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Exposure to manganese, fetal growth, and neurodevelopment in children living in agricultural communities in Costa Rica and California

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By

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Abstract

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There is a growing concern about excess manganese (Mn) exposure in pregnant women and children. Recent studies have reported adverse health effects in children living near Mn mining and/or transformation plants or drinking water contaminated with Mn. This dissertation focuses on environmental exposures to Mn in pregnant women and children living near agricultural fields treated with Mn-containing fungicides in Costa Rica and California, and their effects on fetal growth, length of gestation, and children's neurodevelopment.

Chapter 1 provides a general introduction to human exposure to Mn and highlights the background, significance and specific aims for each study/chapter.

Chapter 2 focuses on the environmental and lifestyle factors associated with Mn concentrations in pregnant women living near banana plantations with extensive aerial spraying of Mn-containing fungicide mancozeb in Costa Rica. For these analyses, Mn concentrations were measured in repeated blood and hair samples collected from 449 pregnant women enrolled in the Infants' Environmental Health Study (ISA). Mean blood Mn and geometric mean hair Mn concentrations were 24.4 µg/L (8.9-56.3) and 1.8 µg/g (0.05-53.3), respectively. Blood Mn concentrations were positively associated with gestational age at sampling ($\beta = 0.2$; 95% CI: 0.1, 0.2), number of household members ($\beta = 0.4$; 95% CI: 0.1, 0.6), and living in a house made of permeable and difficult-to-clean materials ($\beta = 2.6$; 95% CI: 1.3, 4.0); and inversely related to smoking ($\beta = -3.1$; 95% CI: -5.8, -0.3). Hair Mn concentrations were inversely associated with gestational age at sampling (% change = 0.8; 95% CI: -1.6, 0.0); and positively associated with living within 50 meters of a plantation (% change = 42.1; 95% CI: 14.2, 76.9) and Mn concentrations in drinking water (% change = 17.5; 95% CI: 12.2, 22.8). Findings from these analyses suggest that pregnant women living near banana plantations aerially sprayed with mancozeb may be environmentally exposed to Mn.

In Chapter 3 the association of prenatal blood and hair Mn concentrations with fetal growth and length of gestation in pregnant women and children living near banana plantations sprayed with mancozeb in Costa Rica was examined. Data on blood or hair Mn concentrations and birth outcomes were collected from 380 mother-infant pairs from the ISA study. Linear regression and generalized additive models were used in these analyses to test for linear and nonlinear associations. Mean (± SD) blood Mn concentration was 24.4 ± 6.6 µg/L and geometric

mean (geometric SD) hair Mn concentration was 1.8 (3.2) μg/g. Hair Mn concentrations during the 2nd trimester and averaged over pregnancy were positively related to infant chest circumference (β for 10-fold increase = 0.6 cm; 95%CI: 0.2, 1.1; and β = 0.7 cm; 95%CI: 0.2, 1.2). Similarly, mean maternal hair Mn concentrations during pregnancy were associated with increased chest circumference (β for 10-fold increase = 1.32 cm; 95% CI: 0.54, 2.09) in infants whose mothers did not have gestational anemia but not in infants of mothers who had gestational anemia (β = 0.24 cm; 95% CI: -0.57, 1.05; $p_{\mathrm{INT}} = 0.09$). Mean maternal blood Mn concentrations during pregnancy were associated with decreased chest circumference in infants whose mothers were living below the poverty line (β for one-unit increase = -0.06 cm; 95% CI: -0.12, -0.01), but not in those living above poverty (β = 0.02 cm; 95% CI: -0.06, 0.09; $p_{\mathrm{INT}} = 0.08$). Mean maternal hair Mn concentrations were positively associated with length of gestation in infants born to women living below the poverty line (β for 10-fold increase = 4.11 days; 95% CI: 0.47, 7.75) but negatively associated in those living above poverty (β = -3.17 days; 95% CI: -7.93, 1.58; $p_{\mathrm{INT}} = 0.03$). In contrast to findings from previous studies, no linear or nonlinear associations of Mn concentrations with lowered birth weight or head circumference were observed. Nevertheless, hair Mn concentrations were related to larger chest circumference in infants born to women without gestational anemia and longer gestational durations in women living below the poverty line, while blood Mn concentrations were associated with smaller chest circumferences. The clinical significance of larger chest circumference, in the absence of an association between hair Mn concentrations and other measures of fetal growth in this study, is unknown. Inconsistencies between studies could be due to differences in the study population, sample size, time of biological sampling, and sources of Mn exposure.

In Chapter 4 the neurodevelopmental effects of early life exposure to Mn, indicated by prenatal and postnatal dentine Mn levels in children's deciduous teeth, were examined in school-age children living near agricultural fields treated with Mn-containing fungicides in California. Participants in these analyses included 247 children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a prospective cohort study in an agricultural area of California. Data on attention, cognition, memory, and motor functioning were collected from children at ages 7, 9, and 10.5 years. Generalized linear models and generalized additive models were used to test for linear and nonlinear associations, and generalized estimating equation models to assess longitudinal effects. Higher prenatal and postnatal dentine Mn levels were associated with improved cognitive abilities and fine motor coordination at 7, 9, and 10.5 years. Higher prenatal dentine Mn levels were associated with poorer attention at ages 7, 9, and 10.5 but only in boys and children born to mothers with higher lead exposure during pregnancy or gestational anemia. Higher postnatal dentine Mn levels were associated with better immediate and delayed memory at 9 and 10.5 years. Longitudinal models showed a weak association between postnatal dentine Mn levels and better cognitive outcomes at 7 and 10.5 years. These associations were all linear, and no threshold was observed. Previous studies have reported associations between childhood Mn exposure and negative neurodevelopmental effects, but in this study, small and positive relationships between postnatal dentine Mn and fine motor coordination, memory, and cognition were observed in children aged 7, 9, and 10.5 years. Disparities in findings between studies could be due to differences in the study design, timing of Mn measurements, exposure pathways, and/or exposure matrix.

Finally, Chapter 5 highlights the major findings for each chapter/study, conclusion, and future directions.

Dedication

To my parents, my sister, and my husband. For your unconditional love and support.

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List of abbreviations

ADHD: Attention Deficit Hyperactivity Disorder
AUC: Area Under the Curve
BASC-2: Behavior Assessment Scale for Children, Second Edition
BMI: Body Mass Index
CADS: Conners' ADHD/DSM-IV Scales, Parent and Teacher versions
CARTA: Costa Rica Airborne Research and Technology Applications
CAVLT-2: Children's Auditory Verbal Learning Test, Second Edition
CES-D: Center for Epidemiologic Studies Depression Scale
CHAM1: Initial CHAMACOS cohort (recruited on October 1999-October 2000)
CHAM2: Second CHAMACOS cohort (recruited on September 2009- August 2011)
CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas
CI: Confidence Interval
CPT-II: Conners' Continuous Performance Test II, Version 5
DAP: Dialkylphosphate
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition
EBDC: Ethylene Bisdithiocarbamates
ETU: Ethylenethiourea
GAM: Generalized Additive Models
GEE: Generalized Estimating Equations
GLM: Generalized Linear Models
GM: Geometric Mean
GPS: Geographic Positioning System
GSD: Geometric Standard Deviation
HOME: Home Observation for Measurement of the Environment
IAEA: International Atomic Energy Agency
ICC: Intraclass Correlation Coefficient
ICP-MS: Inductively Coupled Plasma Mass Spectrometry
IQ: Intelligence Quotient
ISA: Infants' Environmental Health study ('*Infantes y Salud Ambiental*' in Spanish)
LA-ICP-MS: Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry
LMP: Last Menstrual Period
LOD: Limit of Detection
Mn: Manganese
NEPSY-II: A Developmental NEuroPSYchological Assessment, Second Edition
NIST: National Institute of Standards and Technology
NS: Not Statistically Significant
OP: Organophosphorous or organophosphate
PBDE: Polybrominated Diphenyl Ether
PPVT: Peabody Picture Vocabulary Test
SD: Standard Deviation
SRM: Standard Reference Materials
WISC-IV: Wechsler Intelligence Scale for Children, Fourth Edition
WRAVMA: Wide Range Assessment of Visual Motor Ability

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Chapter 1:
Background and significance

1. Background

1.1. Human exposure to manganese

1.1.1. Sources of exposure

Manganese (Mn) is a naturally occurring element found in air, soil, water, and food (i.e., grains, nuts, cereals, soy-based products, and tea). It is commonly used in iron and steel production, manufacture of dry-cell batteries and glass, textile bleaching, and tanning of leather (ATDSR 2012). It is also an important component of ethylene bisdithiocarbamate (EBDC) fungicides (FAO 1980), the fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT), and contrast agents used in magnetic resonance imaging (ATDSR 2012).

