Award Number: W81XWH-05-1-0239

TITLE: MANGANESE RESEARCH HEALTH PROJECT (MHRP)

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REPORT DATE: February 2013

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE
February 2013

2. REPORT TYPE
Final Addendum

3. DATES COVERED
1 January 2012 – 31 January 2013

4. TITLE AND SUBTITLE

MANGANESE RESEARCH HEALTH PROJECT (MHRP)

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-05-1-0239

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Vanderbilt University
Nashville, TN 37203

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Inhalation of high manganese (Mn) concentrations may result in serious irreversible neurological disease (manganism). The air exposure level associated with an increased risk of acquiring subtle neurological disturbances is currently not known. Welding fumes contain Mn. In this study 332 subjects have been examined with neurobehavioral methods, of whom 137 are currently exposed welders and 137 are referents. Among these, 63 welders and 65 referents were examined with the same methods around six years earlier. Also 34 patients diagnosed with manganism (17 also examined six years earlier) and 25 with idiopathic Parkinson's disease (PD) were examined. Positron Emission Tomography examinations were carried out in eight PD-patients, eight manganism cases and six referents. Currently the results from the study are under publication. The first paper was published this year. A second manuscript will probably be submitted during March 2013.

15. SUBJECT TERMS
Manganese, neurotoxicology, welders, manganism, idiopathic Parkinson's disease

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	17	**19b. TELEPHONE NUMBER** *(include area code)*

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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Introduction

Manganese (Mn) is an essential trace element in humans, but inhalation of high Mn concentrations has been associated with irreversible neurological disease (manganism). Welding fumes may contain high amounts of Mn, and cases of manganism among welders are reported every year in Russia. Welders are by number the most important group of workers occupationally exposed to Mn. Exposure to Mn at lower concentrations can result in subtle motor disturbances. The exposure level associated with an increased occurrence of such disturbances is currently not sufficiently known.

Neurobehavioral tests were applied, parameters for iron status determined, and an extensive exposure assessment carried out in this study. PET-scan examinations were carried out in a limited number of subjects. The main objective is to assess the usefulness of selected neurobehavioral tools in an epidemiological study, in order to investigate their sensitivity for detecting subtle neurological functional changes.

Body

The study was approved on October 20, 2006, by the appropriate regional ethical committee in Norway (The Regional Committee for Medical Research Ethics, Southern Norway (REK Sør)) and on December 25, 2006, by the appropriate ethical committee in Russia (I.P.Pavlov State Medical University- St. Petersburg). A final approval of the project protocol by the DOD was received on January 16, 2007. A contract was signed between the National Institute of Occupational Health (Norway) (NIOH) and Vanderbilt University (USA) on January 31, 2007, to carry out “A Study of the Nervous System in Welders”. In the letter from Vanderbilt University Medical Center to the NIOH dated March 8, 2007, the fully executed original of the contract was received, this date representing the start of the project.

One main objective of the study was to follow-up welders and referents at least 3 years after the initial neurobehavioral examinations. This aim was fulfilled by the re-examination of 63 welders and 65 referents out of previously examined 96 welders and 96 referents. All subjects were invited. The main reason for not participating was that they had quit their job and moved. Another objective was to follow up with neurobehavioral methods as many as possible of 27 previously examined patients diagnosed of having welding related manganism. Seventeen patients could be re-examined with the same neurobehavioral tests as previously used.

In addition, according to study plan, new participants were recruited. Because fewer subjects than anticipated could be followed up, some more new subjects were included in order to reach the planned size of the study, in total 74 new current welders and 72 new referents. Thus, altogether 137 welders and 137 referents were examined with neurobehavioral methods, which was within the number targeted to be examined. In addition, 17 new patients were included in the study. The plan was to include 25, but due to some refusals this figure was not quite reached. Finally, according to the study protocol 25 patients with idiopathic Parkinson’s disease (PD) was planned to be examined with the same neurobehavioral test battery. This number was achieved. In all, the target number of subjects to be included in the study was reached.

