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Manganese exposure and cognitive deficits: A growing concern for manganese neurotoxicity☆

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ABSTRACT

This symposium comprised five oral presentations dealing with recent findings on Mn-related cognitive and motor changes from epidemiological studies across the life span. The *first* contribution highlighted the usefulness of functional neuroimaging of the central nervous system (CNS) to evaluate cognitive as well as motor deficits in Mn-exposed welders. The *second* dealt with results of two prospective studies in Mn-exposed workers or welders showing that after decrease of Mn exposure the outcome of reversibility in adverse CNS effects may differ for motor and cognitive function and, in addition the issue of plasma Mn as a reliable biomarker for Mn exposure in welders has been addressed. The *third* presentation showed a brief overview of the results of an ongoing study assessing the relationship between environmental airborne Mn exposure and neurological or neuropsychological effects in adult Ohio residents living near a Mn point source. The *fourth* paper focused on the association between blood Mn and neurodevelopment in early childhood which seems to be sensitive to both low and high Mn concentrations. The *fifth* contribution gave an overview of six studies indicating a negative impact of excess environmental Mn exposure from air and drinking water on children's cognitive performance, with special attention to hair Mn as a potential biomarker of exposure. These studies highlight a series of questions about Mn neurotoxicity with respect to cognitive processes, forms and routes of exposure, adequate biomarkers of exposure, gender differences, susceptibility and exposure limits with regard to age.

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1. Introduction

Manganese (Mn) is an essential element and is required for adequate functioning of the human central nervous system (CNS). Food is the traditional source of Mn intake in humans and homeostatic mechanisms regulate blood Mn (B-Mn) at the required levels, which vary throughout the life span and are different for women and men. The concentration of Mn in blood is higher in women of childbearing age than in men and increases several-fold during pregnancy in mother and child. The high concentration of B-Mn in the neonate decreases slowly in the early years of life. Later in life, men and post-menopausal women have similar B-Mn concentrations. Increased oral intake or inhalation of Mn may lead to neurotoxic effects. In 1837, the full-blown clinical syndrome of Mn neurotoxicity, currently known as manganism, was described for the first time in workers with excessive occupational exposure to airborne Mn (air-Mn) (Couper, 1837).

☆ Xi'an Conference: Summary of Parallel Symposium-12 (ICOH), June 9, 2011. Chair: Harry A. Roels, Université catholique de Louvain, Brussels, Belgium. Co-chair: Rosemarie M. Bowler, San Francisco State University, CA, USA.

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Because of the obvious parkinsonian features of manganism, clinical diagnosis and epidemiologic research of Mn neurotoxicity have focused until the 1980s almost exclusively on motor/movement deficits resulting from Mn-induced disruptions in the basal ganglia (striatum, globus pallidus). In the 1990s, studies on early neurobehavioral manifestations in Mn-exposed workers focused primarily on early motor function deficits, whereas results from cognitive testing were inconsistent. More recently, especially since 2000, dose-related cognitive deficits have been reported from studies in welders occupationally exposed to Mn-containing fumes (Bowler et al., 2003, 2006, 2007a,b; Ellingsen et al., 2008) and from population-based investigations in children and adults environmentally exposed to Mn from a variety of sources including airborne particulate from industrial activities and mining, drinking water from aquifers, and Mn-based pesticides (Hudnell, 1999; Wasserman et al., 2006). These studies raise a series of new questions regarding Mn neurotoxicity with respect to cognitive processes, forms and routes of exposure, adequate biomarkers of exposure, gender differences, susceptibility and exposure limits with regard to age.

This symposium comprised five oral presentations dealing with recent evidence on Mn-related cognitive changes from epidemiological studies across the life span. 1) Dr. *Yangho Kim* highlighted the usefulness of functional neuroimaging of the CNS to evaluate cognitive and motor deficits in Mn-exposed welders; 2) Dr. *Harry Roels* showed by prospective studies in Mn-exposed workers and welders that, after a reduction of Mn exposure, the reversibility of adverse CNS effects may differ for motor and cognitive function, and addressed the issue of a reliable biomarker for air-Mn exposure; 3) Dr. *Rosemarie Bowler* presented results of an ongoing study assessing the relationship between environmental airborne Mn exposure and neurological and neuropsychological effects in adult Ohio residents living near a Mn point source; 4) Dr. *Birgit Claus Henn*'s paper, presented by Dr. David Bellinger, focused on the association between B-Mn and neurodevelopment in early childhood, which seems to be sensitive to both low and high Mn concentrations; 5) Dr. *Donna Mergler* gave an overview of studies indicating a negative impact of excess environmental Mn exposure from air and drinking water on children's cognitive performance.

2. Occupational exposure to manganese

2.1. Functional neuroimaging in welders exposed to manganese

Concern is rapidly growing as to Mn-induced cognitive neurotoxicity, while brain neuroimaging of Mn neurotoxic effects is shifting from morphological to functional features. As neuroimaging, such as magnetic resonance spectroscopy (MRS), functional MRI (fMRI), and diffusion tensor imaging (DTI), has become widely used in clinical research, we applied these neuroimaging techniques to evaluate changes in cognitive function in Mn-exposed welders.

The study participants were male welders, aged 40 years or older, who welded full-time in their current employment in a factory, each with more than 5 years of welding experience. The control group consisted of age-matched non-welding male production workers from the same factory. B-Mn was used as a biomarker of internal Mn exposure and neurobehavioral tests in combination with functional neuroimaging (MRS, fMRI, and DTI) were performed with the focus on cognitive deficits (verbal memory).

Proton-MRS showed that the ratios of N-acetylaspartate (NAA)/total creatine (tCr), Glx including both glutamine and glutamate (Glx)/tCr, and total choline (tCho)/tCr in both the anterior cingulate cortex (ACC) and parietal white matter (WM) did not differ significantly between 35 welders and 20 controls (Chang et al., 2009). However, the myo-inositol (mI) levels in the ACC, but not in parietal WM, were significantly lower in welders compared to controls. Further, in the frontal brain lobe mI/tCr ratio was significantly positively correlated with verbal memory scores and negatively with B-Mn. We suggest that depletion of mI/tCr in welders may reflect a possible glial cell effect rather than a neuronal effect associated with long-term exposure to Mn and a concomitant cognitive decline in the Mn-exposed welders.