The most important source of Mn for the general population is diet. However, elevated environmental exposures may occur through inhalation of the combustion products of MMT in gasoline (Zayed et al. 1999), inhalation of dust emissions from industrial sources (Menezes-Filho et al. 2011) and Mn mines (Riojas-Rodriguez et al. 2010), or through ingestion of water from wells with high concentrations of natural Mn or contaminated by industrial waste (Hozyasz and Ruszczynska 2004; Cockell et al. 2004; Ljung and Vahter 2007; Jan et al. 2010). Recent studies suggest that agricultural workers and agricultural communities may be exposed to Mn through use of EBDC fungicides, mancozeb and maneb (21% Mn by weight) (Canossa et al. 1993; Gunier et al. 2013; Takser et al. 2004). Nevertheless, Mn exposure from EBDC fungicides has rarely been studied and biological monitoring of exposure relies almost exclusively on urinary ethylenethiourea (ETU), the main metabolite of EBDCs in humans (Colosio et al. 2002). An Italian study of seven pesticide applicators measured both urinary ETU and Mn before and after a three-day exposure to mancozeb, and reported a significant increase in urinary Mn over the exposure period (Canossa et al. 1993). In a study of 149 pregnant women from Canada, those who reported pesticide (unspecified) applications less than one kilometer from their residence had higher blood Mn concentrations than those who did not report applications (Takser et al. 2004). Additionally, a study in California found higher prenatal Mn concentrations in deciduous teeth of children whose mothers during pregnancy were farmworkers, lived with a farmworker, had higher agricultural use of Mn-containing fungicides within three kilometers of their home, or had higher Mn dust loadings in their home (Gunier et al. 2013)

In Costa Rica, mancozeb is the most commonly used pesticide (Valcke et al. 2005) and approximately 4.5 million kilograms of this fungicide are applied annually in agriculture, with about 1.3 million kilograms sprayed aerially in banana plantations (Ramirez 2010). In the United States, about 2.7 million kilograms of mancozeb and maneb are used in agriculture every year (EPA 2011). In the Salinas Valley of California, agricultural use of EBDC fungicides averages 110,000 kg per year and more than 90% is applied at ground level on lettuce crops (CDPR 2014). Agricultural fields in Costa Rica and in the Salinas Valley are frequently sprayed with mancozeb and/or maneb (although the latter is no longer registered for use) and are often surrounded by villages, sometimes with just a few meters of distance between the fields, houses, and schools (Castillo 2000), posing a potential environmental health threat for communities living nearby. In addition, many women living near the fields work in agriculture during pregnancy and it is uncertain how many of them bring their children to the fields or how much pesticide residues they or other agricultural workers bring home on their work clothes and shoes.

1.1.2. Toxicokinetics of Mn

Absorption of ingested Mn is highly controlled through the hepatic portal system (ATDSR 2012); in adults, only 3 to 5% of Mn from food is absorbed (ATDSR 2008; Davidsson et al. 1988; Davidsson et al. 1989). Notably, absorption of Mn in the gastrointestinal tract increases with low concentrations of iron, probably because both iron and Mn are absorbed by the same carrier transport system (Mena et al. 1969).

Airborne Mn is considered one of the most hazardous routes of exposure because, when small particles are inhaled, Mn can enter into the body through the lungs (Vitarella et al. 2000) or access the brain directly through the olfactory bulb (Dorman et al. 2002; Elder et al. 2006; Leavens et al. 2007), evading first pass homeostatic excretory mechanisms (Dorman et al. 2002). Animal studies have shown that Mn can also enter the brain by crossing the microvascular endothelial cells of the blood-brain barrier (Aschner and Gannon 1994) and by crossing the choroid plexuses into cerebrospinal fluid (Murphy et al. 1991; Bock et al. 2008). Brain Mn uptake appears to be mediated by calcium permeable channels (Crossgrove and Yokel 2005) and that brain Mn efflux is diffusion mediated (Yokel et al. 2003). Because of carrier-mediated brain influx but not efflux, Mn is slowly cleared and accumulates in brain tissue with repeated excessive exposures (Lai et al. 1999; Yokel 2009).

The relative amounts absorbed in the nasal mucosa, lung, and gastrointestinal tract following inhalation of Mn in dust are not accurately known (ATDSR 2012), but about 60% of the absorbed Mn is removed from the blood by the liver where it is conjugated with bile and then excreted in the feces (Mena et al. 1969; Davis et al. 1993; Malecki et al. 1996). Mn is also eliminated through urine, pancreatic fluids, breast milk, and hair but the proportions of these elimination routes remain unknown (Aschner et al. 2005). Rodent and primate studies have shown that Mn accumulates in the brain, liver, kidney, and testes (Dorman et al. 2005; Dorman et al. 2006; Tapin et al. 2006) Mn accumulation in the lung in a dose-dependent manner has also been reported (Salehi et al. 2003).

Human studies have shown that maternal blood Mn concentrations increase over the course of pregnancy (Takser et al. 2004; Bradman et al. unpublished results; Spencer 1999; Tholin et al. 1995; Hambridge and Droegemueller 1974). It has been hypothesized that this increase may be related to physiological factors, such as Mn mobilization caused by higher estrogen and progesterone levels (Das and Chowdhury 1997), and increased intestinal absorption of Mn (Kirchgessner et al. 1982). In addition, several studies have reported that Mn concentrations in cord blood and newborns sera are on average two to three times higher than maternal blood concentrations (Takser et al. 2004; Vigeh et al. 2008; Yazbeck et al. 2006; Smargiassi et al. 2002). These findings suggest that Mn plays an important physiological role in fetal development requiring elevated Mn. However, the extent to which environmental exposures may further increase Mn concentrations during pregnancy and early postnatal life and cause adverse health effects is not well understood.

1.1.3. Biological monitoring of Mn exposure

To date, there is no consensus on which is the best biomarker of exposure to Mn. Urinary Mn has been used in multiple occupational studies, but it has been recently argued that it may have limited use as a direct measure of exposure because the primary route of Mn excretion is via the biliary system (Smith et al. 2007; Laohaudomchok et al. 2011). Blood Mn has been frequently used as a biomarker of exposure in occupational and population-based studies (Mergler et al. 1999; Takser et al. 2003). However, Mn concentrations in blood are homeostatically regulated by the hepatic portal system and only reflect short-term exposures (ATDSR 2008; Smith et al. 2007). Hair Mn has also been used in epidemiologic studies and it has been thought to reflect the excess Mn in the body (Bouchard et al. 2007; Baldwin et al. 1999; Saner et al. 1985). Two main limitations of using hair Mn as a predictor of Mn body burden include the exogenous contamination that hair is susceptible to (ATDSR 2012; Eastman et al. 2013) and the variability in hair metal concentrations between individuals due to differences in hair characteristics and personal habits (Sturaro et al. 1994; Chojnacka et al. 2006; Kempson and Lombi 2011). More recent biomarkers used to assess Mn exposure include saliva (Olmedo et al. 2010; Czegeny et al. 2001) and toenails (Laohaudomchok et al. 2011; Grashow et al. 2014); however, laboratory methods for these matrices are less developed.

Evidence suggests that available biomarkers may have a limited ability to assess prenatal exposure to the fetus. Because Mn is incorporated in the developing teeth in an incremental pattern, the distribution of Mn in deciduous teeth may provide information on environmental exposures to Mn during fetal development and early childhood (Arora et al. 2012). Dentine, unlike deciduous enamel, can provide reliable information on the developmental timing of exposures to Mn that occur between the second trimester of pregnancy (13-16 weeks gestation, when incisors start forming) and 10-11 months after birth (when molars stop developing) (Arora et al. 2012). Additional advantages of using shed teeth as biomarkers include a non-invasive collection and excellent storage stability.

1.2. Fetal growth and manganese

Mn is an important cofactor for enzymes necessary for bone formation and metabolism (ATDSR 2012). However, several animal studies have reported that Mn overexposure during gestation is associated with decreased fetal size and weight (Colomina et al. 1996; Sanchez et al. 1993; Treinen et al. 1995). Mn at toxic concentrations is believed to cause oxidative stress that leads to impairment of cellular antioxidant defenses, mitochondrial dysfunction (Keen et al. 2000; HaMai and Bondy 2004), and fetal growth abnormalities (Zota et al. 2009). To date, only a small number of studies in pregnant rodents exposed to elevated doses of Mn orally or subcutaneously has not observed any association with decreased fetal size and weight (Gray and Laskey 1980; Molina et al. 2011; Pappas et al. 1997; Torrente et al. 2002) and one of them found an increase in fetal body weight (Betharia and Maher 2012). Nevertheless, differences between animal studies may simply reflect different dosing parameters (i.e., frequency, magnitude, and timing).

Numerous studies have examined the effects of prenatal Mn exposure on birth outcomes in humans, but their findings are inconsistent (Chen et al. 2014; Eum et al. 2014; Guan et al. 2013; Takser et al. 2004; Vigeh et al. 2008; Zota et al. 2009; Osada et al. 2002; Xu et al. 2011; Yu and Cao 2013). Three studies have reported inverse U-shaped associations between maternal blood Mn concentrations at delivery and birth weight (Chen et al. 2014; Eum et al. 2014; Zota et al. 2009). A recent study did not observe an association between maternal blood Mn concentrations at delivery and birth weight, but found significant nonlinear relationships of Mn concentrations with head and chest circumference (Guan et al. 2013). The only epidemiological study has been published to date on the relationship between blood Mn concentrations measured at multiple time points during pregnancy and birth outcomes did not find any significant associations (Takser et al. 2004). No studies have examined the association between hair Mn concentrations and birth outcomes.

1.3. Neurobehavioral development and manganese

Mn is an important constituent of metalloenzymes that protect the central nervous system against oxidative damage (Aschner and Aschner 1991; Hussain and Ali 1999) and non-enzyme complexes that block the synthesis of oxygen-free radicals (Cheton and Archibald 1988). However, evidence suggests that the protective mechanisms of Mn against oxidative injury may be overwhelmed in cases of excessive exposure to this metal (ATDSR 2008; Ali et al. 1995; Santos et al. 2012). Other potential mechanisms of Mn neurotoxicity include disruption of mitochondrial metabolism (Zwingmann et al. 2003) and alteration of cellular iron metabolism (Kwik-Uribe and Smith 2006). *In vitro* and animal studies have shown that excess Mn targets brain dopaminergic (DA) systems, and possibly glutamatergic and GABAergic systems (Torrente et al. 2002; Crooks et al. 2007; Guilarte et al. 2008; Gwiazda et al. 2002; Kern and Smith 2011; Lai et al. 1984; Lazrishvili et al. 2009; McDougall et al. 2008; Mutkus et al. 2005; Reichel et al. 2006; Tran et al. 2002; Zhao et al. 2009).

