View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Harvard University - DASH

DIGITAL ACCESS TO SCHOLARSHIP AT HARVARD
DASH.HARVARD.EDU

HARVARD LIBRARY
Office for Scholarly Communication

Maternal and Cord Blood Manganese Concentrations and Early Childhood Neurodevelopment among Residents near a Mining-Impacted Superfund Site

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation	Claus Henn, Birgit, David C. Bellinger, Marianne R. Hopkins, Brent A. Coull, Adrienne S. Ettinger, Rebecca Jim, Earl Hatley, David C. Christiani, and Robert O. Wright. 2017. "Maternal and Cord Blood Manganese Concentrations and Early Childhood Neurodevelopment among Residents near a Mining-Impacted Superfund Site." Environmental Health Perspectives 125 (6): 067020. doi:10.1289/EHP925. http://dx.doi.org/10.1289/EHP925.
Published Version	doi:10.1289/EHP925
Citable link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:34651965
Terms of Use	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP925.

Maternal and Cord Blood Manganese Concentrations and Early Childhood Neurodevelopment among Residents near a Mining-Impacted Superfund Site

Birgit Claus Henn,[1] David C. Bellinger,[2,3,4] Marianne R. Hopkins,[2] Brent A. Coull,[5] Adrienne S. Ettinger,[6] Rebecca Jim,[7] Earl Hatley,[7] David C. Christiani,[2] and Robert O. Wright[8]

[1]Department of Environmental Health, Boston University School of Public Health, Boston, Massachusetts, USA
[2]Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[3]Department of Neurology, Harvard Medical School and Boston Children's Hospital, Boston, Massachusetts, USA
[4]Department of Psychiatry, Harvard Medical School and Boston Children's Hospital, Boston, Massachusetts, USA
[5]Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
[6]Department of Nutritional Sciences, University of Michigan School of Public Health, Ann Arbor, Michigan, USA
[7]Local Environmental Action Demanded (L.E.A.D.) Agency, Inc., Vinita, Oklahoma, USA
[8]Division of Environmental Health, Icahn School of Medicine at Mount Sinai, New York, New York, USA

Background: Environmental manganese exposure has been associated with adverse neurodevelopmental outcomes among school-aged children; yet, few studies have evaluated prenatal exposure.

Objectives: Our study examines associations between prenatal manganese concentrations and placental transfer of manganese with neurodevelopment in 224 2-y-old children residing near the Tar Creek Superfund Site.

Methods: We collected maternal and cord blood at delivery, measured manganese using inductively coupled plasma mass spectrometry, and assessed neurodevelopment using the Bayley Scales of Infant Development-II. Associations between manganese and mental (MDI) and psychomotor (PDI) development indices were estimated in multivariable models. Placental transfer, approximated by cord/maternal manganese ratio, cord/total manganese ratio (total = maternal + cord), and by joint classification according to high or low (above or below median) maternal and cord manganese, was evaluated as a predictor of neurodevelopment.

Results: Median levels [interquartile ranges (IQR)] of manganese in maternal and cord blood, respectively, were 24.0 (19.5–29.7) and 43.1 (33.5–52.1) μg/L. Adjusting for lead, arsenic, and other potential confounders, an IQR increase in maternal manganese was associated with −3.0 (95% CI: −5.3, −0.7) points on MDI and −2.3 (95% CI: −4.1, −0.4) points on PDI. Cord manganese concentrations were not associated with neurodevelopment scores. Cord/maternal and cord/total manganese ratios were positively associated with MDI [cord/maternal: β = 2.6 (95% CI: −0.04, 5.3); cord/total: β = 22.0 (95% CI: 3.2, 40.7)] and PDI (cord/maternal: β = 1.7 (95% CI: −0.5, 3.9); cord/total: β = 15.6 (95% CI: 0.3, 20.9)). Compared to mother–child pairs with low maternal and cord manganese, associations with neurodevelopment scores were negative for pairs with either high maternal, high cord, or high maternal and cord manganese.

Conclusions: Maternal blood manganese concentrations were negatively associated with early childhood neurodevelopment scores in our study. Findings highlight the importance of understanding maternal exposures during pregnancy and factors influencing placental transfer. https://doi.org/10.1289/EHP925

Introduction

Manganese (Mn) is a trace essential element, necessary for physiologic processes such as neuronal function (Prohaska 1987; Sloot and Gramsbergen 1994), protein and energy metabolism, bone growth (Aschner and Aschner 2005; Hurley 1981), and enzyme activation (Erikson and Aschner 2003). During fetal and neonatal development, there is an increased need for manganese due to its critical role in brain function and skeletal development (Hurley 1981). Manganese crosses the placenta via active transport (Yoon et al. 2009), likely reflecting fetal nutrient demand. However, excess or accumulated manganese exposure can be neurotoxic and has been associated with deficits in cognition and motor function (Sanders et al. 2015; Zoni and Lucchini 2013). Little is known about how manganese transfer from the mother is regulated. The way in which manganese is partitioned in the maternal/fetal unit may be an important factor in fetal development (Kopp et al. 2012).

Concerns about heightened potential sensitivity to manganese neurotoxicity during fetal and early life compared to adulthood have recently been raised. Research is complicated by the increased fetal demand for manganese during development, as well as the unique physiology of the fetus and infant in which rapid growth makes it susceptible to nutrient deficiency. Increasing maternal blood manganese levels during pregnancy may by a physiologic response to this fetal demand, but the optimal range of manganese levels has not been determined and it remains unclear at what level maternal blood manganese may become harmful to the fetus. Manganese crosses the blood–brain barrier in the fetus at a higher rate than in adults, based on animal experimental data (Cahill et al. 1980; Kostial et al. 1978; Takeda et al. 1999). Because of the developing brain's high oxygen and energy consumption, it is sensitive to oxidative stress and damage from free radicals that can result from elevated manganese exposure (Blomgren and Hagberg 2006; Buonocore et al. 2001; Ikonomidou and Kaindl 2011). Given that neurodevelopment occurs as a cascade of well-timed, regulated events, exposure to toxic insults can cause damage at any stage, which may impair subsequent processes and result in developmental disability (Nowakowski and Hayes 1999).

Environmental manganese exposure has been associated with various neurodevelopmental outcomes among school-age children (Bouchard et al. 2011; Khan et al. 2012; Oulhote et al. 2014a; Wasserman et al. 2006; Wright et al. 2006). Relatively

Address correspondence to B. Claus Henn, Boston University School of Public Health, Department of Environmental Health, 715 Albany St., Boston, MA 02118 USA. Telephone: (617) 638-4653. Email: bclaus@bu.edu

Supplemental Material is available online (https://doi.org/10.1289/EHP925).

R.J. and E.H. were employed by the Local Environmental Action Demanded (L.E.A.D.) Agency, Inc., Vinita, OK, USA (http://www.leadagency.org/), at the time this study was conducted and are currently volunteers for the agency. The other authors declare they have no actual or potential competing financial interests.

Received 4 December 2015; Revised 20 November 2016; Accepted 30 November 2016; Published 28 June 2017.

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

few studies, however, have evaluated neurotoxic effects of prenatal manganese exposure. An umbilical cord serum manganese concentration greater than 5.0 μg/L was associated with poorer performance on neurobehavioral tests in a population-based study of 933 3-d-old neonates in China (Yu et al. 2014). An inverted U-shaped association was reported between maternal blood manganese at delivery and mental and psychomotor development scores among 232 6-mo-old Korean infants with no known exposure source (Chung et al. 2015). Prenatal exposure estimated in deciduous teeth was not associated with mental and psychomotor development scores among 197 six- to 12-mo-old Mexican-American children living in an agricultural area of California where use of Mn-containing fungicides is common (Gunier et al. 2015). In the same cohort, prenatal tooth manganese was associated with poorer behavioral scores in 248 school-age children, but positively associated with scores on tests of memory and cognition (Mora et al. 2015). High (>75th percentile) cord blood manganese concentrations were associated with worse cognitive, language, and overall neurodevelopment scores among 230 2-y old Taiwanese children with no known exposure source (Lin et al. 2013). Cord blood manganese levels were inversely associated with performance on psychomotor tests among 126 3-y-olds in France, whereas no association was observed with maternal blood manganese (Takser et al. 2003). Most prior studies, except Takser et al. (2003), relied on a single biomarker of prenatal manganese exposure, typically cord blood or serum.