According to the objectives of the study, PET scan of the brain was carried out in 22 subjects, of whom six were referents, eight had PD and eight were former welders with diagnosed welding related manganism. A further objective was to assess exposure in order to study potential relations between exposure and neurobehavioral effects. To fulfil this aim personally collected welding aerosol samples (in the thoracic aerosol fraction) was collected among the currently exposed welders, in all 237 air samples. Seven welders refused to carry sampling equipment. These aerosol samples were analysed with respect to their solubility in a simulating lung fluid

(Hatch solution). In addition, for the purpose of exposure assessment, whole blood, serum and urine samples were analysed for a range of relevant elements in all subjects (e.g. Mn, Fe, Cr, Ni, V). A final aim of the study was to assess iron status biomarkers. Thus, serum ferritin and serum hepcidin were analysed. Because these biomarkes are sensitive to inflammation, high sensitivity CRP was analysed to assess potential confounding. According to protocol, the biomarker of alcohol consumption, carbohydrate deficient transferrin in serum, was also measured.

In the original proposal, the concentrations of serum prolactin and inhibin B were suggested to be measured. This was not regarded as relevant to study at that time by the MHRP committee. Thus, these markers were therefore not measured. The iron isotope ratio was planned to be measured in 30 whole blood samples. However, due to lack of funding, this was not done.

Enrollment of participants to the study was closed early in 2011. All collected samples have been analyzed, and the laboratory work was completed early 2012. According to the legislation in Norway, the study records and database have been anonymized in order to protect the information collected from the participants (according to our data protection act). Currently scientific reports from the study are being written. The first paper was printed early 2013, and the second manuscript is currently by the co-authors for approval.

During enrollment of participants, no adverse events or unanticipated problems involving risks to participants were identified. No subjects have withdrawn from the study and no complaints were received. No substantial deviations, except some software problems at the study start have occurred. This resulted in a delay in the enrolling of participants. We have followed the literature to assess whether new information has been published during the study time. The study protocol has not been changed, and there are also no changes in the conflict of interest status.

After the original contract was received, preparations for examining the participants were started. Due to software problems, the start of the data collection was delayed. Altogether 137 welders and 137 age-matched referents were examined with neurobehavioral tests in a cross-sectional designed study. Among these participants, 63 welders and 65 referents were examined with the same neurobehavioral test battery around six years earlier. Thus, this part of the investigation has a prospective study design. Further, 25 patients with newly diagnosed idiopathic Parkinson's disease (PD) and 34 patients diagnosed with manganism were examined. The latter group consists of former welders, of whom 17 had been examined around six years earlier. In 22 subjects, of whom eight had PD, eight had manganism and six were referents, PET scan examinations were carried out. Whole shift air samples for the determination of welding fume components were collected among the welders. Samples of whole blood, serum and urine were collected. The air samples were dissolved in Hatch solution (artificial "lung lining fluid") prior to analysis. All laboratory analysis has been completed.

The mean age of all examined welders was comparable to that of the referents (Table 1). The level of B-Mn in whole blood is substantially higher in the welders. Also the concentrations of Mn in urine and in serum are significantly higher in the welders.

Table 1 Background variables among all welders and referents examined in 2008/2009

	Welders (N=137) Mean[#] (range)	**Referents (N=137) Mean (range)**
Age	39.9(19-70)	40.1(19-70)
Years of welding	16.6(1-45)	-

B-Mn (µg/L)	13.9(5.9-40.3)	8.2(4.1-13.9)[†]

[#] Arithmetic mean; [†] Fourteen subjects declined blood sampling

Table 2 shows some key data of the welders and the referents who were examined in 2002/2003 and re-examined in 2008/2009. Data from the original group that was examined in 2002/2003 have been published (Ellingsen et al., 2006; Ellingsen et al., 2007; Ellingsen et al., 2008).The follow up time was nearly identical in the two groups, being slightly shorter than six years. The referents were older than the welders. The contrast in B-Mn between these welders and the referents is quite large, the mean concentration difference being 5.6 µg/L. At the first examination the difference was 1.6 µg/L. The results from the air samples support the impression from the blood samples of higher exposure at follow up when compared to the baseline.

Table 2 Background variables recorded in the subjects that were examined in 2002/2003 and followed up in 2008/2009. Data at follow up.

	Welders (N=63) Mean[#] (range)	**Referents (N=65) Mean (range)**
Age	42.7(26-70)	45.8(22-70)
Months of follow up	70.8(59-90)	70.7(61-80)
Years of welding	19.5(7-45)	-
B-Mn (µg/L)	13.6	8.0[†]

[#] Arithmetic mean; [†] Eight subjects declined blood sampling

Altogether 25 subjects diagnosed with PD and 34 subjects (all welders) diagnosed with welding related manganism were examined (Table 3). One PD patient had in the medical history information about a cerebral ischemic event, and was thus excluded. These subjects underwent the same neurobehavioral test battery as the welders and referents. Manganism is regarded as a disease of people at younger ages, while PD patients are mostly diagnosed at an age above 60 years of age. It is therefore no surprise that the manganism patients in this study were on average younger than the PD patients.