Rodent and primate studies have shown that early Mn exposure results in behavioral and cognitive deficits including hyperactivity (Tran et al. 2002; Golub et al. 2005; Kern et al. 2010; Lown et al. 1984), maladaptive emotional-social behaviors (Golub et al. 2005), behavioral disinhibition (Kern et al. 2010; Calabresi et al. 2001), and deficits in learning (Tran et al. 2002; Kern et al. 2010), memory (Betharia and Maher 2012; Tran et al. 2002; Kern et al. 2010; Blecharz-Klin et al. 2012; Vezer et al. 2005) and (less consistently) motor function (Torrente et al. 2002; Betharia and Maher 2012; McDougall et al. 2008; Tran et al. 2002; Beaudin et al. 2013) with a few studies showing greater effects in males than in females (Betharia and Maher 2012).

Few epidemiological studies have examined the association between *in utero* Mn exposure and neurodevelopment, but higher *in utero* Mn concentrations measured in blood or teeth have been associated with attention problems (Takser et al. 2003; Ericson et al. 2007), behavioral disinhibition (Ericson et al. 2007), impaired non-verbal memory (Takser et al. 2003), and poor cognitive and language development (Lin et al. 2013) in toddlers and preschoolers, and with externalizing behavior and attention problems (Ericson et al. 2007) in school-age children. Similarly, early postnatal Mn exposure has also been associated with poor language development in toddler boys (Rink et al. 2014), and behavioral problems in school-age boys and girls (Ericson et al. 2007). Notably, one study of children aged 12-36 months observed an inverted U-shaped relationship between child blood Mn concentrations at 12 months and concurrent mental development (Claus Henn et al. 2010).

2. Statement of research question and specific aims

Mn is an essential nutrient that plays an important role in multiple physiologic processes such as somatic growth, bone formation, and insulin synthesis (ATDSR 2008; Bolze et al. 1985; Keen et al. 2000; Hansen et al. 2006), but adverse health effects can be caused by both deficiency and excess of Mn. For instance, recent epidemiological studies have reported that high Mn concentrations during pregnancy and early postnatal period are associated with impaired fetal growth (Zota et al. 2009) and neurobehavioral deficits in children (Claus Henn et al. 2010). Additionally, animal studies have reported that early exposure to Mn may be related to behavioral and neurological effects later in life, long after cessation of exposure (Kern and Smith 2011).

This dissertation aims to determine whether prenatal and early postnatal Mn exposure are associated with fetal growth and neurodevelopment in two mother-child cohorts living near agricultural fields treated with Mn-containing fungicides in Costa Rica and California. The main hypotheses of this proposal are that women and children in these two cohorts are exposed to Mn at higher than background levels due to the application of Mn-containing fungicides and that women and children with higher Mn body burden during pregnancy and in the early postnatal period are likely to be at an increased risk for abnormal fetal growth and impaired neurodevelopment.

Chapter 2 aims to identify lifestyle, occupational, and environmental factors associated with blood and hair Mn concentrations measured during pregnancy in women living near banana plantations with aerial mancozeb spraying in Costa Rica. Chapter 3 aims to determine whether prenatal hair and blood Mn concentrations are associated with fetal growth and length of gestation in this mother-infant cohort living near banana plantations in Costa Rica. Lastly, Chapter 4 aims to examine the relationship of prenatal and postnatal dentine Mn levels in children's deciduous teeth with attention, cognition, memory, and motor development in children aged 7, 9, and 10.5 years living in an agricultural community in California where large amounts of Mn-containing fungicides are applied.

3. Significance

This dissertation builds upon the Infants' Environmental Health Study (ISA) and Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study populations, unique in their high levels of exposure to Mn-containing fungicides, which offer the possibility of conducting a large-scale investigation of Mn exposure in children living in the vicinity of agricultural fields. The proposed study will improve upon past and current studies of Mn exposure and health effects by examining if exposure to Mn-containing fungicides results in toxic levels of Mn. This research has the potential for high impact by providing Costa Rican and Californian legislators and policymakers with information on the health human effects of Mn exposure and specifically of Mn-containing fungicides. Ultimately, this dissertation will address key knowledge gaps faced by agencies such as World Health Organization that seek to provide an appropriate regulation on usage of Mn-containing fungicides for crop production.

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Chapter 2:
Blood and hair manganese concentrations in pregnant women from the Infants' Environmental Health Study (ISA) in Costa Rica[1]

1. Introduction

Manganese (Mn) is an essential nutrient that plays an important role in multiple physiological processes such as somatic growth and bone formation (ATDSR 2012). Animal and human studies have shown that adverse health effects may result from either deficiency or excess of Mn. For example, epidemiological studies found that both low and high Mn concentrations during pregnancy and early childhood were associated with impaired fetal growth (Zota et al. 2009) and neurobehavioral deficits in children (Claus Henn et al. 2010). In addition, animal studies have reported that early exposure to Mn may be related to behavioral and neurological effects later in life, long after cessation of exposure (Kern and Smith 2011; Beaudin et al. 2013).

Food is the main source of Mn intake for the general population (ATDSR 2012), but Mn absorption in the gastrointestinal tract and presystemic elimination by the liver are closely regulated through homeostatic mechanisms (Roth 2006). Although Mn intake from drinking water is much lower than intake from food (WHO 2011), ingestion of water from wells with high concentrations of natural Mn or contaminated by industrial waste has been associated with increased blood or hair Mn and negative health effects in human populations (ATDSR 2012).

Environmental exposure to Mn may also occur through inhalation of the combustion of anti-knock additives in gasoline (Zayed et al. 1999) or inhalation of dust emissions from industrial sources (Menezes-Filho et al. 2011) and Mn mines (Riojas-Rodriguez et al. 2010). Airborne Mn can be inhaled and enter the body through the lungs (Vitarella et al. 2000). It may also access the brain directly through the olfactory bulb, bypassing homeostatic regulatory mechanisms (Dorman et al. 2002).

Ethylene bisdithiocarbamate (EBDC) fungicides, mancozeb and maneb, contain approximately 20% manganese by weight (FAO 1980) and may constitute a potential source of exposure to Mn (Gunier et al. 2013; Takser et al. 2004). Nevertheless, Mn exposure from EBDC fungicides has rarely been studied and biological monitoring of exposure relies almost exclusively on urinary ethylenethiourea (ETU), the main metabolite of EBDCs in humans (Colosio et al. 2002). An Italian study of seven pesticide applicators measured both urinary ETU and Mn before and after a three-day exposure to mancozeb, and reported a significant increase in urinary Mn over the exposure period (Canossa et al. 1993). A Mexican study found high accumulation of Mn in soils of banana plantations sprayed with mancozeb (Geissen et al. 2010). In a study of 149 pregnant women from Quebec, those who reported pesticide (unspecified) applications < 1 kilometer from their residence had higher blood Mn concentrations than those who did not report applications (Takser et al. 2004). Additionally, a study of 207 mother-child pairs from California observed that Mn-containing fungicide spraying < 3 kilometers from pregnant women's residence was associated with higher Mn concentrations in their children's deciduous teeth (Gunier et al. 2013).

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In the United States, about 2.7 million kilograms of mancozeb are used in agriculture every year (EPA 2011), mostly via ground spraying. In Costa Rica, approximately 4.5 million kilograms of mancozeb are applied annually for agricultural purposes, with about 1.3 million kilograms sprayed aerially in banana plantations (Ramirez 2010). In the present study, we aimed to: (1) measure blood and hair Mn concentrations in pregnant women living near banana plantations with aerial mancozeb spraying; (2) determine the variability in blood and hair Mn concentrations between and within women during pregnancy; and (3) identify lifestyle, occupational, and environmental factors associated with blood and hair Mn concentrations measured during pregnancy.

2. Methods and materials

2.1. Study area and study population

The Infants' Environmental Health Study (ISA) is a birth cohort study examining the effects of prenatal and early postnatal exposure to pesticides on birth outcomes and neurodevelopment in children who live in Matina County, Costa Rica (van Wendel de Joode et al. submitted) Matina County, located on the Caribbean coast, is divided into three districts with 57 villages, and has a population of approximately 37,700 people (INEC 2011). Banana plantations, the largest crop in Matina with about 14,000 hectares (34% of the land used in agriculture and livestock grazing) (van Wendel de Joode et al. submitted), are aerially sprayed with mancozeb on a weekly basis to protect the plants from Black Sigatoka disease (Geissen et al. 2010).

Pregnant women were recruited for the ISA study through meetings in local schools, communal groups, advertisements, and friends' referrals. Eligible women were ≥ 15 years old, < 33 weeks of gestation, and living in one of the 40 villages of Matina County located within five kilometers of a banana plantation. A total of 480 women were invited to participate in the study and 94% were enrolled between March 2010 and June 2011. All study activities were approved by the Ethical Committee of the Universidad Nacional and written informed consent was obtained from all participants. Additional informed consent was obtained from the parents or legal guardians of participants < 18 years.

2.2. Study interviews

Women were interviewed between one and three times during pregnancy and following delivery. The baseline interview occurred at enrollment or shortly after [mean = 19 weeks of gestation, range = 3-36 weeks]. Women who were enrolled during the first trimester had follow-up visits during the second and third trimesters. Those enrolled during the second or third trimester had follow-up visits during the third trimester. The follow-up interviews took place at approximately 29 weeks (range = 14-40 weeks) and 32 weeks of gestation (range = 25-35 weeks), and after delivery (mean = 7 weeks postpartum, range = 1-38 weeks). Demographic information gathered during the baseline interview included maternal age, educational level, country of birth, and family income. Information on basic dietary intake, agricultural work, medical conditions, medications, household members, and aerial spraying near the home was obtained at each interview. Study interviewers abstracted data from prenatal and delivery medical records that were completed by hospital/clinic personnel and provided to the study participants. Information on maternal hemoglobin levels was abstracted from medical records for a subset of participants ($n = 64$) to assess maternal iron status during pregnancy.