Cognitive fMRI in 23 Mn-exposed welders and 21 controls during the 2-back memory task (Fig. 1) showed activation of the inferior frontal cortex, the basal ganglia (including the putamen), and the bilateral cerebellum, as well as activation of the common memory-related network of frontal and parietal cortical areas including the premotor cortex, the middle frontal cortex, the inferior and superior frontal cortex, the inferior and superior parietal cortex, the precuneus, and the cuneus (Chang et al., 2010). Between-group analysis for the memory task revealed in welders five areas of increased brain activities, i.e. the left (contralateral) primary sensory motor cortex, the right inferior parietal cortex, the anterior and posterior cingulated cortex, the bilateral inferior frontal cortex, and the basal ganglia when compared to controls. However, no region was significantly more activated in controls compared to welders. After controlling for age and educational level, the percentage change in activation of the parietal cortex was associated with the Korean Auditory Verbal Learning Test (K-AVLT), a delayed recall and recognition test. The percentage change in activation of the inferior frontal cortex was significantly associated with scores on the Stroop Color and Error indices. The percentage change in activation of the ACC was significantly associated with recognition on the K-AVLT and forward digit span test results. fMRI with 2-back memory task indicated that Mn-exposed welders are likely to require more neural resources in working memory networks to compensate for subtle deficits in working memory and altered working memory processes.

Direct comparison between 30 welders and 19 controls using investigator-independent Statistical Parametric Mapping (SPM) voxel-wise analysis of DTI metrics revealed in Mn-exposed welders a reduction in fractional anisotropy (FA) in the genu, body, and splenium of the corpus callosum (CC), and the frontal WM (Kim et al., 2011). The pallidal index showed a statistically significant correlation with FA in the genu (left), body, and splenium of the CC. B-Mn showed statistically significant correlations with FA in the genu (left) and body of the CC, and in the frontal WM. Further, marked increases in radial diffusivity (RD), but negligible changes in axial diffusivity (AD), were evident in the genu, body, and splenium of the CC, and the frontal WM. The pallidal index was significantly correlated with RD in the body of the CC. These findings suggest that microstructural changes such as a reduction of FA with increases in RD in the CC and the frontal WM result from a compromised radial directionality of fibers in such areas, primarily caused by demyelination. The correlations between FA in the frontal WM and the Stroop test scores suggest that poor performance on executive functioning is closely associated with lower FA values in the frontal WM.

In conclusion, cognitive deficits associated with Mn exposure have been reported in previous studies testing neuropsychological performance (Zoni et al., 2007). However, the present studies are the first to show 1) cognitive deficits expressed in terms of functional neuroimaging and 2) significant associations between neurobehavioral performances and brain function monitored by neuroimaging. Our studies suggest that functional neuroimaging such as MRS, fMRI, and DTI combined with neuropsychological performances have the potential to elucidate processes of cognitive deficits in humans exposed to Mn.

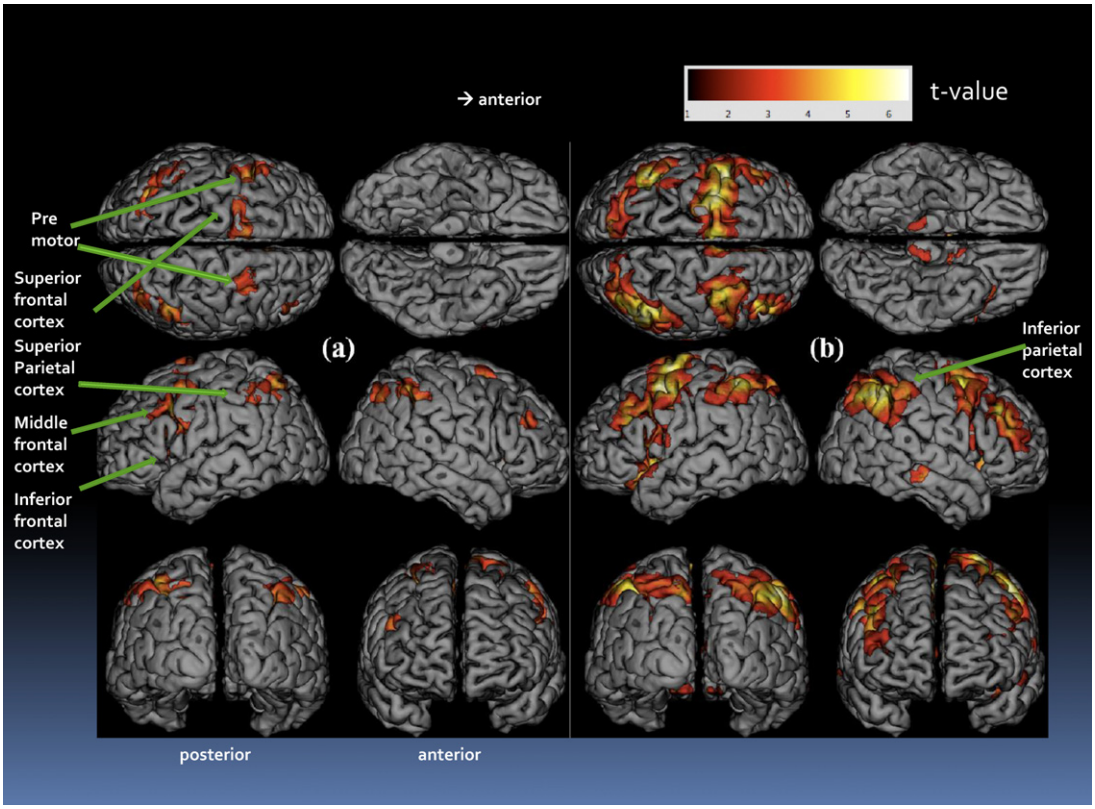

Fig. 1. The activations from within-group analysis in (a) controls and (b) welders in fMRI during the 2-back memory task ($p < 0.05$, false discovery rate used to correct for multiple comparisons).
(From Chang et al., 2010)

2.2. Reversibility outcome of early neurotoxic effects in workers after decrease of exposure and the permissible level of occupational Mn exposure

