Learning slope	−0.000	0.0004	0.69	0.002	0.12	0.86
WRAML						
Story memory	−0.003	0.001	0.01	−0.085	0.044	0.064
Story 1	−0.004	0.001	0.006	−0.11	0.06	0.065
Story 2	−0.004	0.002	0.09	−0.10	0.08	0.22

trials of the CVLT-C ($p = 0.03$), as well as on both the short delay free recall ($p = 0.03$) and long delay free recall trials ($p = 0.005$). They also recalled fewer elements on the WRAML stories ($p = 0.01$). Mn-by-As interaction terms were significant for several scores on the CVLT-C and WRAML story memory (Table 4). As was the case with IQ scores, children for whom both Mn and As hair levels were above the sample medians generally had the lowest adjusted mean scores.

Children's hair As and Mn levels were not significantly associated with their scores on the WRAVMA, the CELF-3, or the CCT. No significant Mn-by-As interactions were found for any of the scores on these tests. Hair Cd levels were not

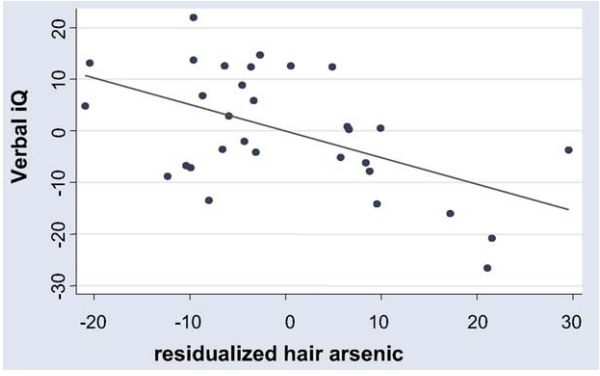

Fig. 1. Verbal IQ vs. hair arsenic level, adjusted for maternal education and sex.

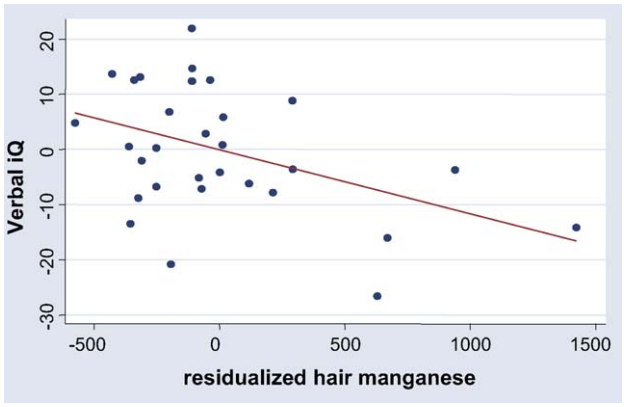

Fig. 2. Verbal IQ vs. hair manganese level, adjusted for maternal education and sex.

associated with children's scores on any of the neuropsychological tests (data not shown).

3.4. Behavior ratings

Parents' ratings of children on the CADS-IV and BRIEF, and teacher's ratings on the CADS-IV, BRIEF, and BASC were not consistently associated with children's hair metal levels, nor were significant associations were found between children's