Gestational age at each interview and sample collection was calculated using the date of the last menstrual period reported by women at study enrollment. When this date was unknown, gestational age was calculated using the gestational age at birth registered in the medical record booklets. We imputed missing values of gestational age (*n* = 3) by randomly selecting a value from the dataset.

2.3. Blood Mn measurements

Venous whole blood (2 mL) was collected into metal-free Vacutainer EDTA tubes (Ref. Number 454036, Greiner Bio-One Vacuette, Monroe, NC) and stored at -20°C until its shipment to the University of California, Santa Cruz, for analysis. Elemental determinations of Mn were performed using high-resolution inductively coupled plasma mass spectrometry (Finnigan XR ICP-MS) (Smith et al. 2007). Analytical accuracy was estimated in > 95%, based on analyses of standard reference materials (NIST SRM 955c, bovine liver) and sample spike-recoveries. The precision of blood Mn measurements, based on triplicate samples analyzed with each analytical batch, was 3.8% relative standard deviation. The analytical limit of detection (LOD) for Mn was 0.003 µg/L; no samples were below the LOD.

Whole blood Mn was measured in 664 blood samples collected from 418 women. A total of 246 women provided two samples during pregnancy and 172 provided only one. The first blood samples were collected at enrollment or shortly after (mean = 21 weeks, range: 3-38 weeks) and the second samples were collected during the follow-up visits (mean = 29.2 weeks, range: 14-40 weeks).

2.4. Hair Mn measurements

Hair samples (~20-30 strands) were collected from the occipital region, within 2 mm from the scalp, and stored in plastic bags at room temperature until their shipment to the Federal University of Bahia, Brazil. Hair samples were cleaned and analyzed as described elsewhere (Menezes-Filho et al. 2009). Briefly, the one-centimeter closest to the scalp was washed for 15 min in 10 mL of 1% Triton X-100 solution in an ultrasonic bath, rinsed several times with Milli-Q water, dried overnight at 70°C and ~10 mg of hair were digested in 2 mL of ultra-pure concentrated nitric acid at 80°C for 2 hours. Acid-digested samples and reference material from the International Atomic Energy Agency (IAEA-085) were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). Reagent blanks were analyzed along with samples in every batch and the intra-batch and batch-to-batch precisions were estimated in 2.4% and 5.9%, respectively. All processed samples and reference materials were analyzed in duplicates and a difference ≤ 10% was considered acceptable. Accuracy in the concentration range of 8.3 to 9.3 µg/g was 102.6% and the analytical LOD for Mn in hair was 0.1 µg/g. Hair samples with Mn concentrations below LOD (*n* = 3) were set at LOD/2.

Mn was measured in 800 hair samples collected from 449 study participants; 351 women provided two hair samples and 98 provided only one. The first hair samples were collected at the enrollment visit (mean = 19 weeks, range: 3-36 weeks) and the second samples were collected during the follow-up visits (mean = 29 weeks, range: 15-40 weeks).

2.5. Drinking water Mn measurements

A convenience sample of 138 participants' houses distributed in 37 villages was selected for drinking water sampling. In houses with water supply (*n* = 92), tap water samples of 10 mL were collected into polypropylene tubes (PerformR™ Centrifuge tubes, Labcon, Petaluma, CA) using the following standardized procedure (adapted from van den Hoven and Slaats) (van den Hoven and Slaats 2006): open the tap for three minutes, reduce flow, and collect sample. In houses with no water supply (*n* = 46), women collected drinking water from wells, community taps, rivers, or bottled water and stored it in large plastic containers. Water samples of 10 mL were collected directly from the plastic containers in these houses. Drinking water samples were stored at -20°C until their shipment to Lund University Hospital, Sweden. Water Mn concentrations were measured in acidified samples (2% HNO_3) using ICP-MS (Thermo X7, Thermo Elemental, Winsford, UK). Analytical accuracy was estimated based on reference material (SLRS-2, Riverine Water Reference Material for Trace Metals, Otawa, CA). The results [mean ± standard deviation (SD)] obtained were 8.9 ± 0.3 (*n* = 14) vs. recommended 10.1 ± 0.3 µg/L. All samples were analyzed in duplicate and the method imprecision (calculated as the coefficient of variation for duplicate preparations measurements) was 2.5%. The analytical detection limit was defined as three times the SD of the blank samples and was estimated as 0.05 µg/L.

2.6. Residential distance from banana plantations

Location of the participants' houses at each of the pregnancy visits was collected using a Global Positioning System (GPS) receiver (Garmin etrex Venture HC, Olathe, KS). Banana plantations within a five-kilometer radius of each participant's house were identified using aerial photos from the Costa Rica Airborne Research and Technology Applications (CARTA) 2005 mission and GPS coordinates of the closest banana plantation were recorded. Houses and closest banana plantations were located on a geocoded map of the Matina County using ArcGIS 10.0 software (Esri, Redlands, CA) and CARTA 2005 mission photos (Figure S1). Plantation locations were measured as static areas of at least four points when possible. Euclidean distances were measured from the point representing the home to the edge of the closest plantation.

2.7. Statistical analysis

Blood Mn concentrations were normally distributed, whereas hair and water Mn concentrations were skewed. Thus, we transformed hair and water Mn concentrations to the $\log_{10}$ scale to normalize the residuals. To assess the within- and between-woman variability and reproducibility of blood Mn and hair Mn concentrations, we calculated the intraclass correlation coefficient (ICC) using mixed-effects models (Rosner 2006).

We ran bivariate and multivariate analyses for blood and hair Mn separately. First, we used linear mixed-effects models with random intercepts to assess the bivariate associations between potential explanatory variables and blood or hair Mn concentrations and to estimate the amount of variability in measured levels explained by the model while accounting for the correlation among repeated measures (Peretz et al. 2002). We also explored potential nonlinear associations between continuous explanatory variables and blood or hair Mn by examining penalized splines in generalized additive models (these did not account for repeated measures). We included potential explanatory variables of Mn exposure in our multivariate models if their p-value in the bivariate analysis was < 0.20. These explanatory variables were added to the multivariate mixed-effects models using manual forward stepwise selection and were retained if significantly associated ($p < 0.10$) with blood or hair Mn. The following variables were significant in the bivariate analyses and therefore considered in the mixed-effects model for blood Mn: age at enrollment, gestational age at each visit, frequency of fruit, vegetable, grain, and tea consumption per week (foods with high Mn content) (ATDSR 2012), pre-pregnancy body mass index (BMI), gestational anemia reported by the women, iron supplementation during pregnancy, smoking during pregnancy, number of household members during pregnancy, agricultural workers in household during pregnancy, occupation at each visit during pregnancy, husband/partner's occupation at each visit during pregnancy, residential distance from banana plantations, source of drinking water in the home, aerial spraying near the home on the day of or the day before each blood sample collection, and washing work clothes of agricultural workers on the day of or the day before each blood sample collection. In the mixed-effects model for hair Mn, we considered education level, marital status, country of birth, percentage of life in Costa Rica at enrollment, gestational age at each visit, frequency of fruit, vegetable, grain, and tea consumption per week; average number of showers per day, number of household members during pregnancy, agricultural workers in household during pregnancy, occupation before pregnancy, husband/partner's occupation before pregnancy, residential distance from banana plantations, source of drinking water in the home, aerial spraying near the home on the day of or the day before each hair sample collection, and washing work clothes of agricultural workers on the day of or the day before each hair sample collection. Missing values for predictors included in the final models (< 5%) were imputed by randomly selecting a value from the dataset. We conducted our final multivariate mixed-effects models for blood and hair Mn with 418 and 449 women, respectively. We performed sensitivity analyses for blood and hair Mn on subgroups for which we had additional variables [e.g., drinking water Mn concentrations ($k = 138$) and housing materials ($k = 275$)] and in the subset of women with both blood and hair concentrations ($k = 411$).

Main effects were considered statistically significant if $p < 0.05$ (two-tailed tests). All analyses were conducted using Stata version 11.2 (StataCorp, College Station, TX).

3. Results

Women in the study were, on average, 24.0 years old (SD = 6.5 years); 81% were born in Costa Rica, 76% were married or living as married, and 60% had a family income below the Costa Rican poverty line (Table 1). Few women had completed high school (3%), and few reported smoking during pregnancy (4%). Approximately 8% of the women worked in agriculture during pregnancy and 74% of them lived with one or more agricultural workers. Twenty-five percent (25%) of the women lived < 50 m away from a banana plantation and about 80% reported drinking water from an aqueduct. Drinking water Mn concentrations were significantly higher in households supplied by well water ($n = 27$; median = 417.0 µg/L) compared to those supplied by the local aqueduct ($n = 102$; median = 3.5 µg/L).

Table 2 presents descriptive statistics for blood and hair Mn concentrations. Blood Mn concentrations ranged from 8.9 to 56.3 µg/L (mean ± SD: 24.4 ± 6.6 µg/L) and hair Mn from 0.05 to 53.3 µg/g [GM (geometric standard deviation, GSD): 1.8 (3.2) µg/g]. Blood Mn concentrations increased significantly with gestational age ($\beta = 0.2$, 95% CI: 0.1 to 0.2; Figure S1A), while hair Mn concentrations showed a small but statistically significant decrease ($\beta = -0.99$, 95% CI: -0.98 to -1.0; Figure S1B). Blood Mn was not correlated with hair Mn concentrations ($r_s = -0.04$, $p = 0.27$, $n = 645$).