Our study examines associations between prenatal manganese exposure and neurodevelopment among young children living near a former mining area in rural Oklahoma. The objectives were *a*) to estimate associations of manganese, measured in paired maternal–infant blood samples, with neurodevelopment at 2 y of age while adjusting for exposures to other metals; and *b*) to explore the role of placental transfer of manganese as a predictor of neurodevelopment.

Methods

Study Participants

Subjects were participants in a prospective birth cohort study of biologic markers of fetal and early childhood exposure to metals, maternal psychosocial stress, and their impact on neurodevelopment. This research was conducted in the area of the Tar Creek Superfund site in Ottawa County, Oklahoma. This Superfund site, a former lead and zinc mining area, contains numerous piles of mine waste enriched in metals that are dispersed throughout the region (ATSDR 2004; Schaider et al. 2007). Study location and objectives have been described elsewhere (Ettinger et al. 2009; Zota et al. 2009). Briefly, pregnant women were recruited during prenatal visits or at delivery from the Integris Baptist Medical Center in Miami, Oklahoma. Mothers and offspring were followed until children were 7 y of age. Eligibility criteria included *a*) giving birth at Integris Hospital; *b*) intention to live within the study area for the next 2 y; *c*) not being currently enrolled in the study with another child; and *d*) having English-language proficiency sufficient to participate in the informed consent process. Eligible mothers received a detailed explanation of study procedures before consenting to participate. The research protocol was approved by the human subjects committees of Integris Baptist Medical Center and Harvard T.H. Chan School of Public Health (HSPH).

The original cohort included 713 mother–infant pairs, who were enrolled between 2002 and 2011. Biomarkers of prenatal manganese exposure data were available for 637 mother–infant pairs (5 pairs missing blood samples; 71 pairs excluded to avoid batch effects inherent in different instruments due to our laboratory's purchase of a new ICP-MS instrument near the end of the study). Neurodevelopment test scores at 2 y of age were available for 225 of these 637 pairs. One subject was excluded from this analysis due to scores that were more than 3 standard deviations below the expected means for both mental and psychomotor development, necessitating referral for intervention. A total of 224 mother–infant pairs were included in this analysis.

Prenatal Manganese Exposure Assessment

Prenatal exposure to manganese was estimated by measuring manganese concentrations in maternal blood and umbilical cord blood samples collected at the time of birth ($\pm$ 12 hr). Blood collection procedures have been detailed elsewhere (Ettinger et al. 2009). Briefly, venous whole blood from mothers and umbilical cord blood from the umbilical vein were collected in trace element–free tubes [BD Vacutainer royal blue top, with K2EDTA #368381 (Becton Dickinson)] following routine clinical procedures by delivery room staff and shipped frozen to the Trace Metals Laboratory at HSPH (Boston, MA). One milliliter of blood was digested with concentrated HNO_3 acid, followed by the addition of hydrogen peroxide and dilution with deionized water. We measured total manganese concentrations in blood with a dynamic reaction cell–inductively coupled plasma mass spectrometer (DRC-ICP-MS; Elan 6,100; PerkinElmer, Norwalk, CT) using previously published methods and quality control measures (Chen et al. 1999; Ettinger et al. 2009). Average recovery of quality control standards for manganese (NIST 1643e, 1 ppb CV, human hair GBW 07601) was 96–104%. Lead and arsenic concentrations were also measured in blood samples and considered as covariates in all analyses given their co-occurrence in environmental media at this site (Zota et al. 2011). The limit of detection (LOD) was 0.02 μg/dL for manganese, lead, and arsenic. All manganese and arsenic measurements were above the LOD. Three (1.3%) lead measurements in cord blood were below the LOD and were assigned a value of one-half the LOD.

Child Neurodevelopment Assessment

Child neurodevelopment was assessed at 2 y of age using the Bayley Scales of Infant Development-II (Bayley 1993). Age-adjusted scores from the Mental Development Index (MDI) and the Psychomotor Development Index (PDI) were used as the primary outcomes. Two trained study personnel administered the test using a standardized protocol and were overseen by a licensed psychologist (D.C.B.) and a graduate student clinical developmental psychologist. All study testers were blinded to the participants' exposure information.

Covariate Assessment

We used interviewer-administered questionnaires at the time of enrollment to collect information on sociodemographic characteristics, including maternal education, race/ethnicity, and smoking and alcohol consumption during pregnancy, as well as potential sources of metals exposure. Information on child's birth weight, head circumference, and gestational age at birth was abstracted from medical records. Gestational age at birth was based on clinical assessment using data from the last menstrual period, the first accurate ultrasound examination during the first trimester, and clinical examination (ACOG 2014). Hemoglobin and hematocrit concentrations were measured in a maternal blood sample collected within 12 hr of admission to labor and delivery according to routine clinical procedures (as well as an extra tube collected at this time for measurement of blood manganese). Maternal IQ

was assessed using the Kaufman Brief Intelligence Test (KBIT) at 6-mo postpartum (Kaufman and Kaufman 1990).

Statistical Analysis

Distributional plots were examined and descriptive statistics were calculated for all variables. Bivariate associations were calculated between all exposures, outcomes, and covariates. The correlation between maternal and cord blood manganese was estimated using Spearman's r correlation coefficient. Characteristics of mother–infant pairs included in all analyses were compared to those excluded from analyses using t-tests for continuous variables and chi-square tests for categorical variables.

We estimated associations between prenatal manganese concentrations and neurodevelopment using multivariable regression. We examined potential nonlinear associations between manganese and neurodevelopment using generalized additive models with penalized splines (constrained to 4 knots). We used a likelihood ratio test comparing models with a smoothed manganese term to models with a linear manganese term to assess linearity of the manganese–neurodevelopment association. To address skewness, we used natural logarithmic-transformed metals concentrations in exposure–neurodevelopment models. We modeled manganese concentrations as continuous $\log_e$-transformed concentrations and compared the 25th to 75th percentile [interquartile range (IQR)]. Neurodevelopment test scores were normally distributed and analyzed as continuous variables.

Potential confounders were selected *a priori* based on previous literature and on established or plausible associations with neurodevelopment (Grandjean and Landrigan 2006; Lanphear et al. 2000; Tong et al. 2007). We included child sex, maternal education (≥12th grade vs. <12th grade), maternal IQ, maternal hemoglobin at time of delivery (g/dL), and concentrations of maternal or cord blood lead and arsenic, centered at the mean of the $\log_e$ distribution and modeled as penalized splines (constrained to 4 knots). Maternal hemoglobin was used as a proxy for iron status, which is a potential confounder given that iron deficiency in the neonate can adversely impact neurodevelopment (Georgieff 2008) and that iron status may influence manganese levels (Gunshin et al. 1997). In sensitivity analyses, we considered the following additional potential confounders because they were associated with $\log_e$-transformed blood manganese levels or neurodevelopment scores in bivariate models ($p < 0.10$): maternal smoking during pregnancy (yes/no), gestational age at birth (weeks), maternal age at time of delivery (years), first-born child (yes/no), annual household income ($20,000–40,000, >$40,000 vs. <$20,000), maternal marital status (married/living with partner vs. never married/separated/divorced), and use of prenatal vitamins. Given the recent emphasis on sex-specific metal effects (Llop et al. 2013) and possible sex-related metabolic differences in manganese regulation (Oulhote et al. 2014b), we explored sex differences in the association between manganese and neurodevelopment by including an interaction term (child sex∗ manganese) in regression models.