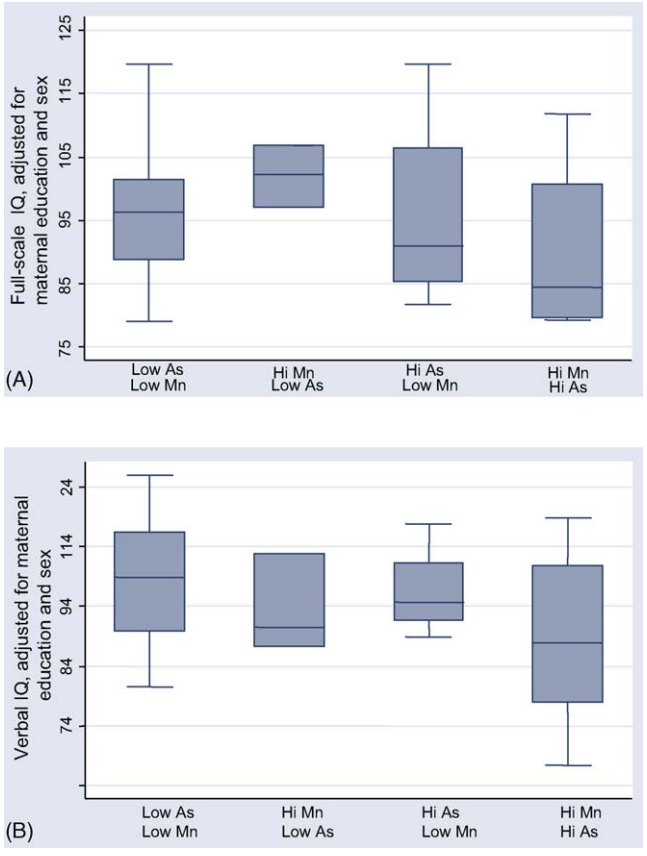

Fig. 3. (A) Boxplots of the adjusted Full-Scale IQ scores of children jointly classified on the basis of median splits of hair arsenic and hair manganese levels. (B) Boxplots of the adjusted Verbal IQ scores of children jointly classified on the basis of median splits of hair arsenic and hair manganese levels.

Table 4
Mean verbal memory subscales by joint As/Mn exposures

Test	Low As/low Mn	High As/low Mn	Low As/high Mn	High As/high Mn	*P*
Trials total	54.4	53	54	47.7	0.04
Short delay free recall	0.3	0.5	−0.2	−0.1	0.03
Long delay free recall	0.3	0.6	−0.2	−0.1	0.01
Story memory	9.1	9	9	6.4	0.03
Story 1	13.2	14	14	10.6	0.001
Story 2	12.3	12	11.7	8.7	0.22

P = interaction *p* value (arsenic × manganese); cells defined by medians of hair manganese and hair arsenic.

hair metal levels and their self-reports on the CDI and BASC (data not shown).

4. Discussion

The major finding of this pilot study is that, in school-aged children, higher hair Mn and As levels, particularly, in combination, were associated with significantly lower scores on an IQ test, as well as on tests of verbal learning and memory.

Although the neurotoxicity of Pb is well established (Bellinger, 2004), relatively little is known about As and Mn neurotoxicity, particularly when exposure occurs during critical developmental periods. Oral administration of As trioxide increases oxidative stress in mouse brain, resulting in reduced glutathione and elevated lipid perioxidation (Chaudhuri et al., 1999; Rao and Avani, 2004). As alters neural networking and leads to an increase in reactive oxygen intermediates in brain explants and neuronal cell cultures (Chattopadhyay et al., 2002a). Prenatal exposure to As causes apoptosis and necrosis of fetal neurons (Chattopadhyay et al., 2002b). Behavioral deficits have also been observed in As-exposed animals. Rats exposed as fetuses to As via maternal drinking water make more errors on a delayed alternation task (Rodriguez et al., 2002). Recent epidemiologic literature suggests that exposure to environmental As can produce neurocognitive deficits in children. Urinary As levels were inversely related to verbal IQ among 39 children living near a lead arsenate smelter, even after adjustment for blood lead level (Calderon et al., 2001). In an ecological study of Taiwanese adolescents, residence in an area with higher water As levels was associated with lower scores on tests of pattern memory and switching attention (Tsai et al., 2003). Wasserman et al. (2004) reported a strong inverse association between drinking water As levels and covariate-adjusted Full-Scale IQ and Performance IQ scores in a sample of 201 children in Bangladesh.