Table 3 Background variables among former welders diagnosed with manganism and patients diagnosed with idiopathic Parkinson's disease (PD).

	Manganism (N=34) Mean[#] (range)	**PD (N=24) Mean (range)**
Age	**59 (47-77)**	**69 (49-84)**
Years of welding	**23.7(7-33)**	**-**
B-Mn (µg/L)	**9.8 (4.2-22.4)**	**10.2[†] (3.7-19.0)**

[#] Arithmetic mean; [†] Three subjects missing

This study is quite large, and the results will be published scientifically in a series of papers. One paper has been published, one will probably be submitted during the next month, and one manuscript is under preparation. A summary of main results is shown below.

SUBSTUDY 1 - The bioavailability of manganese in welders in relation to its solubility in welding fumes

This sub-study of solubility data of Mn and Fe in personally collected welding fume samples in relation to individual concentrations of Mn in biological samples have now been published (Ellingsen et al., 2013). 237 whole-shift welding fume samples were collected. Seven welders refused to carry air sampling equipment. The welding fume was dissolved in Hatch solution to assess the solubility of Mn (and iron) in a simulated lung lining fluid. Only around 12% of the Mn mass contained in the welding fume particles was found to be soluble in the Hatch solution and iron was substantially less soluble than Mn. This is important, because animal studies have shown that intratracheal instillation of soluble $MnCl_2$ or inhalation of soluble $MnSO_4$ resulted in substantially higher striatal Mn concentrations in rats compared to the less soluble MnO_2 or Mn_3O_4. We observed statistically significantly higher B-Mn, U-Mn and S-Mn in the welders as compared to the referents. The highest Pearson's correlation coefficients between Mn in air samples and Mn in biological samples were obtained for the associations between B-Mn and S-Mn and exposure to soluble Mn two days before the blood sample collection. U-Mn was most highly correlated with soluble air Mn collected the day before the biological sampling. Multiple linear regression analysis also indicated that "years of welding" was associated with B-Mn and S-Mn. Simultaneous exposure to Fe had no statistically significant impact on these associations. The correlations between the Mn concentrations in the biological fluids were all statistically highly significant among the welders, the Pearson's r being between 0.63 and 0.69. The corresponding non-significant associations in the referents were between 0.06 and -0.07. These results suggest that the mobility of Mn between the different compartments are quite different in the welders when compared to the referents. In particular the association between S-Mn and U-Mn could suggest that S-Mn in the welders are bound to ligands that can be filtered in the kidney glomeruli, and that these complexes may be smaller in the welders than in the referents.

SUBSTUDY 2 - A neurobehavioral study of manganese exposed welders (tentative title)

The manuscript from this substudy will tentatively be submitted during March 2013. This substudy describes neurobehavioral functions in 137 welders currently exposed to the geometric mean (GM) air concentration of 214 $\mu g/m^3$ (range 1–3230) of total Mn in the welding fume, of which 22 $\mu g/m^3$ (GM) was soluble in the artificial lung fluid Hatch solution. The welders were compared to 137 referents. The welders score significantly poorer than the referents on the Grooved Pegboard, Finger Tapping, Simple Reaction Time (SRT) and possibly the Maximum Frequency tests. They also reported more subjective symptoms. Alcohol consumption was assessed using the serum biomarker carbohydrate deficient transferrin (sCDT). Significant associations between sCDT and neurobehavioral performance was observed for several tests, and welders with sCDT above the upper reference limit (indicating a daily consumption of at least 60-80 gram ethanol) had substantially poorer performances on the Grooved Pegboard test, Finger Tapping test and SRT. No such effects of high sCDT was observed in the referents, indicating a strong interaction between high sCDT and exposure to Mn. Self-reported alcohol consumption was not associated with the neurobehavioral test results. A statistically significant difference in the SRT and Grooved Pegboard test remained after excluding all subjects with sCDT above the normal level, but the difference between the groups was smaller. These welders also reported more subjective symptoms than the referents. We suggest that sCDT should be measured in future neurobehavioral studies of occupationally Mn exposed populations.