The toxicokinetics and toxicodynamics of inhalation exposure to Mn in the occupational setting depend to a great extent on particle size distribution [respirable particulate: <10 μm (welding fume); inhalable particulate: up to 100 μm (dust aerosol)]. Reversibility outcomes of early Mn-induced CNS effects in workers are rarely investigated for subclinical basal ganglia-related perturbations (motor inefficiency), cognitive deficits (e.g. working and delayed memory problems), and mood disturbances. The nature of these effects, the Mn exposure levels at which they occur, and their potential for progression or reversibility are of particular clinical and regulatory interest (MRC-IEH/IOM: Institute for Environment and Health/Institute of Occupational Medicine, 2004). There are only two well-designed prospective studies dealing with the outcome of early neurotoxic effects in occupational Mn exposure settings where exposure decreased or ceased over time. First, there is the 8-year longitudinal study in a Belgian dry-alkaline battery plant which showed that the extent of improvement of hand-eye coordination was inversely proportional to the magnitude of the cumulative air-Mn exposure of the past (Roels et al., 1999). The results suggest that, beyond a certain level of air-Mn, the impact of the neurotoxic action of Mn on the brain has reached a point of no return rendering complete reversibility of early neurotoxic effects no longer possible. The other follow-up study was carried out in Canadian ferromanganese smelter workers 14 years after cessation of exposure and suggested persistent deficits for certain neuromotor functions, cognitive flexibility, and adverse mood states, while recovery occurred for other functions (Bouchard et al., 2007b). More follow-up studies in scenarios where air-Mn decreased or ceased over time are needed to fine-tune regulatory preventive measures focusing separately on respirable and inhalable Mn particulate. On the other hand, a reliable biomarker of exposure to Mn would facilitate preventive control of the exposure on the individual basis.

This summary gives an overview of the main results of three studies: 1) an 8-year follow-up of hand-eye coordination (HEC) in a cohort of 92 dry-cell battery workers exposed in Duracell-Belgium to Mn-dioxide dust aerosol (inhalable Mn particulate); 2) a re-testing after 3.5 years of neuromotor and neuropsychological functioning in a cohort of 26 confined space welders (respirable Mn particulate) of the San Francisco Bay Bridge; and 3) a separate study in 28 indoor welders (Belgium) exploring the potential of plasma Mn (P-Mn) as a reliable biomarker of Mn exposure in welders, because P-Mn is the compartment of Mn in the blood which is prone to homeostatic control and the most readily available fraction for Mn uptake in the organs (e.g. brain).

At the baseline study, the battery workers had on average 5.3 years of Mn inhalation exposure (mean: 1 mg Mn/m^3, inhalable particulate) and 25% of them showed decreased HEC performance in comparison to a control group ($n = 101$) (Roels et al., 1992). According to the cumulative past exposure and job title the cohort was composed of three groups (low, medium, and high exposure). For each exposure group, the annual average of the inhalable air-Mn levels decreased over nearly 8 years of follow-up from 0.47 to 0.16 mg Mn/m^3 for the low exposure group ($n = 23$), from 0.82 to 0.24 mg Mn/m^3 for the medium exposure group ($n = 55$), and from 2.94 to 0.74 mg Mn/m^3 for the high exposure group ($n = 14$). Over the same period, the HEC precision scores of the low exposure group raised to control values, whereas only limited improvement was shown in the medium and high exposure groups (Roels et al., 1999). The complete reversibility of the HEC precision scores in the

low exposure group allowed us to suggest a NOAEL (no-observed-adverse-effect level) of 0.16 mg Mn/m^3 for inhalable Mn-dioxide dust (Table 1A).

The San Francisco Bay Bridge welder cohort (n = 43) had on average 1.5 years of confined space welding (mean: 0.21 mg Mn/m^3, >90% respirable particulate). In the high exposure phase, some of them exhibited *locura manganica* (Mn madness), but at the baseline study all were diagnosed with neuromotor and neuropsychological deficits (Bowler et al., 2007b). At follow-up, 3.5 years later we were able to re-test a group of 26 welders of the original welder cohort and showed that most scores of the neuropsychological tests (including cognitive tests) improved significantly. The UPDRS scores did not improve significantly, the CATSYS test (Danish Product Development, 1996) results showed significant worsening, while for the other neuromotor tests the performances either remained *status quo* (fingertapping) or improved significantly (grooved pegboard, graphomotor tremor) (Table 1B). This suggests differential intrinsic vulnerabilities of the brain loci involved with Mn neurotoxicity. As the Mn exposure of the Bay Bridge welders frequently exceeded the Cal-OSHA TLV of 0.20 mg Mn/m^3 at baseline [88 Mn-air samples out of 159 non-short-term personal air samplings], a more stringent preventive measure is recommended for confined space welding (Bowler et al., 2011a).

Table 1
Reversibility of neuropsychological, neuromotor, and neurological effects in two groups workers with reduced exposure to airborne Mn since the baseline studies.

(A) Dry-alkaline battery plant workers about 8 years after baseline study in 1987

Exposure group	Mn air (mg/m^3)		HEC precision score as % of control value[a]	
	1987	1994/95	1987	1994/95
Low (n = 23)	0.471	0.158[b]	90	100[b]
Medium (n = 55)	0.818	0.241	83	90
High (n = 14)	2.941	0.744	71	83

(B) San Francisco Bay Bridge welders 3.5 years after baseline study in 2005

Performance variable	Change of test performance with respect to baseline study[c]
Neuropsychological tests	
Full IQ	=
Verbal IQ	++
Cognitive flexibility/executive function	++
Information processing speed	=
Visuo-spatial memory (Rey-Osterrieth)	
Immediate recall	+
Delayed recall	++
Visuo-motor tracking speed	+
Verbal skills	+ to +++
Neuromotor tests	
Motor dexterity (dominant hand)	
Fingertapping	=
Grooved pegboard (fine motor dexterity)	+
Graphomotor tremor	+
CATSYS[d]	
Postural hand tremor	−
Body sway intensity	−
UPDRS scores[e]	
Activities of daily living	(+)
Motor	(+)
Bradykinesia	(+)

[a] Hand-eye coordination (HEC), mean score in controls: 1987, 69.3 (n = 101); 1994/95, 68.5 (n = 39).
[b] At 100% reversibility: NOAEL in mg/m^3 for inhalable Mn-air particulate.
[c] Significant worsening; =, *status quo*; (+), not significant improvement; +, significant improvement; ++, large significant improvement; +++, very large significant improvement.
[d] CATSYS tests (Danish Product Development, 1996).
[e] Unified Parkinson's Disease Rating Scale.