Some participants were missing data on one or more key potential confounders (i.e., maternal IQ, maternal hemoglobin, maternal and cord arsenic levels). We used multiple imputation to impute missing values using chained equations with the MI procedure in SAS (SAS Institute Inc., Cary, NC, USA) (van Buuren 2007; White et al. 2011). We assumed data were missing at random, that is, that the missingness did not depend on the unobserved data. We generated 40 imputed data sets. In the imputation process, we included all exposure and outcome variables and covariates thought to be related to the process causing the missing data (see Table S1, for a list of variables). We combined results from models fit with the multiply imputed data sets by applying Rubin's formula (Rubin 2004) in R (R Foundation for Statistical Computing).

We explored the hypothesis that the efficiency with which manganese is transferred from mother to fetus is an important determinant of neurodevelopment, as has been posited previously (Kopp et al. 2012). We evaluated cord/maternal blood manganese ratio as well as cord/total blood manganese ratio (total = maternal + cord manganese) as predictors of neurodevelopment. Ratios were calculated using untransformed manganese concentrations. We also clustered mother–infant pairs into four groups, based on blood manganese concentrations dichotomized at the medians of the two distributions: *a*) low maternal/low cord, representing concordant low exposures; *b*) low maternal/high cord; *c*) high maternal/low cord; and *d*) high maternal/high cord, representing concordant high exposures. Using multivariable regression models, we estimated the associations between this categorical variable, a crude representation of placental transfer, and neurodevelopment. Models with ratios and with clustered mother–infant pairs were adjusted for the same set of *a priori* covariates as manganese–neurodevelopment models (i.e., child sex, maternal education, maternal IQ, maternal hemoglobin at time of delivery, and concentrations of maternal or cord blood lead and arsenic).

We conducted sensitivity analyses to evaluate the robustness of our findings. *a*) We ran models using complete cases only and compared results with those using the imputed datasets. *b*) We examined the influence of extreme values of prenatal manganese concentrations by fitting models with and without outliers identified using the generalized extreme Studentized deviate (ESD) many-outlier procedure, set to identify up to 10 outliers (Rosner 1983). *c*) We examined the potential confounding effects of additional sociodemographic characteristics (listed above) by rerunning models including these variables. For all statistical tests, the significance level was set at 5%. We conducted statistical analyses using SAS (version 9.4; SAS Institute Inc.) and R (version 3.1.2; R Foundation for Statistical Computing).

Results

Table 1 shows characteristics of mother–infant pairs included in all analyses, as well as those excluded from analyses due to missing exposure and/or neurodevelopment data. Included pairs ($n = 224$) differed from excluded pairs ($n = 489$) on several characteristics: mothers included were older at time of delivery, had higher household incomes, were more likely to be married/living with partner and have at least a high school education, had higher blood arsenic levels, and were less likely to have smoked or have had smokers in the household during pregnancy. For study participants, the average age ± SD at time of neurodevelopment assessment was 2.1 ± 0.1 y. MDI scores ranged from 54 to 128 (mean ± SD = 98.8 ± 15.9); PDI scores ranged from 57 to 133 (mean ± SD = 105.3 ± 12.2). Among included pairs, characteristics of participants with complete data on all variables included in the imputation process ($n = 122$) were similar to those of participants with incomplete data ($n = 102$; see Table S2).

Median (25–75th percentile) manganese concentrations in maternal and cord blood were, respectively, 24.0 (19.5–29.7) and 43.1 (33.5–52.1) μg/L. The median manganese concentration in cord blood was nearly twice the median concentration in maternal blood, and the median (25–75th percentile) cord/maternal manganese ratio was 1.8 (1.4–2.3). Manganese levels were in the range of levels reported in other studies of mother–infant pairs (Figure 1). Maternal and cord blood manganese levels were correlated (Spearman rho = 0.39, $p < 0.0001$, $n = 224$), though in a nonlinear manner (Figure 2). At maternal

Table 1. Descriptive characteristics of included versus excluded Tar Creek mother–infant pairs.

Characteristics	Included ($n=224$)[a]			Excluded ($n=489$)[b]		
	n (%)	Mean ± SD or Median (IQR)	Range	n (%)	Mean ± SD or Median (IQR)	Range
Prenatal exposure measures, median, IQR[c]						
Maternal blood Mn (μg/L)	224	24.0 (19.5–29.7)	8.0–117.4	484	22.5 (18.6–29.0)	8.8–80.9
Maternal blood Pb (μg/dL)	224	0.6 (0.4–0.9)	0.06–3.0	484	0.6 (0.4–0.9)	0.03–3.1
Maternal blood As (μg/L)*	222	1.8 (1.1–2.8)	0.2–8.2	482	1.4 (0.9–2.2)	0.1–24.1
Cord blood Mn (μg/L)	224	43.1 (33.5–52.1)	5.4–139.1	481	41.2 (32.1–51.9)	8.5–104.5
Cord blood Pb (μg/dL)	224	0.4 (0.3–0.6)	0.01–3.9	481	0.4 (0.3–0.6)	0.03–4.9
Cord blood As (μg/L)	221	2.3 (1.7–3.3)	0.2–10.6	474	2.4 (1.8–3.5)	0.1–29.0
Child characteristics						
Female sex	91 (40.6)			234 (48.1)		
First-born child	86 (38.6)			189 (38.7)		
Birth weight (g), mean ± SD	224	3,404.7 ± 477.1	2,296–4,874	486	3,331.8 ± 472.0	1,361–4,734
Gestational age at birth (weeks), mean ± SD	224	39.0 ± 1.3	34–41	483	39.1 ± 1.4	26–42
Preterm birth (<37 wk)	9 (4.0)			24 (5.0)		
Head circumference at birth (cm), mean ± SD	215	34.6 ± 1.8	27.9–40.6	467	34.4 ± 1.7	26.7–43.2
Delivery type: cesarean section[d]	64 (29.8)			120 (25.4)		
Maternal characteristics						
Marital status: married or living with partner*	174 (77.7)			281 (59.4)		
Education: ≥12th grade*	191 (85.3)			335 (68.9)		
Smoked during pregnancy: yes*	56 (25.0)			190 (38.9)		
Any smokers in household: yes*	50 (28.3)			116 (45.5)		
Race/Ethnicity						
White	154 (69.4)			312 (66.2)		
Native American	52 (23.4)			115 (24.4)		
Other (including Hispanic)	16 (7.2)			44 (9.3)		
Annual household income*						
<$20K	59 (37.3)			174 (55.6)		
$20K–$40K	54 (34.2)			99 (31.6)		
$40K–$70K	37 (23.4)			31 (9.9)		
>$70K	8 (5.1)			9 (2.9)		
Hemoglobin at delivery, mean ± SD	220	11.7 ± 1.4	6.6–15.7	481	11.8 ± 1.3	3.6–17.2
Anemia at delivery: yes[e]	56 (25.4)			119 (24.7)		
Use of prenatal vitamins: yes*	147 (65.6)			282 (57.7)		
Age at delivery (years), mean ± SD*	224	25.8 ± 5.8	14–43	486	23.9 ± 5.2	15–44
IQ, mean ± SD	186	101.8 ± 17.3	52–135	120	99.6 ± 14.3	62–134