SUBSTUDY 3 - Iron status in welders (tentative title)

This is a manuscript in preparation. It is based on unpublished results of serum ferritin and soluble serum transferrin receptor in addition to the whole blood iron isotope ratio measured in 2003, and the measurement of ferritin, hepcidin and CRP (acute phase reactant) and sCDT in the present study. In addition trace elements known to be transported through the divalent metal transporter 1 (Mn, Fe and Cobalt) were determined in urine, serum and whole blood. The results are preliminary, but it appears that being exposed to quite substantial airborne amounts of iron in the welding aerosol has hardly any impact on the biological concentrations of ferritin, hepcidin and transferrin receptor in serum of the welders. The increase in serum ferritin between 2003 and the current study (six years of extra welding) was similar in the welders and the referents that were followed up. Preliminary results indicate an impact of CRP and sCDT on the serum ferritin concentrations. These preliminary results are a bit surprising, given the high iron concentrations measured in the welding fumes. However, as shown in substudy 1 the solubility of iron is very low. Also the deposition efficiency of this kind of weldings fumes (MMA and MIG welding) is regarded as low (around 15%).

SUBSTUDY 4 - A follow up study of neurobehavioral effects in welders

This substudy describes the results from the follow up of 63 welders and 65 referents examined nearly six years after the first examination with the same neurobehavioral test battery. The welders' current exposure was much higher at follow up compared to the first examination six years previously. The statistical analysis have not been completed, but preliminary results indicate that there may be a selection out of work related to welders with poorer test performance at baseline. The higher exposure at follow up makes it difficult to attribute effects to six additional years of exposure or to the higher ongoing exposure. Tests results for fine motor speed and dexterity, static steadiness and possibly finger tapping deteriorated during follow up more among the welders than among the referents in addition to their increased reporting of subjective symptoms. The Finger Tapping test, which can reveal bradykinesia, appeared to pick up welders with disease. There appears to be an interaction between deterioration due to exposure and the alcohol consumption biomarker CDT in serum.

SUBSTUDY 5 – A neurobehavioral comparison of patients diagnosed with idiopathic PD or manganism

Again, these results are preliminary, and further statistical calculations are required. The results indicate substantial differences in neurobehavioral test performance between the two groups of patients. Crudely it can be summarized that the PD patients had more tremor, but also different quality of tremor when compared to the manganism patients. This can be described mainly as a shift in the tremor frequency pattern towards lower frequencies, a more regular (pathological) tremor, and a smaller dispersion in the tremor frequencies. The mangansim patients, on the other hand, had more postural sway. There is apparently more side difference in the clinical manifestations in the PD-patients as compared to the manganism patients with respect to neurobehavioral performance.

Substudy 6 – Positron Emission Tomography in patients with idiopathic PD or manganism

Eight patients with PD, eight diagnosed with manganism and six referents were examined with PET-scan. The data treatment has not yet been completed, but preliminary results indicate that the manganism patients predominantly have reduced glucose activity in the head of the nucleus caudatus. This is also the case in the PD patients, but they also have alterations in other brain areas such as thalamus and amygdalae. It could be of some interest that the preliminary data indicate an association between certain neurobehavioral test scores and the reduction of glucose activity in the nucleus caudatus.

Key Research Accomplishments

137 welders, 137 referents, 34 patients diagnosed with welding related manganism and 25 patients with newly diagnosed idiopathic Parkinson's disease (PD) have been examined with neurobehavioral methods. Recruitement to the study has been completed.

Eight patients with manganism, eight with PD and six referents were examined with PET-scan.

237 personal whole shift welding fume samples has been collected.

Blood and urine samples were collected in 315 and 314 subjects, respectively.

Welding fumes were dissolved in a simulated lung lining fluid (Hatch solution). Welding fume components were determined by ICP-MS (soluble in Hatch solution) and ICP-OES (non-soluble in Hatch solution).

Metal analysis in urine, whole blood and serum with ICP-MS has been completed.

Relevant biomarkers in serum have been analyzed.

One paper has been published. One manuscript will be submitted during March 2013.

Reportable Outcomes

Conference abstracts

Ellingsen DG. Neurobehavioral performance of Parkinson's disease patients and welders with the diagnosis of manganism (preliminary results). The Manganese Health Research Program Showcase Conference. New discoveries – New directions. Juni 24-25, 2009. Washington DC, Lansdowne Resort, USA.

Ellingsen DG. Manganese neurotoxicity in welders. 9th ISTERH 2011. Trace elements in health and disease: Essentiality, Toxicity. Rixos Premium Hotel, Belek, Turkey, 16.-21. October 2011.