Most of the airborne Mn particulate in welding fume is in the respirable fraction. Therefore, the bioavailability of Mn from respirable particulate is likely to be higher than for coarser dust exposure. As whole blood Mn is not a reliable biomarker for the characterization of individual Mn exposure, the utility of measuring plasma Mn as a biomarker of Mn exposure was examined in a group of 28 welders (Hoet et al., 2011). Ambient air-Mn exposure was determined by personal full-shift measurements on Monday and Tuesday, and on the same days, blood samples were collected before and after the welding shift. Air-Mn varied from 1.3 to 729 μg/m^3 (GM 27.7), while the P-Mn concentration increased on average by 33% compared to the mean value in controls (1.5 μg/L). On Monday after the welding shift, P-Mn correlated well on an individual basis with air-Mn above 10 μg/m^3. In spite of similar air-Mn exposure on Tuesday, the relationship between air-Mn and aftershift P-Mn strikingly differed, in that the inflection was less obvious and the slope of the regression line (P-Mn aftershift/log air-Mn) for a doubling of log air-Mn was 2.3 times lower than on Monday. On Monday (the first day of the workweek), a P-Mn value of 2 μg/L could distinguish air-Mn exposure above or below 20 μg/m^3 with a sensitivity of 69% and a specificity of 82%. This indicates that P-Mn is a promising biomarker of current exposure to Mn in welders and lends biological plausibility to the intended change for the Mn TLV-TWA of 20 μg/m^3 recently proposed by ACGIH for respirable Mn particulate.

3. Environmental exposure to manganese

3.1. Relationships between airborne manganese exposure, anxiety, and neuropsychological and neurological test results in adults

Exposures to high levels of Mn in fumes and dust in the workplace are a known risk for deteriorating psychiatric health, declining cognitive ability, and the onset of movement disorders similar to Parkinson's disease (Bowler et al., 2007a,b; Couper, 1837; Feldman, 1999). Findings from studies of exposures to environmental air-Mn are less conclusive and existing community studies of Mn exposure have focused more on movement disorders, rarely including a comprehensive neuropsychological assessment and standardized scales of affect and mood (Bowler et al., 1999). Here we provide a brief overview of an epidemiologic study in environmentally Mn-exposed adults which aimed to examine 1) whether there is a relationship between air-Mn exposure and neuropsychological test scores, neurological function, and generalized anxiety, and 2) whether there is an association between generalized anxiety and neuropsychological and neurological function.

Marietta, OH (USA) is a community with elevated air-Mn from industrial emissions from Eramet Marietta, Inc., a major U.S. ferro- and silicomanganese producer since the early 1950s (U.S. EPA, 1984, 2010). Mount Vernon, OH, was selected as a "comparison" town for its demographic similarity (U.S. Census Bureau, 2001a,b) and low number of major industries (U.S. EPA, 2011). A random sample of 100 exposed and 90 comparison residents, aged 30–75 years, participated in this study (for inclusion/exclusion criteria, see Bowler et al., 2011b).

A comprehensive neuropsychological test battery was administered, including (but not limited to) tests of category fluency (Animal Naming), processing speed (WAIS-III Digit Symbol Coding), visuospatial learning (NAB Shape Learning), and visuospatial memory (Rey-Osterrieth Complex Figure Test). The Symptom Checklist-90-Revised (SCL-90-R) (Derogatis, 1992) was included as measure of mood, and a generalized anxiety composite score was computed as the arithmetic mean of the Anxiety, Phobic Anxiety, and Obsessive-Compulsive scales. The Bradykinesia and Motor scales of the Unified Parkison's Disease Rating Scale (UPDRS)

were administered (Goetz et al., 2003). Blood levels of Mn, Pb, Hg, and Cd were measured, along with ferritin and hepatic enzymes (ALT and GGT) in serum. Air-Mn exposure indices were modeled for Marietta residents using the AERMOD modeling system (U.S. EPA, 2009). Model inputs included 2001 air-Mn emission data, terrain and meteorologic data.

Outcome, biomarker, and exposure variables were compared between the two towns using Student's *t*-test, ANCOVAs, and Fisher's exact test. For Marietta, a hazard quotient (HQ) was computed by dividing the modeled air-Mn concentration by the U.S. EPA reference concentration (RfC) of 0.05 μg Mn/m^3 and a cumulative exposure index (CEI) was computed as the product of air-Mn with years of residence. For analyses, natural logarithm transformations of values for B-Mn, CEI, and HQ were used, as the arithmetic values were not normally distributed. Multiple regression models were computed using outcome variables and indices of Mn exposure (ln B-Mn, ln CEI, ln HQ), and using generalized anxiety and outcome variables.

The exposed participants resided on average 4.75 miles (range 1–11) from the Mn point source. Their modeled residential air-Mn estimate ranged from 0.04 to 0.96 μg/m^3 (on average 0.18 μg/m^3). The group means of B-Mn were similar for the exposed (9.65 μg/L) and comparison (9.48 μg/L) participants, while the groups did not differ significantly on blood Pb, Hg, or Cd, nor on levels of serum ferritin or hepatic enzymes.

There were no significant differences between the towns on neuropsychological test scores. For the Mn-exposed group, ln CEI significantly predicted performance on Animal Naming ($\beta = -0.20$, $p = 0.04$) and Digit Symbol Coding ($\beta = -0.23$, $p = 0.02$). Ln HQ in the exposed group significantly predicted performance on Animal Naming ($\beta = -0.19$, $p = 0.05$), NAB Shape Learning ($\beta = -0.22$, $p = 0.03$), Rey-Osterrieth Immediate Recall ($\beta = -0.20$, $p = 0.05$), and Rey-Osterrieth Delayed Recall ($\beta = -0.25$, $p = 0.01$). The exposed group reported higher SCL-90-R generalized anxiety than the comparison group ($p = 0.035$). Generalized anxiety in the exposed group was positively related to ln CEI ($p = 0.002$) and ln HQ ($p = 0.049$), and to worse scores on Animal Naming ($p = 0.003$), Rey-Osterrieth Immediate Recall ($p = 0.005$), and Rey-Osterrieth Delayed Recall ($p = 0.001$).