An ICC value of 0.44 was observed for blood Mn suggesting greater within- than between-woman variability in concentrations (Table 2). In contrast, an ICC value of 0.58 for $\log_{10}$-

transformed hair Mn concentrations indicated that only 42% of the variability of hair Mn was due to intra-individual variability.

3.1. Factors associated with blood Mn

In the bivariate analyses, blood Mn concentrations were lower among women who smoked, who had a pre-pregnancy BMI < 18.5 kg/m^2, worked in agriculture during pregnancy, lived < 50 m from a banana plantation, and somewhat lower in women who drank water from a well during pregnancy and who reported aerial spraying near their home the day before the blood specimen was collected (Table 1), but higher in women who lived with ≥ 2 agricultural workers during pregnancy, reported gestational anemia, and took iron supplementation during pregnancy (data not shown). Blood Mn concentrations were not associated with frequency of fruit, vegetable, grain, and tea consumption, or with hemoglobin levels (data not shown). In the subset of women for whom information on house materials was available, blood Mn concentrations were higher among those who lived in houses with walls and/or floors made with permeable and difficult-to-clean materials such as wood and plastic (Table 1).

Figure 1 summarizes the results from the multivariate mixed-effects model for blood Mn (Table S1, Model 1). Blood Mn concentrations were positively and significantly associated with gestational age at sample collection ($\beta = 0.2$, 95% CI: 0.1 to 0.2) and number of household members during pregnancy ($\beta = 0.4$, 95% CI: 0.1 to 0.6), after adjusting for other variables. Conversely, women who smoked ($\beta = -3.1$, 95% CI: -5.8 to -0.3), worked in agriculture during pregnancy ($\beta = -1.9$, 95% CI: -3.9 to 0.1), or reported aerial spraying near their home the day before each blood sample collection ($\beta = -1.0$, 95% CI: -2.1 to 0.1) had lower blood Mn concentrations.

When we ran the same multivariate model in the subset of women for whom we had information on housing materials ($k = 275$ women), aerial spraying near the home the day before each blood specimen collection was no longer associated with blood Mn ($\beta = -0.8$, 95% CI: -2.0 to 0.4; data not shown). Similarly, when housing characteristics were included in the multivariate model (Table S1, Model 2), we observed that aerial spraying near the home the day before each sample collection was not associated with blood Mn ($\beta = -0.7$, 95% CI: -2.0 to 0.5), and that women who lived in houses with permeable walls and/or floors made of difficult-to-clean materials had higher blood Mn concentrations ($\beta = 2.6$, 95% CI: 1.3 to 4.0) compared to women who did not live in such houses.

3.2. Factors associated with hair Mn

Hair Mn concentrations were significantly higher among women who were married and born outside of Costa Rica in the bivariate analyses (Table 1). In addition, women whose husband or partner worked in agriculture before and during pregnancy, women who worked in agriculture before pregnancy, lived with ≥ 1 agricultural workers during pregnancy, drank water from a well during pregnancy, lived < 50 m from a banana plantation, or reported aerial spraying near their home or washing work clothes on the day of the hair sample collection also had higher Mn hair concentrations. Hair Mn concentrations were not associated with frequency of fruit, vegetable, grain, or tea consumption, pre-pregnancy BMI, average number of showers per day, gestational anemia reported by the women, iron supplementation during pregnancy, or hemoglobin levels (data not shown). In the subset of women with Mn concentrations measured in drinking water samples, hair Mn concentrations were positively and significantly associated with water Mn concentrations ($r_s = 0.48$, $p < 0.001$, $k = 138$).

The multivariate mixed-effects model for hair Mn confirmed the findings of the bivariate analyses (Figure 2; also Table S2, Model 1). Gestational age at sample collection was negatively associated with hair Mn concentrations, with a 0.8% (95% CI: -1.6 to 0.0) decrease in hair Mn per one-week increase in gestational age. Hair Mn concentrations were 29.6% (95% CI: 3.2 to 62.8) higher among women born outside of Costa Rica compared to women born in the country, 25.1% (95% CI: 1.6 to 54.0) higher among pregnant women who worked in agriculture before pregnancy compared to women who did not, 128.6% (95% CI: 78.9 to 192.0) higher in women who drank water from a well compared to women who drank water from an aqueduct, and 23.9% (95% CI: 5.0 to 46.2) higher in women who reported aerial spraying near their home on the day of the hair sample collection compared to women who did not. Hair Mn concentrations were also positively associated with the number of people living in the house during pregnancy, increasing 4.8% with each additional household member (95% CI: 0.4 to 9.4). In addition, Mn concentrations in hair were 42.1% (95% CI: 14.2 to 76.9) higher in women who lived < 50 m from a banana plantation compared to women who lived 50 to 600 m away from a plantation.

When we restricted our analysis to the subset of women for whom we had information on drinking water Mn concentrations ($k = 138$), hair Mn concentrations were no longer associated with occupation before pregnancy (% change = 11.1, 95% CI: -26.3 to 67.4), number of household members (% change = 2.6, 95% CI: -5.1 to 11.0), and aerial spraying near the home the day of the hair sample collection (% change = 12.0, 95% CI: -17.5 to 52.1; data not shown). Then, when we included drinking water Mn concentrations in the multivariate model and excluded source of drinking water (because they were strongly associated: $p < 0.001$; Table S2, Model 2), we found a 17.5% (95% CI: 12.2 to 22.8) increase in hair Mn concentrations per μg/L-increase in drinking water Mn concentrations.

Sensitivity analyses using only the subset of women for whom we had both hair and blood Mn concentrations ($k = 411$), did not show substantial differences in the associations between potential explanatory variables and blood Mn. However, hair Mn concentrations were no longer significantly associated with maternal country of birth (% change = 20.2, 95% CI: -6.4 to 54.5) or maternal occupation before pregnancy (% change = 20.7, 95% CI: -3.9 to 51.6; data not shown).

Final multivariate mixed-effects models explained between 8% and 14% of the variability in blood Mn concentrations (Table S1) and between 14% and 30% of the variability in hair Mn concentrations (Table S2).

4. Discussion

In this study of Costa Rican pregnant women living near banana plantations sprayed with Mn-containing fungicides, associations between blood Mn concentrations and occupational and environmental factors were inconsistent, with number of household members and permeable and difficult-to-clean house materials showing positive associations, while occupation at each visit during pregnancy and nearby aerial spraying the day before the blood specimen collection showing negative associations. In contrast, hair Mn concentrations were positively related with several occupational and environmental factors such as occupation before pregnancy, number of household members, nearby aerial spraying on the day of the hair sample collection, and drinking water Mn concentrations, and inversely associated with distance between residences and banana plantations.

Blood Mn concentrations in this population were higher than most concentrations reported in pregnant women from other countries (Table S3) (Zota et al. 2009; Kopp et al. 2012; Yu and Cao 2013; Claus Henn et al. 2011; Rudge et al. 2009; Abdelouahab et al. 2010; Lin et al. 2010; Ljung et al. 2009; Rollin et al. 2009) including women living near agricultural fields treated with pesticides (Figure S3) (Takser et al. 2004; Bradman et al. unpublished results). Hair Mn concentrations in this population were higher than those reported in several studies of pregnant women, including from France (Takser et al. 2003) and the U.S. (Hambridge and Droegemueller 1974). However, these studies used different hair cleaning methods in the pre-analytical phase compared to our study, so the across-study comparison of hair Mn concentrations could reflect analytical differences. A study of non-pregnant women in Brazil living near a ferromanganese alloy plant, that used the same hair cleaning procedure used in the ISA study, observed approximately two-fold higher hair Mn concentrations than those found in our study population (GM = 3.5 and 1.8 μg/g, respectively) (Menezes-Filho et al. 2011). While several studies have shown associations between hair Mn concentrations and environmental Mn exposures (Menezes-Filho et al. 2009; Eastman et al. 2013), the susceptibility of hair to external contamination remains an issue of concern that may impact its utility as biomarker of exposure (ATDSR 2012; Eastman et al. 2013). Currently, there is no definitive method for distinguishing between exogenously derived Mn contamination versus metabolically incorporated Mn in hair (Eastman et al. 2013; Stauber and Florence 1989). Therefore, it remains possible that hair Mn concentrations in this population reflect Mn body burden, external contamination from airborne or waterborne Mn, or a mixture of both.

Blood Mn concentrations measured in repeated samples collected during pregnancy showed only a moderate level of within-subject correlation (gestational age-adjusted ICC = 0.44), indicating that one blood measurement does not accurately represent Mn body burden during gestation. Conversely, we found a relatively high within-person correlation for repeated measures of hair Mn concentrations (gestational age-adjusted ICC = 0.58), suggesting that one hair measurement may represent Mn body burden during pregnancy better than one blood measurement. This is not unexpected, given that a one-centimeter section of hair, as analyzed in this study, would integrate circulating Mn concentrations over the one-month duration of hair growth, while a single blood measurement would reflect body Mn dynamics on the order of days (Smith et al. 2007).

Consistent with previous studies in pregnant women (Figure S3) (Takser et al. 2004; Bradman et al. unpublished results; Hambridge and Droegemueller 1974; Spencer 1999; Tholin et al. 1995), we observed a significant increase in blood Mn concentrations with increasing gestational age, specifically between the first and third trimesters of gestation. This increase in blood Mn during pregnancy could be related to an increase in Mn intestinal absorption (Tholin et al. 1995; Kaludin and Ganovski 1981; Kirchgessner et al. 1982) or changes in Mn metabolism (Tholin et al. 1995). It could also result from physiological factors such as differences in tissue Mn mobilization due to increased estrogen and progesterone concentrations during pregnancy (Takser et al. 2004; Das and Chowdhury 1997).