Ellingsen DG, Zibarev E, Kusraeva Z, Berlinger B, Chashchin M, Chashchin V, Thomassen Y. Biological monitoring of manganese in welders in relation to the solubility of welding fume components. Enviromin 2012, Loskop Dam, Forever Resort, South Africa, March 11-15.

Ellingsen DG, Bast-Pettersen R, Zibarev E, Chashchin M, Kusraeva Z, Thomassen Y, Chashchin V. Tremor in welders and parkinsonism patients in relation to alcohol consumption and exposure to manganese. Neuroh Conference (ICOH-SCNP 2013), Cape Town, South Africa, March 24-27, 2013. (Accepted).

Scientific papers

Ellingsen DG, Zibarev E, Kusraeva Z, Berlinger B, Chashchin M, Bast-Pettersen R, Chashchin V, Thomassen Y. The bioavailability of manganese in welders in relation to its solubility in welding fumes. Environ. Sci.: Processes Impacts;2013:15,357–365.

Manuscripts in preparation

Dag G Ellingsen, Zarina Kusraeva, Rita Bast-Pettersen, Evgenij Zibarov, Maxim Chashchin, Yngvar Thomassen, Valery Chashchin. A neurobehavioral study of manganese exposed welders (Title may be changed).

Conclusion

The enrollment of subjects to the study has been completed. Altogether 332 subjects have been examined with neurobehavioral methods. The laboratory analyses have been completed. The statistical analyses have mostly been finalized. Writing of scientific papers and other reporting has commenced.

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Ellingsen DG, Dubeikovskaya L, Dahl K, Chashchin M, Chashchin V, Zibarev E, Thomassen Y. Air exposure assessment and biological monitoring of manganese in welders. J Environ Monit 2006;8:1078-1086.

Ellingsen DG, Chashchin V, Haug E, Chashchin M, Tkachenko V, Lubnina N, Bast-Pettersen R, Thomassen Y. An epidemiological study of reproductive function biomarkers in male welders. Biomarkers 2007;12:497-509.

Ellingsen DG, Konstantinov R, Bast-Pettersen R, Merkurjeva L, Chashchin M, Thomassen Y, Chashchin V. A neurobehavioral study of current and former welders exposed to manganese. NeuroToxicology 2008;29(1):48-59.

Award Number W81XWH-05-1-0239
PI: Joseph Graziano, PhD
Progress Report, Febuary 12, 2013

This study has been completed and will be submitted for publication within the next ten days. A poster describing our findings was presented at the 2012 annual meeting of the NIEHS Superfund Research Program:

Motor function in Bangladeshi young adults exposed to manganese and arsenic in households wells

Abstract

The neurotoxicity of manganese (Mn) in adults with occupational inhalation exposure is well established. Exposure is linked to a Parkinson-like condition, with weakness, anorexia, apathy, slowed speech, emotionless facial expression, and slow movement of the limbs. Most studies of the consequences of environmental Mn exposure on neurobehavioral functioning consider young children. In our investigations of children in Bangladesh, where exposure to both Mn and arsenic (As) in household wells is elevated, we have described Mn- induced neurotoxicity in children with regard to intellectual, academic, and behavioral functioning. We have now examined motor functioning in 182 young adults (100 male and 82 female, 18-21 year-olds). By design, half of each gender was consuming water with < 500 µg/L Mn and half above. Motor function was assessed using the Bruninks-Oseretsky Test (BOT-2) which includes a total score of overall motor proficiency (TMC) and four subscales: fine manual control (FMC), manual coordination (MC), body coordination (BC) and strength and agility (SA). In analyses adjusted for As, gender, maternal education, serum ferritin and Hgb, log-transformed Mn in blood was inversely related to Body Coordination ($B=-4.32$, $p < .05$). We also found that identified individuals exposed to WMn> 500 µg/L had decreased Strength and Agility scores ($B=-0.78$, $p< .05$). In analyses stratified by gender, associations were consistently stronger for males. Neither blood As nor water As contributed significantly to measures of motor function. Results for young men are consistent with those based on investigations of occupational Mn exposure, and should elevate concerns for environmental exposures for populations of young adults.