The Mn-exposed group had higher scores than the comparison group on the UPDRS Bradykinesia scale ($p = 0.048$) and Total Motor scale ($p = 0.034$). In the exposed group, generalized anxiety was significantly related to UPDRS Activities of daily living ($p < 0.001$), Bradykinesia ($p = 0.004$), and Total Motor ($p = 0.027$).

Although no differences in neuropsychological test scores were found between the groups, the observed relationships of CEI and HQ with generalized anxiety, category fluency, processing speed, visuospatial learning and memory suggest the existence of some association of anxiety states and neuropsychological function with environmental air-Mn exposure at the Marietta site. It remains an open question whether these associations are due to direct neurotoxic effects of air-Mn or to the participants' concern about the potential health effects of air pollution. These results highlight the importance of assessing both neuropsychological function and anxiety in population groups environmentally exposed to Mn.

3.2. Blood manganese concentrations and postnatal neurodevelopment

Manganese is an essential metal that has been shown to be neurotoxic to humans at increased uptake. Adverse neurologic and neuropsychological effects have been reported in occupational settings with high-level exposure, as well as among adults and children following low-level environmental exposure. Reported neuropsychological/cognitive effects include impaired learning and recall, lower cognitive performance, motor function deficits, and behavioral effects (Santos-Burgoa et al., 2001; Wright et al., 2006; Bouchard et al., 2007a; Wasserman et al., 2006; Rodriguez-Agudelo et al., 2006). Among children, previous studies have focused on effects of exposure to children over age five. Effects on neurodevelopment from early childhood Mn exposure, however, have not been studied. This is a period of rapid brain development and includes the initiation of the response to sensory input following fetal life (Nowakowski and Hayes, 1999). Therefore, the early postnatal period may be a critical time in which to study Mn exposure, as timing of exposure often determines the dose–response curve for toxicity.

We used a prospective study design to examine whether early-life Mn exposure is associated with neurodevelopmental effects among children. Mother–infant pairs were enrolled during pregnancy or at 1-month postpartum from prenatal clinics and maternity hospitals in Mexico City. For this analysis, we examined 448 children who were followed from birth to the age of 3 years. Archived blood samples collected at 1 and 2 years of age were analyzed for Mn concentrations (B-Mn) with a dynamic-reaction-cell inductively coupled plasma-mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, CT). We assessed child neurodevelopment at 6-month intervals (i.e., at 12, 18, 24, 30, and 36 months of age) using the Bayley Scales of Infant Development-II (Spanish version), an age-adjusted test of development. Scores from the Mental Development Index (MDI; assessing cognition) and Psychomotor Development Index (PDI; assessing motor development) were used as the primary outcomes.

We examined potential nonlinearity in the association between B-Mn and neurodevelopment using penalized splines in generalized additive models. In adjusted multivariable regression models, we modeled B-Mn as a continuous variable (for linear associations) or used indicator variables for quintiles of the B-Mn distribution (for nonlinear associations). Neurodevelopment scores were approximately normally distributed and were modeled as continuous outcomes. We fit separate multivariable regression models for each B-Mn measurement and for each time point of the neurodevelopment assessment (i.e., 12-month B-Mn predicting 12-, 18-, 24-, 30-, and 36-month Bayley scores; 24-month B-Mn predicting 24-, 30-, and 36-month Bayley scores). We also made use of all the repeated measures of Bayley scores in linear mixed effects models.

At 12 and 24 months of age, the mean (SD) B-Mn concentrations were 24.3 (4.5) μg/L and 21.1 (6.2) μg/L, respectively. 12- and 24-month B-Mn levels were correlated (Spearman correlation = 0.55) showing a decline of B-Mn over time [$\beta = -5.7$; 95% confidence interval (95% CI): −6.2 to −5.1], which is consistent with evidence of declining B-Mn with increasing age (Spencer, 1999; Chan et al., 1992; Collipp et al., 1983). We observed a nonlinear association between 12-month B-Mn and 12-month MDI ($p = 0.04$). The smoothed plot (Fig. 2) of this association estimates the highest MDI score of 97.7 points at a B-Mn of 24.4 μg/L. At the 5th and 95th percentiles of B-Mn (18.1 μg/L and 32.5 μg/L, respectively), MDI scores were estimated to be 93.9 points (95% CI: 91.7–96.0) and 94.2 points (95% CI: 91.6–96.8), respectively. Given the evidence of nonlinearity for the effect of 12-month B-Mn on neurodevelopment, we fit a regression model with indicator variables for each quintile of B-Mn. Higher 12-month MDI scores were estimated among children in B-Mn quintile-3 (22.5–25.1 μg/L) compared to children in the lowest B-Mn quintile (15.3–20.1 μg/L) ($\beta = 4.8$; 95% CI: 1.6–8.1). We subsequently fit regression models comparing MDI of the lowest and highest B-Mn quintiles to the middle three quintiles (i.e., quintiles 2–4 collapsed) in order to further describe Mn effects on MDI at the upper and lower ends of the B-Mn distribution. Lower 12-month MDI scores were observed among children in the lowest and highest B-Mn quintiles compared to children in the middle three quintiles (Table 2).