In contrast to blood Mn, we found a small but significant decrease in hair Mn concentrations between the first and third trimesters of gestation (GM = 1.9 and 1.7 μg/g, respectively). A similar finding was reported in a study of 20 pregnant women in Colorado (means at II and III trimester = 0.2 and 0.1 μg/g, respectively) (Hambridge and Droegemueller 1974). We can only speculate as to the reason why hair Mn concentrations decrease as blood Mn concentrations increase over gestation. One hypothesis is that fetal Mn requirements increase during pregnancy, resulting in a decrease in Mn excretion from the body and into hair (similar findings have been reported in relation to zinc) (Carbone et al. 1992). Another hypothesis is that hair dynamics change over the course of pregnancy (e.g., increased hair growth due to higher estrogen concentrations) (Gizlenti and Ekmekci 2013).

Blood Mn concentrations did not show consistent associations with environmental and occupational factors: some characteristics were positively associated with blood Mn concentrations (e.g., number of household members and living in a house made of permeable and harder-to-clean walls and/or floors), but others were negatively associated (e.g., occupation in agriculture during pregnancy and aerial spraying near the home the day before the blood specimen collection). These inconsistent findings could be due to the homeostatic mechanisms that closely regulate blood Mn concentrations (Roth 2006), or due to changes in Mn mobilization from brain or bone deposits due to increased fetal Mn requirements (Melgar et al. 2008). Variations in iron metabolism, liver function, and bone metabolism could also explain the observed results (Davis et al. 1993; Hauser et al. 1994; Jarvinen and Ahlstrom 1975; Layrargues et al. 1998; Mena et al. 1969; Spahr et al. 1996).

We observed significantly lower blood Mn concentrations in the few women who smoked during pregnancy compared to those who did not smoke. Similar findings have been previously reported in pregnant women from a study in Canada (Takser et al. 2004; Das and Chowdhury 1997) and in adult smokers from a national study in Korea (Lee and Kim 2011). This negative association could result from the decrease in estrogen levels linked to cigarette smoking and its consequent effect on Mn mobilization (Das and Chowdhury 1997; Bernstein et al. 1989). Further studies are required to determine the mechanisms by which smoking affects blood Mn concentrations.

Hair Mn concentrations were associated with several environmental characteristics such as residential distance from banana plantations, aerial spraying near the home on the day of the hair sample collection, source of drinking water in the home, and drinking water Mn concentrations. Studies of children exposed to Mn have reported higher hair and teeth Mn concentrations in relation to sources of environmental airborne exposure (Gunier et al. 2013; Menezes-Filho et al. 2009; Eastman et al. 2013). For example, children who lived near or directly downwind from a ferromanganese alloy plant in Brazil had higher hair Mn concentrations compared to children who lived farther away (Menezes-Filho et al. 2009). Similarly, Italian children living near an active ferroalloy plant had significantly higher hair Mn concentrations compared to children living near a historic but currently inactive plant (Eastman et al. 2013). More recently, California children whose mothers lived within three km from an agricultural field treated with Mn-containing fungicides during pregnancy had higher teeth Mn concentrations compared to children whose mothers lived farther away (Gunier et al. 2013). These findings suggest that higher hair Mn concentrations observed in women living < 50 m from a banana plantation compared to women who lived farther away could be due to drift associated with applications of mancozeb, and this drift could result in higher Mn body burden and/or external contamination from airborne Mn.

We observed a positive and significant relationship between hair Mn and drinking water Mn concentrations in this population, consistent with other studies (Agusa et al. 2006; Bouchard et al. 2007; Bouchard et al. 2011; He et al. 1994; Kondakis et al. 1989). Mn concentrations in well water were significantly higher than in water supplied by the national water system or local aqueduct and, although relatively few women drank water from wells (*n* = 72), their hair Mn concentrations were significantly higher than those of women who drank water from other sources. Mn concentrations in well water could be naturally occurring or reflect residues from mancozeb spraying (Melgar et al. 2008). It is possible that women who drank water with high Mn concentrations also washed their hair with this water and, consequently, contaminated their hair with waterborne Mn (Eastman et al. 2013).

It is noteworthy that women born outside of Costa Rica had significantly higher hair Mn concentrations compared to those born in the country. These differences are partially explained by the strong association between country of birth and residential distance from plantations, husband/partner's occupation before and during pregnancy, and aerial spraying near the home the day before or the day of the hair sample collection (van Wendel de Joode et al. submitted). However, country of birth may also be a proxy for unmeasured explanatory variables, since it remained significant in the multivariate model, even after adjusting for other related variables. Occupation in agriculture before pregnancy, but not during pregnancy, was also associated with increased hair Mn concentrations. This association could be due to the fact that hair integrates exposures over longer time frames rather than recent exposures (Smith et al. 2007; Schroeter et al. 2011).

This study has several limitations. Food is the main source of Mn in the general population, but we did not measure Mn concentrations in food. In addition, we did not measure indicators such as bone metabolism, iron status, liver function, and hormonal secretions that would have allowed us to better understand the variations observed in blood Mn concentrations. We did not quantify Mn concentrations in indoor air or house dust, so we were not able to examine how well blood and hair Mn concentrations correlated to air and dust Mn concentrations. We did not collect information on meteorological conditions to better estimate pesticide drift and airborne exposure to mancozeb and Mn. Therefore, we could not directly examine the contribution of different pathways of Mn exposure and some level of exposure misclassification is expected.

Our findings suggest that pregnant women who live near banana plantations aerially sprayed with mancozeb may have higher blood and hair Mn concentrations than those reported in most studies of pregnant women. Hair Mn concentrations were associated with several environmental characteristics, including residential distance to banana plantations and drinking water Mn concentrations. In contrast, blood Mn concentrations were not consistently associated with environmental or occupational characteristics. In future analyses, we will examine the relationship between hair and blood Mn concentrations, fetal growth, and children's neurodevelopment in the ISA cohort.

5. Tables and figures

Table 1. Study cohort characteristics and results from bivariate linear mixed-effect models for blood and hair Mn concentrations, ISA study.

	Overall	**Blood Mn (µg/L)** (n = 664, k = 418)		**Hair Mn (µg/g)** (n = 800, k = 449)	
	N **(%)**	**Mean (95%CI)**	p_{ME}	**GM (95%CI)**	p_{ME}
Demographic and pregnancy-related characteristics					
Age (years)					
<18	78 (17.4)	25.3 (24.0, 26.7)	--	1.8 (1.4, 2.2)	--
18-24	208 (46.3)	24.4 (23.6, 25.2)	0.24	1.7 (1.5, 2.0)	0.86
25-29	84 (18.7)	23.0 (21.7, 24.3)	0.02	1.7 (1.3, 2.1)	0.71
30-34	42 (9.4)	24.7 (22.8, 26.6)	0.60	2.0 (1.4, 2.7)	0.63
≥35	37 (8.2)	24.9 (22.9, 27.0)	0.75	2.1 (1.5, 2.9)	0.43
Education level					
≤6th grade	235 (52.3)	24.4 (23.6, 25.2)	--	1.9 (1.6, 2.2)	--
7-11th grade	201 (44.8)	24.4 (23.6, 25.2)	0.99	1.7 (1.5, 2.0)	0.26
Completed high school	13 (2.9)	23.3 (20.1, 26.6)	0.54	1.2 (0.6, 2.0)	0.10
Marital status					
Single	109 (24.3)	24.6 (23.4, 25.8)	--	1.5 (1.2, 1.8)	--
Married/living as married	340 (75.7)	24.3 (23.6, 25.0)	0.66	1.9 (1.7, 2.1)	0.07
Country of birth					
Costa Rica	364 (81.1)	24.3 (23.6, 24.9)	--	1.6 (1.5, 1.8)	--
Other Central American countries	85 (18.9)	24.8 (23.5, 26.1)	0.49	2.4 (1.9, 2.9)	0.004
Family income[a]					
Above poverty line	169 (40.6)	24.1 (23.2, 25.0)	--	1.7 (1.4, 2.0)	--
Below poverty line and above extreme poverty	170 (40.9)	24.4 (23.5, 25.4)	0.61	1.8 (1.5, 2.1)	0.52
Below extreme poverty line	77 (18.5)	24.1 (22.7, 25.5)	0.98	1.9 (1.5, 2.4)	0.33
Language at home					
Spanish only	417 (92.9)	24.5 (23.9, 25.1)	--	1.8 (1.6, 2.0)	--
Spanish and other language	32 (7.1)	23.2 (21.2, 25.3)	0.25	1.8 (1.3, 2.6)	0.90
Parity[a]					
0	154 (35.6)	24.3 (23.3, 25.2)	--	1.7 (1.5, 2.0)	--
≥1	279 (64.4)	24.4 (23.7, 25.1)	0.87	1.8 (1.6, 2.0)	0.71
Smoking during pregnancy					
No	431 (96.0)	24.5 (23.9, 25.1)	--	1.8 (1.6, 2.0)	--
Yes	18 (4.0)	21.4 (18.6, 24.1)	0.03	1.6 (1.0, 2.6)	0.70
Occupational characteristics					
Agricultural workers in household during pregnancy					
0	117 (26.0)	23.7 (22.6, 24.8)	--	1.5 (1.2, 1.8)	--
1	241 (53.7)	24.4 (23.6, 25.1)	0.34	1.8 (1.6, 2.1)	0.07
≥2	91 (20.3)	25.3 (24.0, 26.5)	0.06	2.0 (1.6, 2.5)	0.03
Occupation before pregnancy					
None/not agriculture	339 (75.5)	24.5 (23.8, 25.1)	--	1.6 (1.5, 1.8)	--
Agriculture	110 (24.5)	24.1 (22.9, 25.2)	0.58	2.3 (1.9, 2.8)	0.002
Occupation during pregnancy[b]					
None/not agriculture	412 (91.8)	24.5 (23.9, 25.1)	--	1.7 (1.6, 1.9)	--
Agriculture	37 (8.2)	22.4 (20.5, 24.4)	0.04	2.2 (1.6, 3.0)	0.22