Intro

The neurotoxicity of manganese (Mn) in adults with occupational inhalation exposure is well established. Exposure is linked to a Parkinson-like condition, with weakness, anorexia, apathy, slowed speech, emotionless facial expression, and slow movement of the limbs. Most studies of the consequences of environmental Mn exposure on neurobehavioral functioning consider young children. In our investigations of children in Bangladesh, where exposure to both Mn and arsenic (As) in household wells is elevated, we have described Mn- induced neurotoxicity in children with regard to intellectual, academic, and behavioral functioning. We have now examined motor functioning in 182 young adults (100 male and 82 female, 18-21 year-olds). By design, half of each gender was consuming water with < 500 µg/L Mn and half above. Motor function was assessed using the Bruninks-Oseretsky Test (BOT-2) which includes a total score of overall motor proficiency (TMC) and four subscales: fine manual control (FMC), manual coordination (MC), body coordination (BC) and strength and agility (SA). In analyses adjusted for As, gender, maternal education, serum ferritin and Hgb, log-transformed Mn in blood was inversely related to Body Coordination ($B=-4.32$, $p < .05$). We also found that identified individuals exposed to WMn> 500 µg/L had decreased Strength and Agility scores ($B=-0.78$, $p< .05$). In analyses stratified by gender, associations were consistently stronger for males. Neither blood As nor water

As contributed significantly to measures of motor function. Results for young men are consistent with those based on investigations of occupational Mn exposure, and should elevate concerns for environmental exposures for populations of young adults.

Methods

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. Prior to beginning recruitment for this study, we obtained approval from Institutional Review Boards at Columbia University and from the Bangladesh Medical Research Council.

Participants

Between March and September of 2010, we recruited 182 young adults 18-21 years old (100 males and 82 females) into our protocol, based on their Mn home well water concentrations, as assessed at age 8-10 years. By design, half of each gender had been consuming water with < 500 µg/L Mn and half above that.

Recruitment

WMn data for household wells, measured when the children were approximately 8-10 years, was available from our HEALS cohort central database (xx); we generated a list of 3,825 households with young adult children. During home visits to potential participants, field staff verified their current address, the Mn status of their well water, and checked eligibility criteria. Eligible participants had to have been living in the same household when initial recruitment of their parents into the HEALS study had occurred. In addition, participants had to have been drinking from the designated well for at least the past year, they had to report being free of acute or chronic diseases, of physical disability or vision problems, and that they were neither pregnant nor have given birth in the past 6 months. We recruited one participant from each well.

Once eligibility was verified, study procedures were explained to the parents and the participant, informed consent was obtained from participants and a new water sample was obtained. At this visit, the field team also collected socio-demographic information for the household and made an appointment for the participant to appear at the field clinic in the next few days.

At the field clinic, motor function was assessed using the Bruninks-Oseretsky Test, Second version (BOT-2) (Pearson Corporation, San Antonio, Texas). Blood samples were collected for the measurement of manganese (BMn), arsenic (BAs), selenium (BSe) and lead (BPb). Each participant was given a small amount of money and a card entitling him/her to health care at our study medical clinic in appreciation for his/her participation.

Measures

Water sample collection and measurement of Mn and other exposures: Initial information on WMn was based on baseline data collected in one of the two waves of enrollment of parents into the HEALS Cohort (i.e., either 2000-2002, or 2006-2008). Field sample collection and laboratory procedures are described elsewhere in detail (1-3). Similar procedures were followed for measurement of current well-water exposures.

Water samples were analyzed by high-resolution inductively-coupled plasma mass spectrometry (HR ICP-MS) as previously described (3).

Motor function:

Bruninks Oseretsky Test Version2 (BOT-2):

The Bruninks Oseretsky Test (BOT-2) is an individually-administered test that measures a wide range of motor skills in young persons, standardized on a US nationally representative sample of more than 1500 4-21 year olds. It is the most widely used standardized measure of motor proficiency, with excellent psychometrics: reliability (0.90-0.97) and validity (positive predictive value ~88%) (4-8). It has been used successfully in studies of individuals with Developmental Coordination Disorder (6-8); scores for children with Developmental Coordination Disorder are between one and two standard deviations below norms (5). The BOT-2 uses a composite structure organized around both the muscle groups and the limbs involved in movement, generating four subscale scores and a summary, Total Motor Composite (TMC). (Figure 1).

Translation and training

Test materials were translated into Bangla and then back translated into English. Study testers were trained by an experienced psychometrician (GW). Fifty participants were tested in initial feasibility studies and were not included in this analysis. Testers were blind to exposure characteristics of study participants.

Questionnaire data on covariates:

During home visits, the field team collected socio-demographic information including living conditions, maternal and paternal education and occupation, participants' education and occupation history other potential covariates (e.g., birth order, sibship size). A general assessment of participant's health was assessed during the home visit by field interviewers, using a standard set of questions.