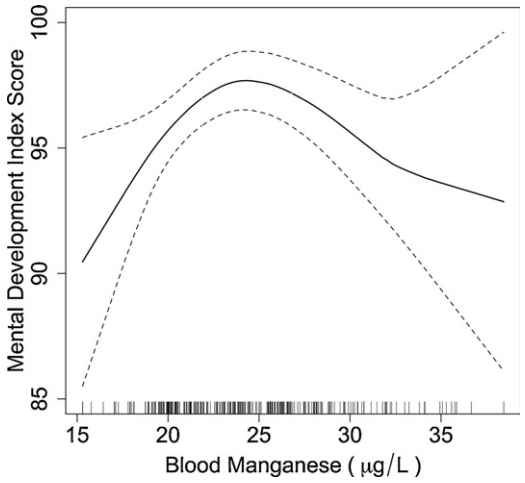

Fig. 2. Penalized spline for 12-month blood manganese (μg/L) predicting 12-month MDI, controlling for sex, 12-month blood lead, 12-month hemoglobin, gestational age, maternal IQ, and maternal education, among 270 children. The solid line represents the estimate; dotted lines represent the 95% confidence interval. Vertical lines on the *x*-axis represent the distribution of blood manganese.
(From Claus Henn et al., 2010)

Mixed effects models of repeated outcome measures demonstrated lower MDI scores among children with 12-month B-Mn in the lowest quintile ($\beta = -2.4$; 95% CI: −4.3 to −0.5) and somewhat lower scores in the highest quintile ($\beta = -0.9$; 95% CI: −2.8 to 1.1), compared with children in the middle three quintiles. There was no association of 24-month B-Mn with the 24- to 36-month MDI scores at any time point, or in repeated measures models. There was no association of 12- or 24-month B-Mn with PDI scores at any time point.

In summary, we observed an inverted U-shaped association between 12-month B-Mn and concurrent mental development, suggesting that both low and high B-Mn levels may have adverse neurological effects. The nonlinear association is consistent with Mn as both an essential nutrient and a toxicant. Mn protects against oxidative stress at physiologic levels, while at high levels Mn itself acts as an oxidant. Because of the association for 12-month B-Mn but not for 24-month B-Mn, it is possible that 12 months is a sensitive time point for Mn effects on cognition. The suggestive evidence of a nonlinear association between B-Mn and neurodevelopment may indicate the need to focus on early childhood, as this appears to be a developmental stage during which children are sensitive to both low and high Mn levels.

3.3. Manganese exposure and children's cognitive performance

There is increasing interest in environmental exposure to Mn and its effects on children's cognitive capacities and behaviors. Here, we examine six studies on cognitive function in school-age children in relation to identified and measured sources and biomarkers of Mn exposure, and pointed out some of the gaps in our knowledge.

Two studies examined children exposed to airborne Mn in the vicinity of Mn mining and/or transformation in Mexico (Riojas-Rodríguez et al., 2010) and Brazil (Menezes-Filho et al., 2011). In Mexico, median air-Mn PM_{10} in the exposed region was 0.13 μg Mn/m^3, while in Brazil, where $PM_{2.5}$ was measured, median air-Mn concentration was 0.11 μg Mn/m^3. Blood and hair Mn (H-Mn) concentrations were higher for Mexican children from the Mn-exposed area ($n = 79$) compared to those ($n = 93$) from a non-exposed region (median B-Mn, 9.5 μg/L and 8.0 μg/L; median H-Mn, 12.6 μg/g and 0.56 μg/g, respectively). For Mn-exposed children in Brazil ($n = 83$), median B-Mn and H-Mn were 8.2 μg/L

Table 2
Crude and adjusted effect estimates from regression models of 12-month blood manganese quintiles predicting Mental Development Index.

Covariates	12-month ($n = 270$)				18-month ($n = 267$)				24-month ($n = 268$)			
	Crude		Adjusted		Crude		Adjusted		Crude		Adjusted	
	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)	Beta	(95% CI)
Mental Development Index												
Blood Manganese, Lowest Quintile[a,b]	−3.08	(−5.84 to −0.32)	−3.35	(−5.99 to −0.70)	−3.30	(−5.99 to −0.62)	−3.40	(−6.07 to −0.74)	−1.26	(−4.98 to 2.46)	−1.51	(−5.18 to 2.17)
Blood Manganese, Highest Quintile[a,c]	−3.30	(−6.06 to −0.55)	−2.84	(−5.51 to −0.17)	0.24	(−2.45 to 2.92)	−0.10	(−2.80 to 2.59)	−0.40	(−4.09 to 3.29)	−0.95	(−4.64 to 2.74)
12-mo Blood Lead (μg/dL)			−0.18	(−0.58 to 0.22)			−0.27	(−0.67 to 0.13)			−0.44	(−1.00 to 0.11)
Child's sex[d]			3.15	(1.07 to 5.24)			1.85	(−0.26 to 3.95)			3.13	(0.25 to 6.02)
Mother's IQ			0.07	(−0.02 to 0.17)			−0.03	(−0.12 to 0.07)			0.02	(−0.11 to 0.14)
Mother's total years of school			0.02	(−0.41 to 0.46)			0.52	(0.08 to 0.97)			0.66	(0.06 to 1.25)
12-Mo Hemoglobin (g/dL)			1.15	(0.34 to 1.96)			−0.11	(−0.93 to 0.71)			−0.24	(−1.36 to 0.88)
Gestational age (wks)			1.03	(0.22 to 1.84)			0.72	(−0.10 to 1.54)			0.25	(−0.88 to 1.39)

From Claus Henn et al. (2010).
[a] Comparison group is children in middle three quintiles of manganese distribution (i.e., quintiles 2, 3, and 4).
[b] Lowest quintile of 12-month blood manganese is <20.2 μg/L
[c] Highest quintile of 12-month blood manganese is >28.0 μg/L.
[d] Child sex coded as: 0 = male, 1 = female.

and 5.83 μg/g, respectively. A previous study in this region (Menezes-Filho et al., 2009) reported a gradient of H-Mn in children living closest to the plant or downwind presenting the highest concentrations (median H-Mn around 30 μg/g), while the median H-Mn in the reference group was 1.2 μg/g. Correlations between B-Mn and H-Mn were weak (Mexico: $r = 0.22$; $p = 0.01$), or inexistent (Brazil: $r = 0.\ 05$; $p = 0.66$). Both studies used the Wechsler Intelligence Scale for Children (WISC-III), validated for their respective countries. The adjusted Full Scale IQ was significantly and inversely associated with H-Mn, but not with B-Mn.