Unlike As and Pb, Mn is an essential nutrient as well as a toxic metal (McMillan, 1999). Although rare, Mn deficiency is possible. The principal route of exposure to Mn is via the diet, although inhalation is an important route of occupational exposure (Barceloux, 1999; Greger, 1999). The health impacts of long-term exposure to environmental Mn are unknown (Davis, 1998). The primary mechanisms of Mn neurotoxicity are also not well understood, but appear to involve oxidative damage to neurons (Aschner, 1999; Verity, 1999) and effects on essential neuronal metabolic enzymes such as aconitase (Zheng and Graziano, 1998). Decreased brain dopamine has been described in chronic manganism, and lesions of the basal ganglia are characteristic (McMillan, 1999). Although the motor system has generally been considered to be the critical target function in Mn neurotoxicity, the spectrum of adverse effects is likely to be considerably broader (Woolf et al., 2002). In epidemiologic studies of adults exposed to Mn-contaminated water, signs of subtle neurotoxicity have been found (Kondakis et al., 1989). Santos-Burgoa et al. (2001) reported that adults' scores on the Mini-Mental Status Examination, a dementia screening test, were inversely associated with blood Mn levels. Bowler et al. (2005) found that welders exposed to Mn vapor had deficits in Full-Scale IQ, Verbal IQ, verbal memory, and visual-spatial skills. In 11–13-year-old Chinese children, exposure to Mn-contaminated drinking water was associated with lower scores on tests of short-term memory, manual dexterity, and visual-perceptual speed (He et al., 1994). A follow-up study of this population reported that higher Mn exposures were associated with lower mathematics and language scores (Zhang et al., 1995). In a small case–control study, children with attention deficit hyperactivity disorder had significantly higher hair Mn levels than did controls (Crinella et al., 1998). Among children exposed to Mn in drinking water, Wasserman et al., (in press) found dose-dependent decreases in children's Full-Scale IQ, Verbal IQ, and Performance IQ scores.

Data on the effects of exposure to chemical mixtures are limited, but the results of some studies suggest that joint exposures to As, Mn, and Pb can act synergistically in producing neurotoxicity. Co-administration of Pb and Mn increases brain Pb levels approximately three-fold, compared to the administration of Pb alone (Chandra et al., 1981; Chiba and Kikuchi, 1984). Joint exposure to Mn and Pb increased brain Pb in five of seven brain regions studied, including the hippocampus (Shukla and Chandra, 1987). Administration of mining waste containing Pb, Mn, and As to rats resulted in elevated levels of brain As and Mn, which were associated with decreased dopaminergic transmitter following depolarization (Rodriguez et al., 1998). Mejia et al. (1997) showed multiplicative rather than additive changes in monoaminergic neurotransmitter levels in the brains of rats exposed to both Pb and As. Compared to rats exposed only to Mn or Pb, rats exposed to both metals show increased spontaneous motor activity (Chandra et al., 1981). In studies of conditioned avoidance responses, exposure to Pb and Mn decreased learning more than did either exposure to Pb or Mn alone,

although the effects appeared to be additive rather than multiplicative (Chandra et al., 1981). Gestational exposure to Pb and Mn combined produces birth weight and brain weight reductions that are greater than those that result from exposure to either metal alone (Chandra et al., 1983). We are not aware of any data on the effects of joint exposure to Mn, As, and Pb on neurodevelopmental outcomes in humans.

The major sources and pathways of exposure to metals in the communities around the Tar Creek Superfund site are not well described, and our group is currently conducting detailed environmental assessments to clarify this. Although it is plausible to assume that the opportunities for exposure to As, Mn, and Cd are increased among children living in this former mining region, the absence of data on population levels of As and Mn in the hair of US children make it difficult to draw conclusions about whether the exposures of the children in our pilot study sample are unusually high. The U.S. EPA lists Pb, Cd, and zinc as the principal pollutants of the Tar Creek site (U.S. EPA, 2003), but high levels of Mn have also been found in the area. The median level of Mn in mine water samples was 1870 μg/l and ranged as high as 15,000 μg/l, several orders of magnitude higher than the U.S. EPA's maximum tolerable level for drinking water of 50 μg/l (Christenson, 1995). Water samples taken from 16 Boone boreholes were also high in Mn, with a mean value of 3318 and a maximum value of 9800 μg/l (State of Oklahoma, 2000). Mn levels as high as 1900 ppm have been found in sediments along Tar Creek downstream from the Superfund site in Miami. Well water samples taken from the Roubidoux aquifer were found to be as high as 4400 μg/l, with 5% of samples exceeding 1910 μg/l (State of Oklahoma, 2000). In another study, conducted by the U.S. Geological Survey, the Mn levels in water from 14 wells in Ottawa County ranged from 150 to 9800 μg/l, with a median value of 3000 μg/l (U.S.G.S., 2002).