Anthropometric data: At the field clinic visit, height and weight, as well as serum ferritin and Hgb, were measured prior to the assessment of motor function.

Results

Demographic and exposure characteristics. Table 1 presents information on demographic and exposure characteristics for all participants and for males and females separately. The average participant was 19 years old and had completed approximately 8 years of schooling; females had completed two further years of school, relative to males. In contrast, their parents had completed on average only 3 years of school. Only 6% were already married. While males were expectably taller and heavier, there were no differences across gender in BMI, nor were there gender differences in house type or paternal occupation. Baseline WMn was significantly higher for females than for males. Scores for Body Coordination and the Total Motor Composite were significantly higher for males than for females.

Table 1 also presents information on current well-water characteristics. By design, all participants were living in the same house as when their baseline well characteristics had been measured (at ages 8-10 years). Of 182 participants, only 8 had switched their primary home well since baseline. Expectably, WAs measured at baseline and current measures were significantly positively correlated [for WAs, $r_{(180)}$=0.92, for WMn, $r_{(180)}$=

0.83, both *p's* <0.001], and correlated with each other also [at both Baseline and current assessment $r_{(180)}$=0.53, *p's* <0.001].

Associations between exposure and motor function. Table 2 presents results of logistic regression examining associations between As and Mn measured in blood, and subscale and total scores on the BOT2. Before adjustment for variables other than gender, and both exposures, BMn was significantly negatively related to the Body Coordination subscale [B= -4.32, p < .05]. This association persisted when we examined Model 2, which further adjusted for hemoglobin, serum ferritin, and maternal education [B=-4.96, p < .05]. Altogether the features in Model 2 explained 9% of the variance in Body Coordination scores.

When we considered associations with current well-water concentrations of WAs and WMn and motor outcomes, based on the same models that were examined for the blood measures, WMn was consistently significantly related to only the Strength/Agility subscale, in both Model 1 [B=-0.22, p < .05] and in Model 2 [B=-0.22, p < .05], explaining 3-4% of the outcome variance (data not shown).

As, measured in either blood or water was unrelated to any outcome, in either Model 1 or Model 2. When we stratified the sample by gender, and re-examined both models, associations were consistently stronger for males than for females, and the BMn association with Body Coordination for males was considerably increased [for males only, in Model 2, B=-9.12, p < .01].

Finally, when we considered current WMn stratified as above or below 500 µg/L, those with WMn > 500 µg/L had decreased Strength and Agility scores, in both analyses adjusting only for gender [B=-0.80, p < .05], as well as in those that further considered maternal education and WAs > 10 µg/L [B=-0.78, p < .05].

Conclusions

Occupational exposure to Mn in adults has been consistently associated with a constellation of CNS impairments, including kinetic tremor, rigidity, dystonia, and gait disturbances, with symptoms of Parkinsonism, as well as with problems with working memory concentration and spatial orientation (9).

Because, however, adults with occupational Mn exposure are distinguishable in important ways from those with Parkinsonism (PD), it is likely that Mn neurotoxicity involves distinct pathologic mechanisms, most likely related to reduced dopaminergic functioning. The sensitivity of the dopaminergic system to Mn exposure has been noted in non-human primates (10).

In our earlier work with children in Bangladesh exposed to both As and Mn in well water (11) we found that As measured in blood (but not BMn) was related to poorer scores on BOT-2 subtests of Fine Motor Control and Bodily Control. On the other hand, among the current sample, WMn levels were approximately 30% higher than in that earlier study (946.5 ug/L vs 725.5 ug/L), and current study participants had continued exposure that persisted for 10 years longer than that in our study of children. Thus, continued exposure to Mn, seen both in occupational investigations and in the present study of young adults, may pose emerging risk for motor coordination.

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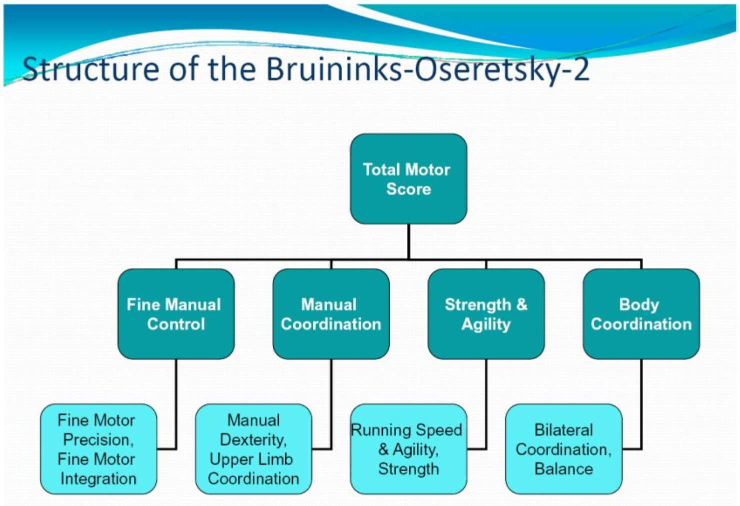