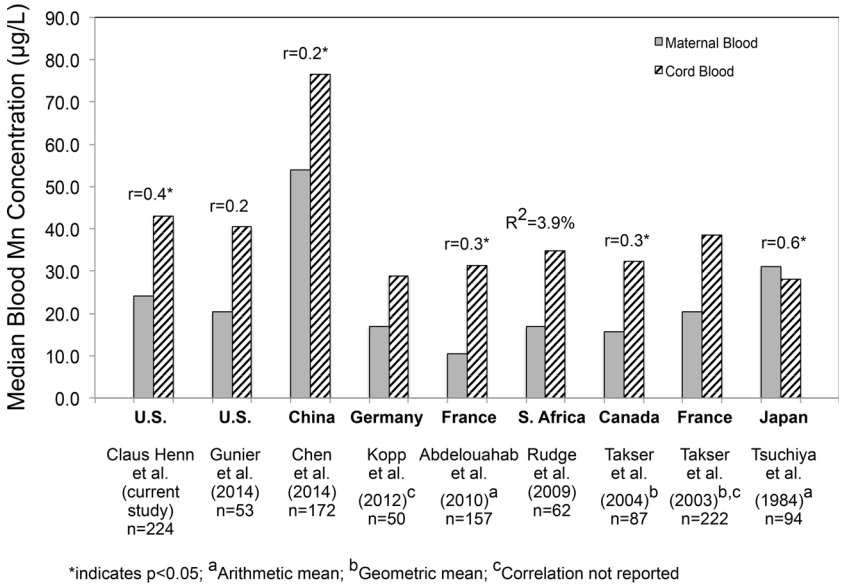
Note: Individuals included in analysis differed from excluded individuals, $p<0.05$. t-tests were conducted on $\log_e$-transformed blood metals concentrations.
[a]Numbers may not sum to total sample size ($n=224$) for some characteristics due to missing data: first-born child $n=1$, delivery type $n=9$, household smokers $n=47$, race/ethnicity $n=2$, income $n=66$, anemia $n=4$. Percentages are based on observations with known values only.
[b]Numbers may not sum to total sample size ($n=489$) for some characteristics due to missing data: sex $n=2$, first-born child $n=1$, marital status $n=16$, education $n=3$, prenatal smoking $n=1$, household smokers $n=234$, race/ethnicity $n=18$, income $n=176$, anemia $n=8$. Percentages are based on observations with known values only. Participants excluded from analyses due to missing blood samples ($n=5$), new ICP-MS instrument error ($n=71$), missing outcome data ($n=412$), and neurodevelopment scores more than 3 SD below expected mean ($n=1$).
[c]Median and interquartile range (IQR, 25–75Th percentiles) reported for blood metals concentrations.
[d]Compared to vaginal or vaginal assisted delivery.
[e]Anemia at delivery defined as maternal hemoglobin <11.0 g/dL at delivery, which is based on the definition from the Centers for Disease Control and Prevention (during 3rd trimester), the World Health Organization (during pregnancy), and the American Congress of Obstetricians and Gynecologists (in 1st and 3rd trimesters; ACOG 2014).

manganese less than 40 μg/L, cord manganese level increased by an estimated 1.1 [95% confidence interval (CI): 0.8, 1.4] μg/L for each 1-μg/L increase in maternal manganese. At maternal manganese levels greater than 40 μg/L, however, a negative association was apparent, although the estimated curve is imprecise in this range because it is based on a limited number of observations ($n=18$).

There was a lack of evidence for a departure from linearity in the associations between $\log_e$-transformed maternal manganese levels and neurodevelopment scores, based on likelihood ratio tests and visual inspection (Figure 3A,B). Maternal manganese was significantly negatively associated with both mental and psychomotor development (Table 2): an interquartile range increase in maternal manganese (10.1 μg/L) was associated with decreases of 3.0 (95% CI: −5.3, −0.7) and 2.3 (95% CI: −4.1, −0.4) points in MDI and PDI scores, respectively.

Visual inspection of smoothed plots of $\log_e$-transformed cord blood manganese with neurodevelopment suggest an increase in MDI and PDI scores at lower concentrations and a leveling off at mid-range concentrations (Figure 3C,D). However, there was a lack of evidence for a departure from linearity based on likelihood ratio tests comparing models with penalized spline terms to models with linear terms (MDI: $p=0.15$; PDI: $p=0.12$). There were no significant associations between $\log_e$-transformed cord blood manganese and MDI or PDI scores (Table 2). Results from exploratory analyses of sex-specific manganese associations, in which we included an interaction term (child sex ∗ manganese) in regression models, were inconclusive (see Table S3).

We modeled associations between neurodevelopment and three proxy measures of the efficiency of placental transfer. In multivariable adjusted models of cord/maternal blood manganese ratio and cord/total blood manganese ratio (total = maternal + cord manganese), MDI and PDI scores were positively associated with both ratios [cord/maternal-MDI: β = 2.6 (95% CI: −0.04, 5.3); cord/maternal-PDI: β = 1.7 (95% CI: −0.5, 3.9); cord/total-MDI: β = 22.0 (95% CI: 3.2, 40.7); cord/total-PDI: β = 15.6 (95% CI: 0.3, 20.9)]. Based on the analysis of clustered mother–infant pairs, pairs with high maternal manganese only (i.e., maternal manganese levels ≥median), high cord manganese only, or high maternal and high cord manganese had lower neurodevelopment scores, compared to mother–child pairs with low maternal and low cord manganese levels (Table 3). PDI scores were significantly



*indicates p<0.05; [a]Arithmetic mean; [b]Geometric mean; [c]Correlation not reported

Figure 1. Comparison of median blood manganese concentrations in maternal–infant pairs by study. Maternal levels measured in blood collected near time of delivery; infant levels measured in umbilical cord blood.

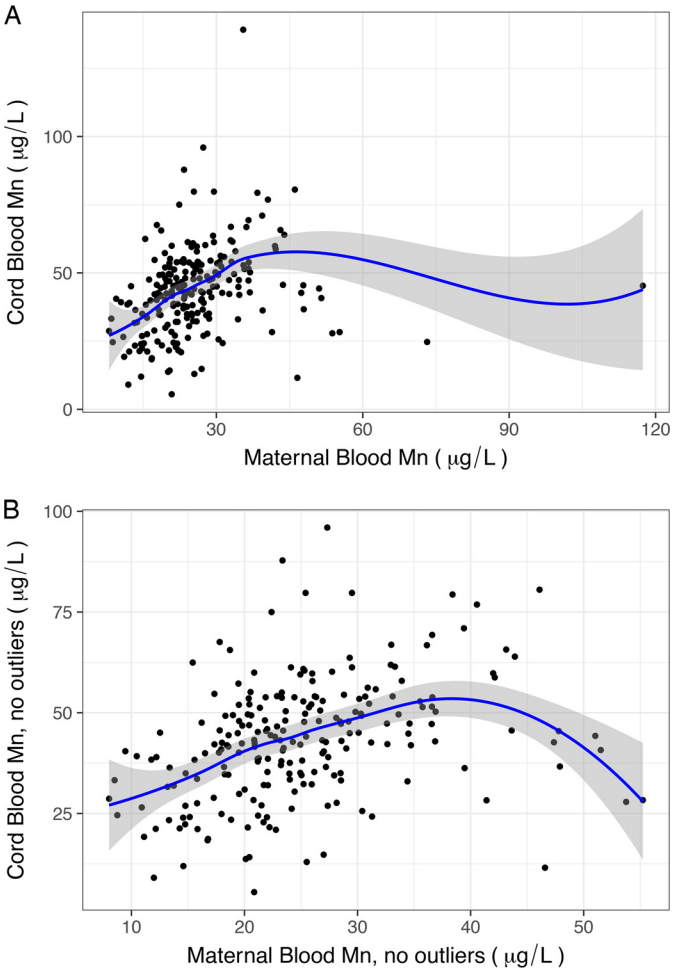


Figure 2. Scatter plots with Loess smoother of maternal blood manganese (µg/L) and cord blood manganese (µg/L). (*A*) All observations. Spearman rho = 0.39, $p < 0.0001$ ($n = 224$); (*B*) Excluding manganese outliers (two maternal: 73.2, 117.4 µg/L; one cord: 139.1 µg/L). Spearman rho = 0.40, $p < 0.0001$ ($n = 221$).

lower among pairs in all three groups, compared to the low concordant exposure group.