There is also evidence of elevated community exposures to As via water, soil, and garden produce (Ecology and Environment, 1996). In 1996, an EPA-sponsored risk assessment concluded that the concentrations of As in water, yard, soil, and garden produce were sufficient to produce a cancer risk in excess of 1×10^{-4} and a hazard quotient greater than 1.0 for non-cancer endpoints.

Several limitations of this pilot study warrant comment. Foremost among these is the limited sample size and weak statistical power, which would be expected to have reduced the likelihood of detecting true associations between hair metal levels and children's outcome scores. On the other hand, the small sample size also limited our ability to evaluate and adjust for potential confounding bias, and thus to distinguish true from artifactual associations.

Second, although we attempted to obtain the results of the children's past blood lead screening tests, records could be located for only three children. Despite the long history of lead mining in the region, children were not routinely screened for lead poisoning until very recently. We were therefore unable to evaluate the role that past lead toxicity might have played in determining the children's test scores, either by itself or in combination with the other metals. Pb levels in the hair samples we obtained did not appear to confound or modify the associations we noted between children's neuropsychological test scores and either their hair As or hair Mn levels. This conclusion must be tempered, however, in light of the limitations of hair Pb as an exposure biomarker.

Third, although we had hoped that recruiting children from a limited number of classrooms, rather than through community-wide advertisements, would reduce the likelihood of a volunteer bias, it is nevertheless possible that families who consented to participate differed from those who did not, perhaps in having particular concerns about the possibility that area pollution had adversely affected their children. This might have affected the distributions of test scores among participants. To our knowledge, biomarkers of As, Mn, and Cd have not been measured in area residents, making it unlikely that the probability of a child's participation was related to known biomarker levels for the metals of interest. Therefore, it is unlikely that the associations we observed were a result of a selection bias.

A fourth limitation pertains to possible exposure misclassification. Hair might not be the tissue that provides the most accurate measure of a child's exposure to the metals of interest. Moreover, hair metal levels measured at school-age might not reflect the appropriate exposure averaging time for the outcomes evaluated. However, such errors are likely to have been non-differential, which would be more likely to obscure true associations than to produce spurious ones.

Fifth, the test battery might not have included assessments of the outcomes that are most sensitive to As, Mn, or Cd, producing, in effect, outcome misclassification. This error would also have biased our effect estimates toward rather than away from the null.

In summary, in a cross-sectional pilot study, we found significant inverse associations between children's neuropsychological function and both hair Mn and As levels. The functional domain most consistently associated with hair metal levels was verbal skills, including memory. Furthermore, evidence of neurotoxicity was most prominent among children in whom hair levels of both Mn and As were greater than the median values in the sample. Further research on both As and Mn, and on combined exposures to As and Mn, are needed to validate these findings and to understand the impact of chemical mixtures on children's neurodevelopment.

Acknowledgements

Supported by a Milton Fund grant from Harvard University to DCB (540-45496-624473-730001). ROW is supported by a grant from the National Institutes of Health, K23 ES00381. This work was also supported in part by a grant from the ATSDR Superfund Reconciliation & Reclamation Act, administered through the Association of Occupational and Environmental Clinics Association (AOEC), Washington D.C. The views expressed in this paper are solely those of the authors, and do not necessarily reflect the views of ATSDR, AOEC, or the NIH.

References

Adams W, Sheslow D. WRAVMA. Wide range assessment of visual motor abilities. Wilmington, DE: Wide Range, Inc; 1995.