Table 1. Sample Characteristics				
	Total sample X (sd) N=182	Male N=100	Female N=82	
Demographics				
Age	19.22 (0.80)	19.26 (0.79)	19.16 (0.81)	
Married[a]	6.04 (11)	5.00 (5)	7.32 (6)	
Education (years)	7.86 (3.39)	6.96 (3.63)	8.96 (2.70)	p< .0001
Mother's education (% None)	50.55	52.00	48.78	
Father's education (% None)	37.91	40.00	35.37	
BMI	20.48 (2.52)	20.60 (2.67)	20.34 (2.34)	
Blood measures				
Blood As (ug/L)	5.93 (4.62)	6.46 (4.60)	5.30 (4.58)	p < .02
Blood Mn (ug/L)	14.06 (3.28)	13.15 (2.64)	15.16 (3.65)	p< .0001
Serum ferritin (ng/dl)	49.58 (28.08)	60.12 (30.02)	37.11 (19.36)	p< .0001
Hemoglobin (g/dL)	12.92 (1.36)	13.72 (1.04)	11.94 (1.01)	p< .0001
Water characteristics				
Baseline Water As	31.54 (46.53)	27.85 (38.45)	36.04 (54.72)	--
Baseline Water Mn	912.33 (886.61)	802.80 (810.16)	1045.90 (959.9)	--
Current Water As (ug/L)	30.69 (51.85)	27.05 (40.68)	35.13 (62.84)	--
Current Water Mn(ug/L)	946.51 (973.03)	726.24 (730.08)	1215.14 (1153.7)	p < 0.01
BOT-2 Standard scores				
Fine Manual Control	29.62 (2.81)	29.43 (2.91)	29.84 (2.69)	--
Manual Coordination	43.69 (6.66)	44.43 (6.46)	42.79 (6.83)	--
Body Coordination	38.65 (5.69)	39.71 (5.60)	37.37 (5.55)	p< .01
Strength and Agility	33.61 (2.15)	33.81 (1.99	33.37 (2.32)	--
Total Motor Composite	33.75 (2.37)	34.22 (2.28)	33.18 (2.36)	p< .01

Table 2. Estimated model parameters for exposure and motor scores

	Fine Motor Coordination	Manual Control	Body Coordination	Strength and Agility	Total Motor Composite
	B (se)	B (se)	B (se)	B (se)	B (se)
Model 1 (n=182)					
Gender	0.26 (0.45)	-1.94 (1.05)m	-1.81 (o.88)*	-0.36 (0.34)	-0.95 (0.36)*
Log BAs	-0.58 (0.32)[m]	-0.58 (0.77)	-0.17 (0.64)	-0.30 (0.25)	-0.28 (0.27)
Log BMn	0.15 (0.97)	1.26 (2.30)	-4.32 (1.91)*	-1.16 (0.74)	-1.13 (0.80)
R^2	.02	.02	.07	.03	.07
Model 2 (n=179)					
Gender	0.64 (0.62)	-1.83 (1.46)	-1.69 (0.85)	0.33 (0.46)	-0.66 (0.51)
Maternal Ed	0.80 (0.43)[m]	1.96 (1.01)[m]	1.70 (0.85)*	0.34 (0.32)	0.78 (0.36)*
Serum ferritin	0.10 (0.12)	0.14 (0.29)	-0.12 (0.24)	0.17 (0.09)[m]	0.05 (0.10)
Hgb	0.12 (0.21)	0.00 (0.49)	0.16 (0.41)	0.22 (0.16)	0.12 (0.17)
Log BAs	-0.53 (0.33)	-0.65 (0.78)	-0.04 (0.65)	-0.23 (0.25)	-0.24 (0.27)
Log BMn	-0.03 (1.01)	1.11 (2.51)	-4.96 (1.99)*	-1.32 (0.76)	-1.33 (0.83)
R^2	.05	.05	.09	.07	.09