Sensitivity Analyses

Sensitivity analyses produced three main findings. *a*) Results from models of maternal and cord manganese with neurodevelopment using complete cases only were similar to results of analyses that included imputed data (Table 2). Adjusted associations between neurodevelopment and joint classification of maternal–infant groups according to high or low (above or below median) maternal and cord manganese were also similar when based only on complete cases (Table 3). *b*) Three subjects were identified with outlying blood manganese levels (two maternal: 73.2, 117.4 µg/L; one cord: 139.1 µg/L; Figure 2) using the generalized ESD many-outlier procedure. When these subjects were excluded, associations between manganese and neurodevelopment remained similar (Table 2), indicating that the few outlying manganese levels did not drive the associations we observed. Further, the correlation between maternal and cord manganese was unchanged (Spearman rho = 0.40, $p < 0.0001$, $n = 221$ vs. for all observations: Spearman rho = 0.39, $p < 0.0001$, $n = 224$) and a nonlinear pattern remained (Figure 2). *c*) With adjustment for additional sociodemographic variables, conclusions were unchanged (Table 2).

Discussion

We estimated inverse linear associations between $\log_e$-transformed maternal blood manganese and early childhood mental and psychomotor development scores. Our findings are generally consistent with other studies that have estimated associations between biomarkers of prenatal manganese and neurodevelopmental outcomes (Lin et al. 2013; Takser et al. 2003; Yu et al. 2014). Our study is unique in that it is among the first to evaluate neurodevelopment outcomes in association with both maternal and infant biomarkers of manganese exposure, and with proxy measures of manganese placental transfer. To our knowledge, only one other study has estimated both maternal and cord blood effects: Takser et al. (Takser et al. 2003) observed inverse associations between cord, but not maternal, blood manganese and

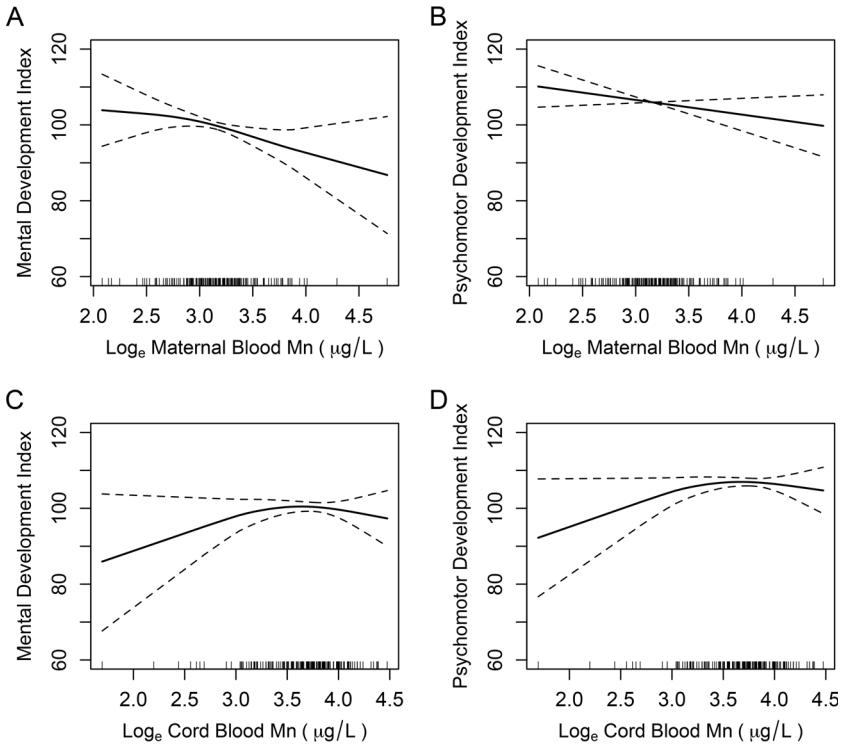


Figure 3. Smoothed associations between blood manganese and child neurodevelopment. Generalized additive models with penalized splines (constrained to 4 knots), adjusted for child sex, maternal education, maternal IQ, maternal hemoglobin, and smoothed $\log_e$ blood lead and arsenic (centered). Models use complete case method ($n = 181$). (*A*) Maternal blood manganese with MDI; (*B*) maternal blood manganese with PDI; (*C*) cord blood manganese with MDI; (*D*) cord blood manganese with PDI.

performance on psychomotor subscales of the McCarthy Scales of Children's Abilities among 126 3-y-olds with prenatal biomarker levels similar in magnitude to our study [geometric mean (range) 20.4 μg/L (6.3–151.2) and 38.5 μg/L (14.9–92.9) in maternal and cord blood samples, respectively]. In contrast, we found that maternal, but not cord, blood manganese was associated with neurodevelopment, as indicated by lower mean MDI and PDI scores, in our study population. Other studies have also reported evidence of adverse effects of cord blood manganese (Lin et al. 2013; Yu et al. 2014), although maternal biomarkers were not assessed.

Median blood manganese levels in our U.S.-based cohort were lower than median values reported for children in China (Chen et al. 2014), but higher than median or mean values reported for study cohorts in Germany, France, South Africa, and Canada (Abdelouahab et al. 2010; Chen et al. 2014; Kopp et al. 2012; Rudge et al. 2009; Takser et al. 2004). In this cohort, the routes of exposure likely include ingestion of manganese in dusts. We have previously reported positive associations between child hair manganese levels and house dust, but not indoor air, yard soil or tap water, in a subset of this cohort (Zota et al. 2015). Our observation that the median manganese level in cord blood was about twice as high as in maternal blood likely reflects active transport of manganese across the placenta (Krachler et al. 1999; Nandakumaran et al. 2016). Our finding is similar to previous reports in whole blood from 62 mother–infant pairs in South Africa (median 16.8 μg/L and 34.9 μg/L in maternal and cord blood samples, respectively) (Rudge et al. 2009) and in

Table 2. Adjusted associations of prenatal manganese biomarkers with neurodevelopment.

	Outcome: MDI			Outcome: PDI		
Models	*n*	β	95% CI	*n*	β	95% CI
Models with $\log_e$ maternal blood manganese[a]						
Using imputed data	224	−3.0	−5.3, −0.7	224	−2.3	−4.1, −0.4
Using complete case method	181	−2.9	−5.5, −0.3	181	−1.6	−3.7, 0.5
Using imputed data, excluding outliers[b]	221	−2.7	−5.2, −0.2	221	−2.4	−4.4, −0.4
Using imputed data, adjusting for additional potential confounders[c]	224	−2.9	−5.3, −0.4	224	−2.3	−4.3, −0.3
Models with $\log_e$ cord blood manganese[a]						
Using imputed data	224	0.5	−1.8, 2.8	224	−0.1	−1.9, 1.8
Using complete case method	181	0.8	−1.8, 3.4	181	0.9	−1.2, 3.0
Using imputed data, excluding outliers[b]	221	0.1	−2.3, 2.5	221	−0.2	−2.1, 1.7
Using imputed data, adjusting for additional potential confounders[c]	224	1.2	−1.8, 3.6	224	0.3	−1.7, 2.2

Note: Adjusted for child sex, maternal IQ, maternal education, maternal hemoglobin, and smoothed $\log_e$ blood Pb and As (centered) in maternal or cord blood.
[a]Scaled to difference between 25th and 75th percentile of blood Mn (maternal: 10.1 μg/L; cord: 18.5 μg/L).
[b]Outlying manganese levels in maternal blood: 73.2, 117.4 μg/L; cord blood: 139.1 μg/L.
[c]Additionally adjusted for maternal smoking during pregnancy, gestational age at birth, maternal age at time of delivery, first-born child, annual household income, maternal marital status, and prenatal vitamin use.