Aschner M. Manganese homeostasis in the CNS. Environ Res 1999;80:105–9.

Barceloux DG. Manganese. J Toxicol Clin Toxicol 1999;37:293–307.

Bellinger DC. Lead. Pediatrics 2004;113(Suppl. 1):1016–22.

Bellinger DC, Adams H. Environmental pollutant exposures and children's cognitive development. In: Sternberg RJ, Grigorenko EL, editors. Environmental effects on cognitive abilities. Mahwah, NJ: Lawrence Erlbaum Associates; 2001. p. 157–88.

Bellinger DC, Bolger M, Goyer R, Barraj L, Baines J. Cadmium. WHO food additives series 46. Safety evaluation of certain food additives and contaminants. Geneva, International Programme on Chemical Safety; 2003, p. 247–305.

Boll T. Children's category test. San Antonio: The Psychological Corporation; 1993.

Bowler RM, Roels H, Mergler D, Koller W, Doty RL. An epidemiologic multidisciplinary study of welders: Early neurotoxic health effects of manganese from welding fumes. Presented at the 9th International Symposium on Neurobehavioral Methods and Effects in Occupational and Environmental Health, Gyeongju, Korea, 26–29 September, 2005.

Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, et al. Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 2001;85:69–76.

Chandra AV, Ali MM, Saxena DK, Murthy RC. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. Arch Toxicol 1981;49:49–56.

Chandra SV, Murthy RC, Saxena DK, Lal B. Effects of pre- and postnatal combined exposure to Pb and Mn on brain development in rats. Ind Health 1983;21:273–9.

Chattopadhyay S, Bhaumik S, Purkayastha M, Basu S, Nag Chaudhuri A, Das Gupta S. Apoptosis and necrosis in developing brain cells due to arsenic toxicity and protection with antioxidants. Toxicol Lett 2002a;136:65–76.

Chattopadhyay S, Bhaumik S, Nag Chaudhury A, Das Gupta S. Arsenic-induced changes in growth development and apoptosis in neonatal and adult brain cells in vivo and in tissue culture. Toxicol Lett 2002b;128:73–84.

Chaudhuri AN, Basu S, Chattopadhyay S, Das Gupta S. Effect of high arsenic content in drinking water on rat brain. Ind J Biochem Biophys 1999;36:51–4.

Chiba M, Kikuchi M. The in vitro effects of zinc and manganese on delta aminolevulinic acid dehydratase activity inhibited by lead or tin. Toxicol Appl Pharmacol 1984;73:388–94.

Christenson S. Contamination of Wells Completed in the Roubidoux Aquifer by Abandoned Zinc and Lead Mines, Ottawa County, Oklahoma. Oklahoma City: U.S. Geological Survey, Water-Resources Investigations Report 1995; 95-4150.

Connors KC. CADS-Parent Version. North Tonawanda, NY: Multi-Health Systems, Inc.; 1997.

Connors KC. CADS-Teacher Version. North Tonawanda, NY: Multi-Health Systems, Inc.; 1999.

Crinella FM, Cordova EJ, Ericson J. Manganese, aggression, and attention-deficit hyperactivity disorder (abstract). Neurotoxicology 1998;19: 468–469.

Davis JM. Methylcyclopentadienyl manganese tricarbonyl: health risk uncertainties and research directions. Environ Health Perspect 1998;106(Suppl. 1):191–201.

Delis DC, Kramer JH, Kaplan E, Ober BA. California verbal learning test-children. San Antonio: The Psychological Corporation; 1994.

Ecology and Environment. Baseline Human Health Risk Assessment of Residential Exposures, Tar Creek Superfund, Ottawa County, Oklahoma. 1996, U.S. EPA Region 6.

Gioia GA, Isquith PK, Guy SC, Kenworthy L. Behavior rating inventory of executive functions. Odessa, FL: Psychological Assessment Resources, Inc.; 2000.

Greger JL. Nutrition versus toxicology of manganese in humans: evaluation of potential biomarkers. Neurotoxicology 1999;20:205–12.