Husband/partner's occupation before pregnancy[a]					
None/not agriculture	152 (35.9)	24.5 (23.5, 25.4)	--	1.4 (1.2, 1.6)	--
Agriculture	271 (64.1)	24.3 (23.6, 25.0)	0.78	2.0 (1.8, 2.3)	<0.001
Husband/partner's occupation during pregnancy[b]					
None/not agriculture	175 (39.0)	23.9 (23.0, 24.8)	--	1.5 (1.3, 1.7)	--
Agriculture	274 (61.0)	24.6 (23.9, 25.4)	0.24	2.0 (1.8, 2.3)	0.001
Environmental characteristics					
Residential distance from banana plantations during pregnancy (meters)					
<50	114 (25.4)	23.6 (22.4, 24.7)	--	2.5 (2.0, 3.0)	--
50-<600	230 (51.2)	24.8 (24.0, 25.6)	0.09	1.5 (1.3, 1.7)	<0.001
≥600	105 (23.4)	24.4 (23.2, 25.5)	0.32	1.8 (1.5, 2.2)	0.02
Source of drinking water in the home					
Aqueduct	354 (78.8)	24.6 (24.0, 25.3)	--	1.5 (1.3, 1.6)	--
Well (banana company or private)	72 (16.0)	23.3 (21.9, 24.7)	0.10	3.7 (3.0, 4.7)	<0.001
Other source (river, rain, tank, bottled water)	23 (5.2)	23.9 (21.5, 26.3)	0.58	2.3 (1.5, 3.3)	0.04
Drinking water Mn concentrations (µg/L)[a]					
1st quartile: 0.0-0.5	36 (26.1)	25.0 (23.2, 26.9)	--	0.7 (0.5, 1.0)	--
2nd quartile: 0.6-9.8	33 (23.9)	23.7 (21.7, 25.7)	0.32	1.5 (1.1, 2.1)	0.002
3rd quartile: 9.9-157.7	35 (25.4)	25.7 (23.7, 27.6)	0.64	2.0 (1.5, 2.8)	<0.001
4th quartile: 157.8-1600.0	34 (24.6)	24.2 (22.3, 26.1)	0.54	3.6 (2.6, 5.0)	<0.001
Wall and floor materials of the house[a]					
Both made of non-permeable and easy-to-clean materials	119 (40.5)	22.5 (21.4, 23.6)	--	1.4 (1.2, 1.8)	--
One or both made of permeable and difficult-to-clean materials	175 (59.5)	25.4 (24.5, 26.3)	<0.001	1.5 (1.3, 1.8)	0.61
Reported aerial spraying near the home[b]					
Day before sample collection					
No	333 (74.2)	24.5 (23.9, 25.2)	--	1.7 (1.5, 1.9)	--
Yes	116 (25.8)	23.6 (22.6, 24.6)	0.10	1.9 (1.6, 2.3)	0.15
Day of sample collection					
No	334 (74.4)	24.4 (23.8, 25.0)	--	1.7 (1.5, 1.9)	--
Yes	115 (25.6)	24.2 (23.1, 25.3)	0.72	2.1 (1.8, 2.5)	0.01
Reported washing work clothes of agricultural workers[b]					
Day before sample collection[a]					
No	335 (80.2)	24.3 (23.7, 25.0)	--	1.7 (1.6, 1.9)	--
Yes	83 (19.8)	24.3 (23.2, 25.5)	0.97	2.0 (1.7, 2.4)	0.16
Day of sample collection					
No	360 (80.1)	24.6 (23.9, 25.2)	--	1.7 (1.5, 1.9)	--
Yes	89 (19.9)	23.6 (22.4, 24.7)	0.13	2.2 (1.8, 2.6)	0.01

Abbreviations: *n*, number of samples; *k*, number of women; CI, confidence interval; ME, mixed-effect models; GM, geometric mean.

[a] Information was missing for several women with at least one blood sample (*k* = 31 for family income, *k* = 15 for parity, *k* = 24 for husband/partner's occupation before pregnancy, *k* = 287 for drinking water Mn concentrations, *k* = 143 for wall and floor materials of the house, and *k* = 26 for washing work clothes the day before sample collection) and for several women with at least one hair sample (*k* = 33 for family income, *k* = 16 for parity, *k* = 26 for husband/partner's occupation before pregnancy, *k* = 311 for drinking water Mn concentrations, *k* = 155 for wall and floor materials of the house, and *k* = 31 for washing work clothes the day before sample collection).

[b] Variables were analyzed as time-varying characteristics in the bivariate and multivariate linear mixed-effects models.

Table 2. Distribution and variability of blood and hair Mn concentrations in the study population.[a]

						Percentile						
Biomarkers	***n***	***k***	**Mean (SD)**	**GM (GSD)**	**Min**	**25th**	**50th**	**75th**	**Max**	σ^2_{btw}	σ^2_{within}	**ICC**
Blood manganese (μg/L)	664	418	24.4 (6.6)	23.5 (1.3)	8.9	20.2	24.0	28.4	56.3	18.49	23.78	0.44
Hair manganese (μg/g)[b]	800	449	3.6 (5.9)	1.8 (3.2)	0.1	0.8	1.6	3.7	53.3	0.14	0.10	0.58

Abbreviations: *n*, number of samples; *k*, number of women; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation; ICC, intraclass correlation coefficient.

[a] Calculations of the variance between- and within-woman and ICC for blood and hair Mn concentrations were adjusted for gestational age.

[b] Variances between- and within-woman and ICC were calculated and reported for $\log_{10}$-transformed hair Mn concentrations.

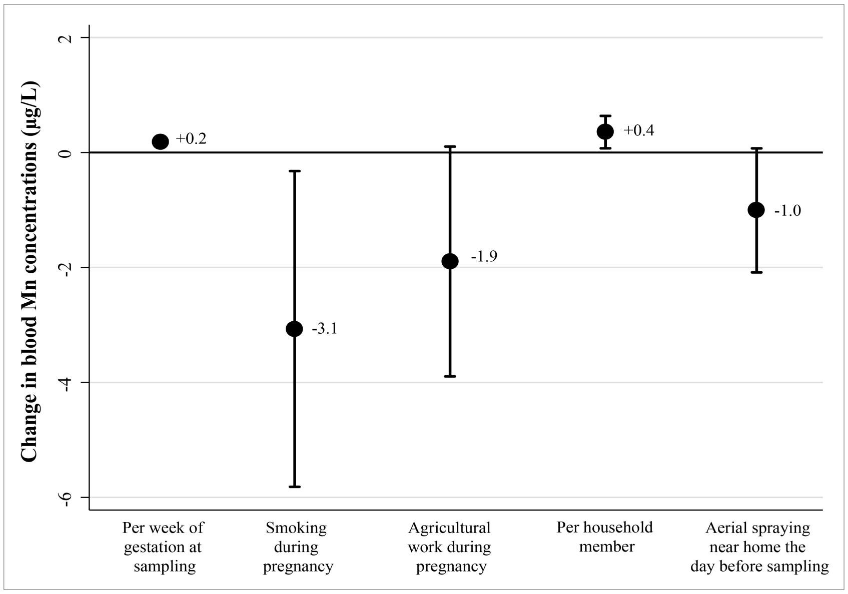

Figure 1. Change in blood Mn concentrations (µg/L) for explanatory variables from multivariate linear mixed-effects model (*n* = 664, *k* = 418). Beta coefficients and 95% confidence intervals.

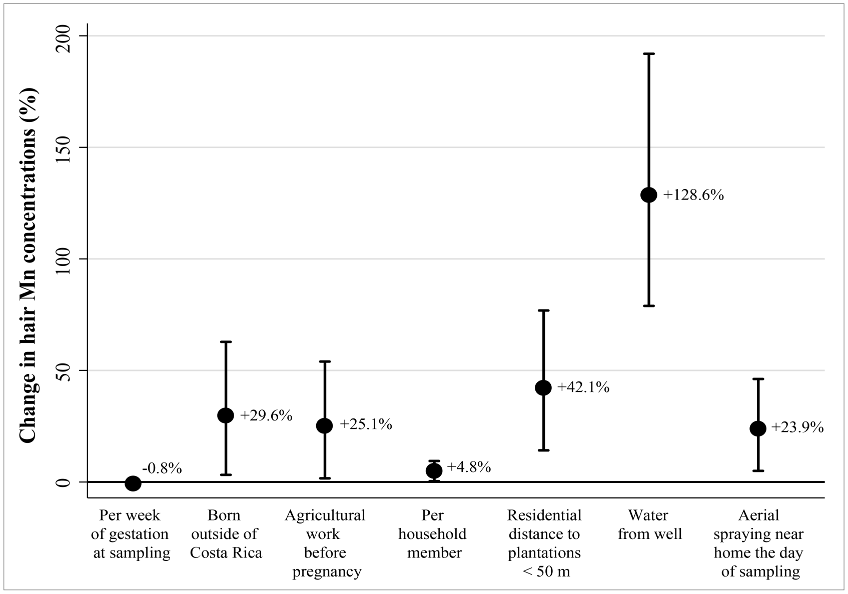

Figure 2. Percentage change in hair Mn concentrations (µg/g) for select explanatory variables estimated from multivariate linear mixed-effects model (*n* = 800, *k* = 449). Percent change [100*(10^(beta coefficient)-1)] and 95% confidence intervals.