Table 3. Adjusted associations between neurodevelopment and joint classification of maternal–infant groups according to high or low (above or below median) maternal and cord manganese.

Joint classification of maternal and cord Mn levels	Outcome: MDI			Outcome: PDI		
	n	β	95% CI	*n*	β	95% CI
Using imputed data						
Low maternal, low cord[a]	71	-	-	71	-	-
Low maternal, high cord	41	−3.7	−9.5, 2.1	41	−6.2	−10.8, −1.5
High maternal, low cord	41	−5.2	−11.0, 0.5	41	−7.0	−11.6, −2.3
High maternal, high cord	71	−7.2	−12.3, −2.1	71	−6.3	−10.4, −2.3
Using complete case method						
Low maternal, low cord[a]	63	-	-	63	-	-
Low maternal, high cord	38	−2.7	−9.0, 3.5	38	−5.4	−10.5, −0.4
High maternal, low cord	37	−5.7	−11.9, 0.5	37	−6.4	−11.4, −1.4
High maternal, high cord	43	−7.4	−13.5, −1.3	43	−4.0	−8.9, 0.9

Note: Models adjusted for child sex, maternal IQ, maternal education, maternal hemoglobin, and smoothed $\log_e$ cord blood Pb and As (centered); groups defined by maternal and cord blood Mn levels. Low indicates <median; high indicates ≥median. Median maternal Mn = 24.0 μg/L; median cord Mn = 43.1 μg/L.
[a]Reference category.

serum from 202 mother–infant pairs in China (median 2.8 μg/L and 4.0 μg/L in maternal and cord serum samples, respectively) (Yu et al. 2013). The Spearman correlation coefficient we observed between maternal and cord blood manganese was in the range of correlation coefficients reported by other studies (Abdelouahab et al. 2010; Chen et al. 2014; Gunier et al. 2014; Takser et al. 2004; Yu et al. 2013), though few studies appear to have allowed for possible nonlinearity (Chen et al. 2014; Gunier et al. 2014).

Our observation that the concentration of manganese in maternal blood, despite generally being lower than the concentration in cord blood, was a stronger predictor of neurodevelopment was unexpected, and the explanation is not apparent. We hypothesized that cord blood would more proximally represent fetal exposure, and therefore be more strongly associated with neurodevelopment. However, given that manganese is a nutrient, relationships are difficult to predict.

It is possible that high maternal manganese levels produce adverse effects on the placenta that are subsequently responsible for poorer neurodevelopment. This might explain differences in results compared to cord blood manganese. The fetus may be protected from direct adverse effects of excess maternal manganese by accumulating manganese in the placenta, but it may be indirectly affected by maternal manganese via placental factors that regulate neurodevelopment. An *in vitro* human placenta study has demonstrated that during normal steady-state exposure, the placenta efficiently transfers manganese to the fetus, but at high-dose maternal exposure the placenta limits transfer to the fetal circulation and accumulates manganese (Miller et al. 1987). This is consistent with the nonlinear relationship we observed between maternal and cord blood manganese levels, whereby levels were positively associated up to approximately 40 μg/L maternal blood, and cord blood levels subsequently leveled off or declined. There was, however, a relatively small number of observations in this higher exposure range. Alternative explanations may include mechanisms that do not involve a causal effect of maternal manganese, and findings should be confirmed in other studies.

Several biological mechanisms for the neurotoxic effects of prenatal manganese exposure have been proposed. Animal studies have reported that maternal developmental exposure in mice affects neurogenesis in the offspring, altering the number of immature granule cells in the hippocampal dentate gyrus and causing neuronal mismigration (Ohishi et al. 2012; Wang et al. 2012). In an experimental study, *in utero* exposure altered epigenetic gene regulation in offspring, which may affect programing of cells involved in neurogenesis (Wang et al. 2013). Manganese neurotoxicity appears to involve oxidative stress and dopaminergic dysfunction (Racette et al. 2012), and a link to adverse neurodevelopmental outcomes through disruption of thyroid homeostasis following alterations to dopamine activity has also been proposed (Soldin and Aschner 2007).

In our exploration of placental transfer, we found that MDI and PDI scores increased as the cord/maternal or cord/(maternal + cord) manganese ratios increased, suggesting that increasing cord manganese levels relative to maternal levels or relative to total (i.e., cord + maternal) levels might be beneficial. We also found that both MDI and PDI scores were lower among mother–infant pairs with either high maternal levels or high cord levels, compared to mother–infant pairs with both low maternal and low cord manganese. Overall, these results are consistent with our finding of a negative association between maternal manganese levels and scores for cognition and psychomotor function at 2 y of age. Further study is necessary, but these preliminary findings suggest that not only are concentrations of manganese in maternal blood predictors of neurodevelopment, but the relative amount of manganese transferred from maternal to fetal circulation may also be important. Factors that influence manganese transfer should be investigated more deeply.

Our study has several limitations. Prenatal maternal blood manganese may not best represent manganese total body burden given that it is a nutrient with a steady-state normal concentration range that is regulated in the body, rather than a xenobiotic that undergoes metabolism and excretion. Physiologic factors such as genetic variability or variability in metabolic function or liver function may contribute to differences in manganese levels (Claus Henn et al. 2011; Zerón et al. 2011). In addition, little is known about how maternal blood manganese levels vary during labor and delivery; therefore, timing of blood sample collection may influence blood manganese levels. However, blood manganese levels also increase in the setting of chronic exposure. For example, blood levels have been correlated with airborne manganese levels (Smith et al. 2007), with an indicator of take-home occupational pesticide exposure in a region where manganese-containing pesticides are commonly applied (Gunier et al. 2014), and with MRI intensity index in the globus pallidus among manganese-exposed children (Kafritsa et al. 1998). Our modest sample size limits the precision in our effect estimates. However, by multiply imputing missing covariate data, an approach that is valid if data are missing at random, we maximized the use of available exposure and outcome data. Finally, there is a possibility of selection bias from loss to follow up if factor(s) related to participation are associated with both manganese levels and neurodevelopment.

This is among the first studies to examine both maternal and infant biomarkers of prenatal manganese exposure in relation to neurodevelopment, which allows for a comparison of biomarkers as well as an exploration of placental transfer. The prospective design of our study strengthens the growing literature on manganese and neurodevelopment, which remains dominated by cross-sectional studies that cannot establish temporality. We collected data on a large number of potential confounders, including co-exposures to lead and arsenic, and results were robust to additional confounder adjustment.

Conclusions

In our U.S. study population, the concentration of manganese in maternal blood at or near the time of delivery was associated with lower neurodevelopment scores at 2 y of age. In addition, we found preliminary evidence suggesting that placental factors may influence associations between prenatal manganese exposure

and neurodevelopmental outcomes. In studies of prenatal manganese exposure, careful consideration should be given to the selection of biomarkers and the role of placental transfer should be evaluated.