The other four studies investigated children whose drinking water contained Mn from anthropogenic sources in China (He et al., 1994) or naturally from the bedrock in Bangladesh (Wasserman et al., 2006, 2011) and Quebec, Canada (Bouchard et al., 2011). The Chinese study used a matched-pair design with 92 exposed and 92 referents; water Mn (W-Mn) for the exposed varied between 241 and 346 μg/L, while the referents' drinking water contained 30–40 μg/L. In the Bangladesh studies, where recruitment was initially carried out with respect to arsenic (As) in drinking water, the 142 children investigated with respect to Mn exposure used water from wells with ≤10 μg As/L and a mean W-Mn concentration of 795 ± 755 μg/L, ranging from 4 to 3908 μg/L (Wasserman et al., 2006). Recruitment of children ($n = 299$) for the second study was on the basis of both As and Mn in well water; average W-Mn was 725.5 ± 730.0 μg/L, ranging from 40 to 3442 μg/L (Wasserman et al., 2011). In Quebec, 362 children were enrolled in a study on the basis of Mn concentrations in municipal wells that used groundwater; mean home tap water Mn was 97 ± 212 μg/L, ranging from 1–2700 μg/L (Bouchard et al., 2011), with 97% of the children using tap water below the WHO recommended concentration of 400 μg Mn/L. Although all of these studies showed an inverse relation between Mn in well water and/or biomarkers of Mn exposure and cognitive performance, they did not use the same tests, nor did they use the same biomarkers of exposure.

The Chinese study reported poorer performance for the Mn-exposed children compared to the non-exposed as to Digit Span, Digit Symbol, Benton Visual Retention, Pursuit Aiming, and Santa Ana, as well as an inverse association between test scores and H-Mn (He et al., 1994). In the first study in Bangladesh, the authors reported an inverse association between Full Scale IQ on the WISC-III and well water Mn concentration, but not with B-Mn (Wasserman et al., 2006). The second Bangladesh study (Wasserman et al., 2011), designed to examine possible synergy between As and Mn exposures, adapted the WISC-IV for this population; B-Mn, but not W-Mn, was significantly associated with several subscales, notably, Perceptual Reasoning and Working Memory. No interaction was observed between the levels of As and Mn in water; H-Mn was not assessed. In the Quebec study, W-Mn and H-Mn were inversely associated with Full Scale IQ assessed using the Wechsler Abbreviated Scale of Intelligence (WASI), with a stronger association for W-Mn (Bouchard et al., 2011).

We combined the results from the 617 children from the Mexico, Brazil and Quebec studies; all used H-Mn as a biomarker of Mn exposure and had a measure of Full IQ. Using multiple linear regression models, we first adjusted Full IQ score for each study separately on mothers' Raven score, mothers' education, *z*-score of height for age and then examined the association with H-Mn, taking into account study, age, and sex. We then stratified for sex and re-did the analyses. The results show an overall decrease in Full IQ of 2.62 points for a 10-fold increase in H-Mn, the interaction term H-Mn and sex is significant ($p = 0.04$) with greater loss estimate of Full IQ in girls compared to boys [−4.19 (95% CI: −6.19 to −2.07) and −1.08 (95% CI: −3.21 to −1.05), respectively]. These results are particularly interesting in light of recent findings showing long lasting neuronal morphological changes in female, but not in male mice following Mn exposure, despite similar striatal Mn accumulation and decline (Madison et al., 2011).

The results of these studies indicate a negative impact of excess environmental Mn on children's cognitive development, although there are still many questions to answer with respect to adequate biomarkers of Mn exposure, other sources of Mn exposure, such as from spraying of Mn-based pesticides like Maneb and Mancozeb, the overall profile of cognitive deficits and the possible role of prenatal excess Mn exposure. There is likewise a need for a program of inter-laboratory calibration for Mn biomarkers, especially H-Mn. Moreover, since Mn is an essential element involved in many vital processes, girls and boys may present different responses.

4. Integrative discussion

According to the WHO's (1981) Environmental Health Criteria for manganese, one may estimate for healthy western adults an average oral intake of Mn via food of about 3.6 mg/day and a GI absorption rate not higher than 5%, which would represent a systemic Mn absorption of 180 μg/day. If the liver had no homeostatic control over the systemic Mn status, the Mn absorbed via the GI tract would lead to a concentration of about 40 μg Mn per liter of circulating blood (assuming 4.5 L of blood for an adult). The Toxicological Profile of Manganese (ATSDR, 2008) reports a range for whole B-Mn from 4 to 15 μg/L. In Europe (Germany), a biological reference value of 15 μg/L is set for whole blood, a threshold which is the presumed 95th percentile of the distribution in the general population (Pesch et al., 2012). However, a representative study on whole blood manganese in adult European or American men and women is still lacking, let alone in children and newborns. Whole blood contains four major compartments of Mn: 66% of Mn is in the red blood cells, 4.4% in the plasma, 23.2% in the white blood cells, and 6.6% in the platelets (Milne et al., 1990). Plasma manganese is considered the most readily biologically available fraction in the blood pool for uptake in the organs. In normal adults without increased exposure to Mn, the P-Mn concentration most likely does not exceed 2 μg Mn per liter plasma (Hoet et al., 2011). To cope with a daily dietary absorption of 180 μg Mn in normal adults, the homeostatic system for Mn must be very effective to maintain the plasma concentration of Mn within a physiologically normal range, which is accomplished by the bile, the main excretion pathway for Mn in humans.

In this symposium, different biomarkers of exposure to Mn were used to characterize individual exposures: B-Mn and P-Mn for exposure in welders, B-Mn and/or H-Mn for environmental exposure to airborne Mn or to drinking water Mn, and the special case of whole blood Mn as a reflection of the newborns' "exposure in *utero*" to the Mn status of pregnant women whose B-Mn rises for physiological reasons. Occupational exposure to Mn occurs usually via inhalation of airborne Mn particulate of which the particle size determines to a large extent the bioavailability of Mn and hence the toxicodynamic outcome. This is particularly the case in welders exposed to welding fume aerosols in which the particulate is more than 90% in the respirable fraction and may reach the alveolar compartment of the lungs. A great part of Mn absorbed via this pulmonary route is conveyed with the blood circulation directly to the brain, thus bypassing the homeostatic control of the liver. Nonrespirable particulate, impacted in the upper airways, travels upwards via the mucociliary escalator, is swollen down and eventually handled as oral intake of Mn. This is the reason why inhalation of welding fumes has a much greater potential of causing neurotoxic effects than inhalation of coarser dust particulate. It is not surprising that the health-based preventive measures of ACGIH recommend different TWA-TLVs for Mn inhalation, i.e. 20 μg/m^3 for respirable and 200 μg/m^3 for