He P, Liv DH, Zhang GG. Effects of high level manganese sewage irrigation on children's neurobehaviour. Chung Hua Yu Fang I Hsueh Tsa Chih 1994;28:216–8 (abstract translated).

Kondakis XG, Makris N, Leotsinidis M, Prinou M, Papapetropoulos T. Possible health effects of high manganese concentration in drinking water. Arch Environ Health 1989;44:175–8.

Kovacs M. Children's depression inventory. North Tonawanda, NY: Multi-Health Systems, Inc.; 2001.

McMillan DE. A brief history of the neurobehavioral toxicity of manganese: some unanswered questions. Neurotoxicology 1999;20:499–507.

Mejia JJ, Diaz-Barriga F, Calderon J, Rios C, Jimenez-Capdeville ME. Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicol Teratol 1997;19:489–97.

Rao MV, Avani G. Arsenic-induced free radical toxicity in brain of mice. Indian J Exp Biol 2004;42:495–508.

Reynolds CR, Kamphaus R. Behavior assessment system for children. Circle Pines, MN: American Guidance Service, Inc.; 1992.

Rodriguez VM, Dufour L, Carrizales L, Diaz-Barriga F, Jimenez-Capdeville ME. Effects of oral exposure to mining waste on in vivo dopamine release from rat striatum. Environ Health Perspect 1998;106:487–91.

Rodriguez VM, Carrizales L, Mendoza MS, Fajardo OR, Giordano M. Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicol Teratol 2002;24:743–50.

Santos-Burgoa C, Rios C, Mercado LA, Arechiga-Serrano R, Cano-Valle F, Wynter RA, et al. Exposure to managanese: health effects on the general population, a pilot study in central Mexico. Environ Res 2001;85:90–104.

Semel E, Wiig EH, Secord WA. CELF. Clinical evaluation of language fundamentals. 3rd ed. San Antonio: The Psychological Corporation; 1995.

Sheslow D, Adams W. WRAML. Wide range assessment of memory and learning. Wilmington, DE: Wide Range, Inc.; 1990.

Shukla GS, Chandra SV. Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. Arch Environ Contam Toxicol 1987;16:303–10.

State of Oklahoma. McCaleb N, Rogers E (co-Chairmen). Governor Frank Keating's Tar Creek Superfund Task Force—Final Report 2000. Oklahoma City, OK: Chat Usage Subcommittee, 2000.

Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 2003;24:667–74.

The Psychological Corporation, WASI, Wechsler abbreviated scale of intelligence, Manual, San Antonio, The Psychological Corporation; 1999.

Tsai SY, Chou HY, The HW, Chen CM, Chen CJ. The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 2003;24:747–53.

U.S.G.S. (Geological Survey). Project Title: Assessment of ground-water flow and recharge in the Boone Aquifer in Ottawa County, Oklahoma, 2002 (available as: http://ok.water.usgs.gov/proj/boone.aquifer.html).

U.S. EPA, 2003. Region 6. Tar Creek (Ottawa County) Oklahoma. Dallas, TX: U.S. Environmental Protection Agency, updated 2/18/03 (available as: http://www.epa.gov/earth1r6/6sf/pdffiles/tarcreek.pdf).

Verity MA. Manganese neurotoxicity: a mechanistic hypothesis. Neurotoxicology 1999;20(2–3):489–97.

Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, et al. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 2004;112:1329–33.

Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect, in press. Available on line at http://ehp.niehs.nih.gov/docs/2005/8030/abstract.html.

Woolf A, Wright R, Amarasiriwardena. Bellinger D. A child with chronic manganese exposure from drinking water. Environ Health Perspect 2002;110:613–6.

Zhang G, Liu D, He P. Effects of manganese on learning abilities in school children. Chin J Prev Med 1995;29:156–8.

Zheng W, Graziano JH. Manganese (Mn) exposure inhibits mitochondrial aconitase activity. Neurotoxicology 1998;19:469 (abstract).