Acknowledgments

The research described in this paper was funded in part by National Institute of Environmental Health (NIEHS)/National Institutes of Health (NIH) grants P42-ES016454, R01-ES016283, P01-ES012874; R01-ES014930, R01-ES013744, P30-ES000002, P30-ES023515, and R00-ES022986. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

References

Abdelouahab N, Huel G, Suvorov A, Foliguet B, Goua V, Debotte G, et al. 2010. Monoamine oxidase activity in placenta in relation to manganese, cadmium, lead, and mercury at delivery. Neurotoxicol Teratol 32:256–261, PMID: 19744554, https://doi.org/10.1016/j.ntt.2009.08.010.

ACOG (American College of Obstetricians and Gynecologists). 2014. Committee opinion no 611: method for estimating due date. Obstet Gynecol 124:863–866.

Aschner JL, Aschner M. 2005. Nutritional aspects of manganese homeostasis. Mol Aspects Med 26:353–362, PMID: 16099026, https://doi.org/10.1016/j.mam.2005.07.003.

ATSDR (Agency for Toxic Substances and Disease Registry). 2004. Tar Creek Superfund site: Report to Congress. Atlanta, GA:ATSDR.

Bayley N. 1993. *Bayley Scales of Infant Development*. 2nd ed. San Antonio, TX: Psychological Corp.

Blomgren K, Hagberg H. 2006. Free radicals, mitochondria, and hypoxia–ischemia in the developing brain. Free Radic Biol Med 40:388–397, PMID: 16443153, https://doi.org/10.1016/j.freeradbiomed.2005.08.040.

Bouchard MF, Sauvé S, Barbeau B, Legrand M, Brodeur MÈ, Bouffard T, et al. 2011. Intellectual impairment in school-age children exposed to manganese from drinking water. Environ Health Perspect 119:138–143, PMID: 20855239, https://doi.org/10.1289/ehp.1002321.

Buonocore G, Perrone S, Bracci R. 2001. Free radicals and brain damage in the newborn. Biol Neonate 79:180–186, PMID: 11275648.

Cahill DF, Bercegeay MS, Haggerty RC, Gerding JE, Gray LE. 1980. Age-related retention and distribution of ingested Mn_3O_4 in the rat. Toxicol Appl Pharmacol 53:83–91, PMID: 7385241.

Chen KL, Amarasiriwardena CJ, Christiani DC. 1999. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. Biol Trace Elem Res 67:109–125, PMID: 10073418, https://doi.org/10.1007/BF02784067.

Chen L, Ding G, Gao Y, Wang P, Shi R, Huang H, et al. 2014. Manganese concentrations in maternal–infant blood and birth weight. Environ Sci Pollut Res Int 21:6170–6175, PMID: 24477335, https://doi.org/10.1007/s11356-013-2465-4.

Chung SE, Cheong HK, Ha EH, Kim BN, Ha M, Kim Y, et al. 2015. Maternal blood manganese and early neurodevelopment: the Mothers and Children's Environmental Health (MOCEH) study. Environ Health Perspect 123:717–722, PMID: 25734517, https://doi.org/10.1289/ehp.1307865.

Claus Henn B, Kim J, Wessling-Resnick M, Téllez-Rojo MM, Jayawardene I, Ettinger AS, et al. 2011. Associations of iron metabolism genes with blood manganese levels: a population-based study with validation data from animal models. Environ Health 10:97, PMID: 22074419, https://doi.org/10.1186/1476-069X-10-97.

Erikson KM, Aschner M. 2003. Manganese neurotoxicity and glutamate-GABA interaction. Neurochem Int 43:475–480, PMID: 12742094.

Ettinger AS, Zota AR, Amarasiriwardena CJ, Hopkins MR, Schwartz J, Hu H, et al. 2009. Maternal arsenic exposure and impaired glucose tolerance during pregnancy. Environ Health Perspect 117:1059–1064, PMID: 19654913, https://doi.org/10.1289/ehp0800533.

Georgieff MK. 2008. The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus. Biochem Soc Trans 36:1267–1271, PMID: 19021538, https://doi.org/10.1042/BST0361267.

Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368:2167–2178, PMID: 17174709, https://doi.org/10.1016/S0140-6736(06)69665-7.

Gunier RB, Arora M, Jerrett M, Bradman A, Harley KG, Mora AM, et al. 2015. Manganese in teeth and neurodevelopment in young Mexican-American children. Environ Res 142:688–695, PMID: 26381693, https://doi.org/10.1016/j.envres.2015.09.003.

Gunier RB, Mora AM, Smith D, Arora M, Austin C, Eskenazi B, et al. 2014. Biomarkers of manganese exposure in pregnant women and children living in an agricultural community in California. Environ Sci Technol 48:14695–14702, https://doi.org/10.1021/es503866a.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, et al. 1997. Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388:482–488, PMID: 9242408, https://doi.org/10.1038/41343.

Hurley LS. 1981. The roles of trace elements in foetal and neonatal development. Philos Trans R Soc Lond, B Biol Sci 294:145–152, PMID: 6118892.

Ikonomidou C, Kaindl AM. 2011. Neuronal death and oxidative stress in the developing brain. Antioxid Redox Signal 14:1535–1550, PMID: 20919934, https://doi.org/10.1089/ars.2010.3581.

Kafritsa Y, Fell J, Long S, Bynevelt M, Taylor W, Milla P. 1998. Long-term outcome of brain manganese deposition in patients on home parenteral nutrition. Arch Dis Child 79:263–265, PMID: 9875025.

Kaufman AS, Kaufman NL. 1990. *Manual for the Kaufman Brief Intelligence Test*. Circle Pines, MN:American Guidance Service.

Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, et al. 2012. Manganese exposure from drinking water and children's academic achievement. Neurotoxicology 33:91–97, PMID: 22182530, https://doi.org/10.1016/j.neuro.2011.12.002.

Kopp RS, Kumbartski M, Harth V, Brüning T, Käfferlein HU. 2012. Partition of metals in the maternal/fetal unit and lead-associated decreases of fetal iron and manganese: an observational biomonitoring approach. Arch Toxicol 86:1571–1581, PMID: 22678741, https://doi.org/10.1007/s00204-012-0869-4.

Kostial K, Kello D, Jugo S, Rabar I, Maljković T. 1978. Influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81–86, PMID: 720306.

Krachler M, Rossipal E, Micetic-Turk D. 1999. Trace element transfer from the mother to the newborn–investigations on triplets of colostrum, maternal and umbilical cord sera. Eur J Clin Nutr 53:486–494, https://doi.org/10.1038/sj.ejcn.1600781.

Lanphear BP, Dietrich K, Auinger P, Cox C. 2000. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. Public Health Rep 115:521–529, PMID: 11354334.

Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH, et al. 2013. *In utero* exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ Res 123:52–57, PMID: 23578827, https://doi.org/10.1016/j.envres.2013.03.003.

Llop S, Lopez-Espinosa MJ, Rebagliato M, Ballester F. 2013. Gender differences in the neurotoxicity of metals in children. Toxicology 311:3–12, PMID: 23632092, https://doi.org/10.1016/j.tox.2013.04.015.

Miller RK, Mattison DR, Panigel M, Ceckler T, Bryant R, Thomford P. 1987. Kinetic assessment of manganese using magnetic resonance imaging in the dually perfused human placenta *in vitro*. Environ Health Perspect 74:81–91, PMID: 3691434.

Mora AM, Arora M, Harley KG, Kogut K, Parra K, Hernández-Bonilla D, et al. 2015. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. Environ Int 84:39–54, https://doi.org/10.1016/j.envint.2015.07.009.

Nandakumaran M, Al-Sannan B, Al-Sarraf H, Al-Shammari M. 2016. Maternal–fetal transport kinetics of manganese in perfused human placental lobule *in vitro*. J Matern Fetal Neonatal Med 29:274–278, https://doi.org/10.3109/14767058.2014.998193.

Nowakowski RS, Hayes NL. 1999. CNS development: an overview. Dev Psychopathol 11:395–417, PMID: 10532616.