inhalable particulate. Chronic overexposure to Mn in the occupational setting may lead to accumulation of this metal in the brain which can be evidenced by MRI and may entail neurotoxic effects as illustrated by the occupational exposure contributions in this symposium. Functional neuroimaging combined with neuropsychological tests showed the potential to elucidate processes of cognitive deficits. Furthermore, neuropsychological cognitive test scores showed significant improvement after Mn exposure ceased or decreased, whereas deficits in most of the motor test scores did not. HEC precision deficit has been shown to be completely reversible only when previous cumulative exposure did not exceed "some critical level". This suggests differential intrinsic vulnerabilities of the brain loci involved with Mn neurotoxicity.

Despite the evidence of inconsistent findings as to the relationship between whole B-Mn and exposure to Mn, B-Mn is still used as biomarker of occupational Mn exposure. It remains still to be elucidated whether it reflects current, recent or long-term exposure. On a group basis, B-Mn is useful to document internal exposure, however, on the individual basis only P-Mn seems to be a promising biomarker of exposure to Mn in welders. As a biomarker of environmental exposure to Mn, either via air or drinking water, B-Mn also showed unusual characteristics. A recent epidemiological investigation comparing adult residents from Marietta (OH), a town with elevated air-Mn (on average 0.18 $\mu g/m^3$) due to industrial emissions, and Mount Vernon (OH), a comparison town, showed similar mean B-Mn concentrations, i.e. 9.65 and 9.48 μg/L, respectively. No differences in neuropsychological test scores were found between the two towns. It remains an open question whether the associations between the cumulative exposure index (based on air-Mn) and several neuropsychological outcomes, particularly generalized anxiety, are due to direct neurotoxic effects of air-Mn or to the concern/fear of the Marietta residents about the potential health effects of air pollution.

The last two contributions of this symposium deal with cognitive performance in six studies on school-aged children and one study on postnatal neurodevelopment in relation to environmental Mn exposure. Increased air-Mn was shown in two children studies, one from Mexico (PM_{10}, median 0.13 $\mu g/m^3$) and one from Brazil ($PM_{2.5}$, median 0.11 $\mu g/m^3$). Adjusted Full Scale IQ was significantly and inversely associated with H-Mn, but not with B-Mn. The other four studies investigated children whose drinking water was contaminated with Mn, one from China (W-Mn: 241–346 μg/L), two from Bangladesh [W-Mn: mean 795 μg/L (4-3908) and mean 726 μg/L (40–3442)], and one from Quebec [(W-Mn: mean 97 μg/L (1–2700)]. Although these studies showed inverse relation between cognitive performance and W-Mn and/or biomarkers of exposure, they are difficult to compare as the cognitive tests used differed and the relationships with regard to the biomarker of exposure (either B-Mn or H-Mn) were inconsistent. An analysis of three children studies combined (Mexico, Brazil, Quebec) was possible as the studies used H-Mn as a biomarker of Mn exposure and had a measure of Full IQ. The study outcome showed an overall decrease in Full IQ of 2.62 points for a 10-fold increase in H-Mn and a significant ($p = 0.04$) interaction term H-Mn × sex with a greater loss of Full IQ in girls compared to boys. Taken together, these studies suggest a negative impact of excess environmental Mn exposure on children's cognitive development, however, consistency as to the association with variables reflecting internal Mn dose needs confirmation. The contribution dealing with early childhood Mn exposure effects on neurodevelopment fills a gap in our current knowledge. The period following fetal life is characterized by rapid brain development and the initiation of the response to sensory input. That the early postnatal period may be critical as to Mn exposure is a valid research hypothesis. However, one should bear in mind that the fetus' environmental exposure to Mn occurs in the womb of the mother and is subjected to physiological demands of Mn for fetal and early postnatal development, e.g. skeleton and brain. Whole B-Mn is increased in pregnant women and the newborn, but the question remains whether the physiologically active fraction of Mn in the blood in the fetal stage is the Mn in the red blood cells, representing the bulk of the blood Mn, or a free circulating form of Mn transiently elevated because of the pregnancy status. Nevertheless, this prospective study showed an inverted U-shaped association between 12-month B-Mn and concurrent mental development, suggesting that both low and high B-Mn levels may have adverse neurological effects.

Environmental exposure to airborne Mn in adults did not seem to reach a sufficient level to entail a critical concentration of Mn accumulation in the brain causing neurotoxic effects. Recently developed pharmacokinetic models for non-human primates and humans (Schroeter et al., 2011) seem to indicate that long-term exposure to respirable particulate above 20 $\mu g\ Mn/m^3$ is expected to lead to Mn accumulation in the globus pallidus. It is thus clear that for airborne Mn only occupational exposures may exceed such an exposure threshold, whereas in the environmental air-Mn exposure studies the air-Mn levels are at least two orders of magnitude lower. With respect to the children's exposure to Mn in drinking water, a daily consumption of 1 L would likely constitute a health risk in those individuals drinking water containing more than 2000 μg Mn/L. According to the Toxicological Profile of Manganese (ATSDR, 2008), the Estimated Safe and Adequate Daily Intake for Mn in children of 4–10 years old has been estimated 1–2 mg/day. In none of the drinking water studies does W-Mn exceed the average concentration of 1 mg/L and in the Quebec study the mean W-Mn did not reach even 0.1 mg/L. With regard to the W-Mn studies in children it remains an open question to what extent potential confounders (e.g. demographic variables) or effect modifying factors (e.g. Fe-deficiency, possible greater GI absorption rate for W-Mn) still influence the outcome of the multiple regression models. Finally, with regard to H-Mn, a prerequisite for its usefulness as a biomarker of W-Mn or air-Mn exposure is the demonstration of unequivocal toxic-kinetic relationships between the external exposure, the Mn dose in the organism (internal exposure) and the excretion rate of Mn with type and growth of hair.

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