Ohishi T, Wang L, Akane H, Shiraki A, Goto K, Ikarashi Y, et al. 2012. Reversible aberration of neurogenesis affecting late-stage differentiation in the hippocampal dentate gyrus of rat offspring after maternal exposure to manganese chloride. Reprod Toxicol 34:408–419, PMID: 22561194, https://doi.org/10.1016/j.reprotox.2012.04.009.

Oulhote Y, Mergler D, Barbeau B, Bellinger DC, Bouffard T, Brodeur MÈ, et al. 2014a. Neurobehavioral function in school-age children exposed to manganese in drinking water. Environ Health Perspect 122:1343–1350, https://doi.org/10.1289/ehp.1307918.

Oulhote Y, Mergler D, Bouchard MF. 2014b. Sex- and age-differences in blood manganese levels in the U.S. general population: National Health and Nutrition Examination Survey 2011–2012. Environ Health 13:87, https://doi.org/10.1186/1476-069X-13-87.

Prohaska JR. 1987. Functions of trace elements in brain metabolism. Physiol Rev 67:858–901, PMID: 3299411.

Racette BA, Aschner M, Guilarte TR, Dydak U, Criswell SR, Zheng W. 2012. Pathophysiology of manganese-associated neurotoxicity. Neurotoxicology 33:881–886, PMID: 22202748, https://doi.org/10.1016/j.neuro.2011.12.010.

Rosner B. 1983. Percentage points for a generalized ESD many-outlier procedure. Technometrics 25:165–172, https://doi.org/10.2307/1268549.

Rubin DB. 2004. *Multiple Imputation for Nonresponse in Surveys*. Hoboken, NJ: Wiley-Interscience.

Rudge CV, Röllin HB, Nogueira CM, Thomassen Y, Rudge MC, Odland JØ. 2009. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of south african delivering women. J Environ Monit 11:1322–1330, https://doi.org/10.1039/b903805a.

Sanders AP, Claus Henn B, Wright RO. 2015. Perinatal and childhood exposure to cadmium, manganese, and metal mixtures and effects on cognition and behavior: a review of recent literature. Curr Environ Health Rep 2:284–294, PMID: 26231505, https://doi.org/10.1007/s40572-015-0058-8.

Schaider LA, Senn DB, Brabander DJ, McCarthy KD, Shine JP. 2007. Characterization of zinc, lead, and cadmium in mine waste: implications for transport, exposure, and bioavailability. Environ Sci Technol 41:4164–4171, PMID: 17612206.

Sloot WN, Gramsbergen JB. 1994. Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia. Brain Res 657:124–132, PMID: 7820609.

Smith D, Gwiazda R, Bowler R, Roels H, Park R, Taicher C, et al. 2007. Biomarkers of Mn exposure in humans. Am J Ind Med 50:801–811, PMID: 17924418, https://doi.org/10.1002/ajim.20506.

Soldin OP, Aschner M. 2007. Effects of manganese on thyroid hormone homeostasis: potential links. Neurotoxicology 28:951–956, PMID: 17576015, https://doi.org/10.1016/j.neuro.2007.05.003.

Takeda A, Ishiwatari S, Okada S. 1999. Manganese uptake into rat brain during development and aging. J Neurosci Res 56:93–98, PMID: 10213480, https://doi.org/10.1002/(SICI)1097-4547(19990401)56:1<93::AID-JNR12>3.0.CO;2-P.

Takser L, Lafond J, Bouchard M, St-Amour G, Mergler D. 2004. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a southwest Quebec population. Environ Res 95:119–125, https://doi.org/10.1016/j.envres.2003.11.002.

Takser L, Mergler D, Hellier G, Sahuquillo J, Huel G. 2003. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 24:667–674, PMID: 12900080, https://doi.org/10.1016/S0161-813X(03)00058-5.

Tong S, Baghurst P, Vimpani G, McMichael A. 2007. Socioeconomic position, maternal IQ, home environment, and cognitive development. J Pediatr 151:284–288, PMID: 17719939, https://doi.org/10.1016/j.jpeds.2007.03.020.

van Buuren S. 2007. Multiple imputation of discrete and continuous data by fully conditional specification. Stat Methods Med Res 16:219–242, PMID: 17621469, https://doi.org/10.1177/0962280206074463.

Wang L, Ohishi T, Shiraki A, Morita R, Akane H, Ikarashi Y, et al. 2012. Developmental exposure to manganese chloride induces sustained aberration of neurogenesis in the hippocampal dentate gyrus of mice. Toxicol Sci 127:508–521, PMID: 22407947, https://doi.org/10.1093/toxsci/kfs110.

Wang L, Shiraki A, Itahashi M, Akane H, Abe H, Mitsumori K, et al. 2013. Aberration in epigenetic gene regulation in hippocampal neurogenesis by developmental exposure to manganese chloride in mice. Toxicol Sci 136:154–165, PMID: 23976782, https://doi.org/10.1093/toxsci/kft183.

Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. 2006. Water manganese exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 114:124–129, PMID: 16393669, https://doi.org/10.1289/ehp.8030.

White IR, Royston P, Wood AM. 2011. Multiple imputation using chained equations: issues and guidance for practice. Stat Med 30:377–399, PMID: 21225900, https://doi.org/10.1002/sim.4067.

Wright RO, Amarasiriwardena C, Woolf AD, Jim R, Bellinger DC. 2006. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. Neurotoxicology 27:210–216, https://doi.org/10.1016/j.neuro.2005.10.001.

Yoon M, Nong A, Clewell HJ III, Taylor MD, Dorman DC, Andersen ME. 2009. Evaluating placental transfer and tissue concentrations of manganese in the pregnant rat and fetuses after inhalation exposures with a PBPK model. Toxicol Sci 112:44–58, https://doi.org/10.1093/toxsci/kfp198.

Yu X, Cao L, Yu X. 2013. Elevated cord serum manganese level is associated with a neonatal high ponderal index. Environ Res 121:79–83, PMID: 23164521, https://doi.org/10.1016/j.envres.2012.11.002.

Yu XD, Zhang J, Yan CH, Shen XM. 2014. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. Environ Res 133:232–238, PMID: 24971720, https://doi.org/10.1016/j.envres.2014.04.012.

Zerón HM, Rodríguez MR, Montes S, Castañeda CR. 2011. Blood manganese levels in patients with hepatic encephalopathy. J Trace Elem Med Biol 25:225–229, PMID: 21975221, https://doi.org/10.1016/j.jtemb.2011.07.003.

Zoni S, Lucchini RG. 2013. Manganese exposure: cognitive, motor and behavioral effects on children: a review of recent findings. Curr Opin Pediatr 25:255–260, PMID: 23486422, https://doi.org/10.1097/MOP.0b013e32835e906b.

Zota AR, Ettinger AS, Bouchard M, Amarasiriwardena CJ, Schwartz J, Hu H, et al. 2009. Maternal blood manganese levels and infant birth weight. Epidemiology 20:367–373, PMID: 19289966, https://doi.org/10.1097/EDE.0b013e31819b93c0.

Zota AR, Riederer AM, Ettinger AS, Schaider LA, Shine JP, Amarasiriwardena CJ, et al. 2015. Associations between metals in residential environmental media and exposure biomarkers over time in infants living near a mining-impacted site. J Expos Sci Environ Epidemiol 26:510–519, https://doi.org/10.1038/jes.2015.76.

Zota AR, Schaider LA, Ettinger AS, Wright RO, Shine JP, Spengler JD. 2011. Metal sources and exposures in the homes of young children living near a mining-impacted Superfund site. J Expos Sci Environ Epidemiol 21:495–505, https://doi.org/10.1038/jes.2011.21.