6. Supporting information

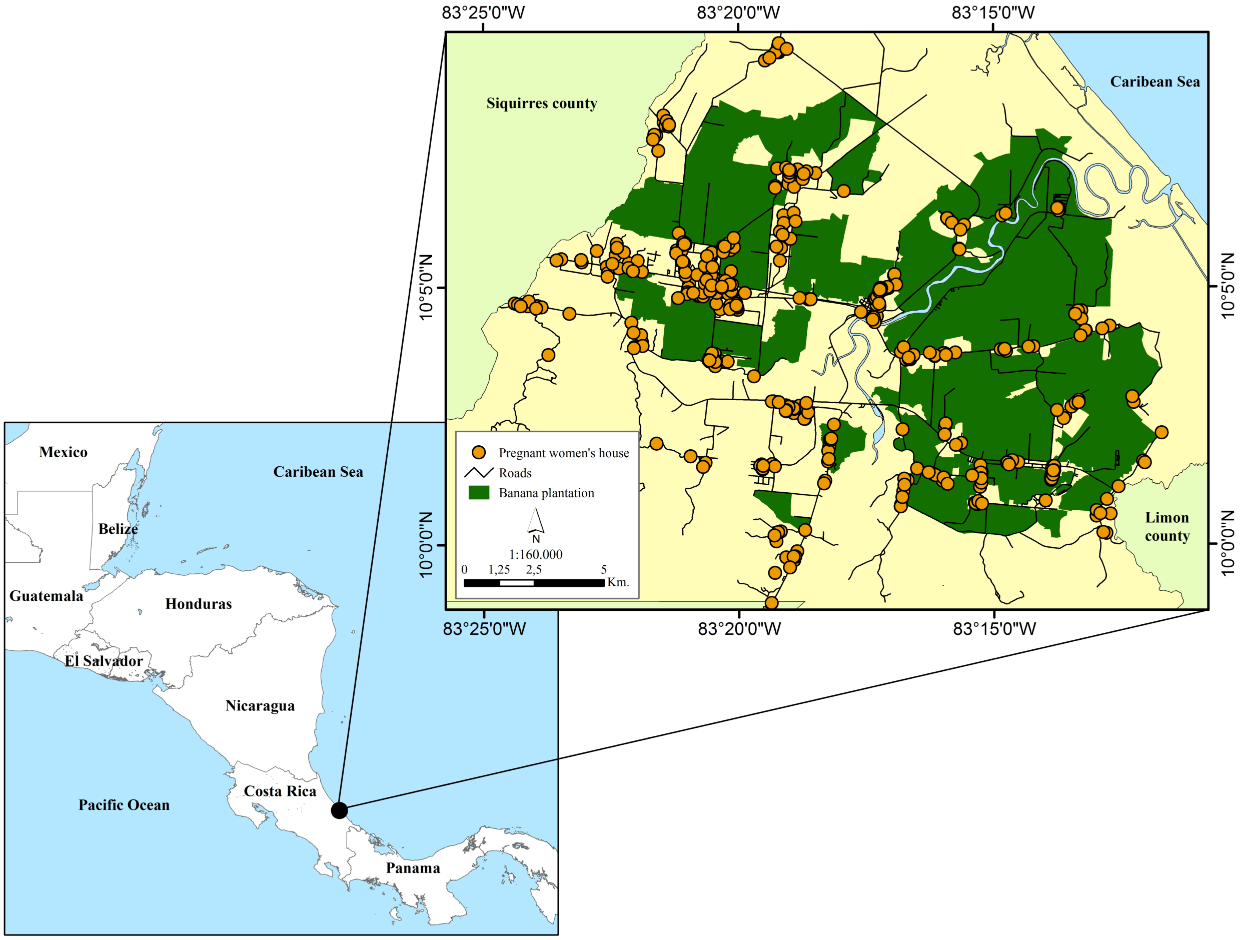

Figure S1. Map of prenatal residential locations and banana plantations in the Matina County, Costa Rica. Data source: Costa Rica Airborne Research and Technology Applications (CARTA) 2005 mission.

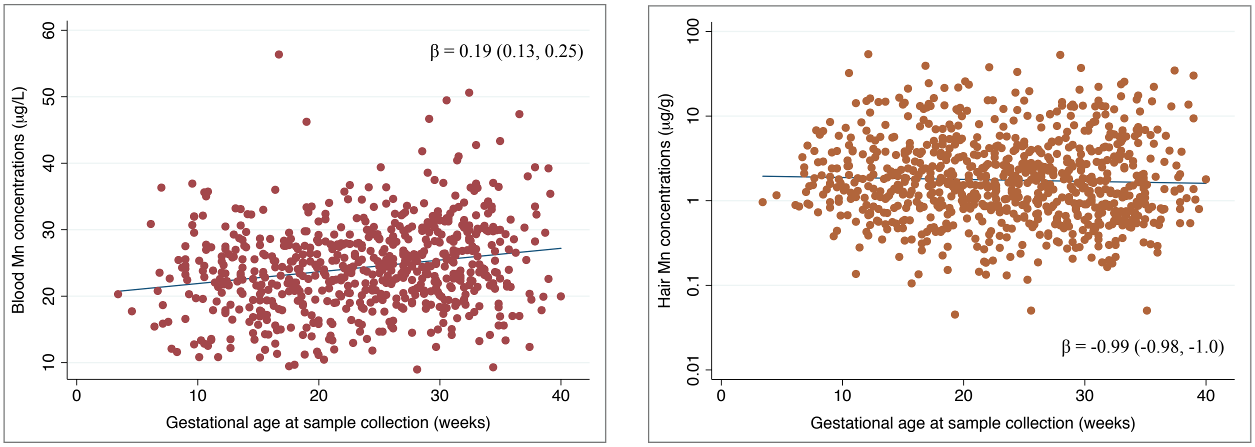

Figure S2. Distribution of (*A*) blood and (*B*) hair Mn concentrations (on the $\log_{10}$ scale) by gestational age at sample collection. Beta coefficients and 95% confidence intervals from unadjusted linear mixed-effects models.

Table S1. Final multivariate linear mixed-effects models for blood Mn concentrations (μg/L) in pregnant women from the ISA study.

Characteristic	**Model 1** (n = 664, k = 418)			**Model 2** (n = 487, k = 275)		
	β (95%CI)	***p*-value**	**Partial R^2**	**β (95%CI)**	***p*-value**	**Partial R^2**
Gestational age at each sample collection (per week)	0.2 (0.1, 0.2)	<0.001	0.04	0.2 (0.2, 0.3)	<0.001	0.04
Smoking during pregnancy						
Yes vs. no	-3.1 (-5.8, -0.3)	0.03	0.01	-3.3 (-6.4, -0.2)	0.04	0.01
Occupation at each visit during pregnancy						
Agriculture vs. none/not agriculture	-1.9 (-3.9, 0.1)	0.06	0.01	-2.1 (-4.5, 0.2)	0.08	0.01
Household members during pregnancy (per person)	0.4 (0.1, 0.6)	0.01	0.01	0.3 (0.0, 0.7)	0.04	0.01
Reported aerial spraying near home the day before each blood sample collection						
Yes vs. no	-1.0 (-2.1, 0.1)	0.07	0.01	-0.7 (-2.0, 0.5)	0.24	0.01
Wall and floor materials of the house	--					
One or both made of permeable and difficult-to-clean materials vs. both made of non-permeable and easy-to-clean materials				2.6 (1.3, 4.0)	<0.001	0.04
R^2 for model			0.08			0.14

Abbreviations: n, number of samples; k, number of women; CI, confidence interval; R^2, coefficient of determination.

Table S2. Final multivariate linear mixed-effects models for hair Mn concentrations (µg/g) in pregnant women from the ISA study.

Characteristic	**Model 1** (n = 800, k = 449)			**Model 2** (n = 259, k = 138)		
	% change[a] (95%CI)	***p*-value**	**Partial R^2**	**% change[a] (95%CI)**	***p*-value**	**Partial R^2**
Gestational age at each sample collection (per week)	-0.8 (-1.6, 0.0)	0.06	0.001	-1.4 (-2.8, 0.0)	0.05	0.001
Country of birth						
Other Central American countries vs. Costa Rica	29.6 (3.2, 62.8)	0.03	0.02	73.1 (8.8, 175.6)	0.02	0.02
Occupation before pregnancy						
Agriculture vs. none/not agriculture	25.1 (1.6, 54.0)	0.04	0.02	6.5 (-27.3, 56.1)	0.75	0.02
Household members during pregnancy (per person)	4.8 (0.4, 9.4)	0.03	0.01	3.9 (-3.5, 11.9)	0.31	0.01
Residential distance to banana plantations						
<50 vs. 50-<600 meters	42.1 (14.2, 76.9)	0.002	0.04	41.7 (-4.1, 109.6)	0.08	0.04
≥600 vs. 50-<600 meters	19.7 (-4.6, 50.2)	0.12		28.4 (-14.3, 92.4)	0.23	
Source of drinking water in the home						
Well vs. aqueduct	128.6 (78.9, 192.0)	<0.001	0.09	--		
Other source vs. aqueduct	37.3 (-7.6, 104.0)	0.12		--		
Reported aerial spraying near home the day of each hair sample collection						
Yes vs. no	23.9 (5.0, 46.2)	0.01	0.01	19.8 (-11.2, 61.7)	0.24	0.01
Drinking water Mn concentrations (per µg/L)[b]	--			17.5 (12.2, 22.8)	<0.001	0.22
R^2 for model			0.14			0.30

Abbreviations: n, number of samples; k, number of women; CI, confidence interval; R^2, coefficient of determination.

[a] Percent change in hair Mn concentrations associated with 1-unit increase (continuous variables) or with a category difference (categorical variables) in the predictor variable specified; % change = 100*(10^(beta coefficient)-1).

[b] Percent change in hair Mn concentrations associated with one µg/L increase in water Mn concentrations; % change = 100*(beta coefficient).

Table S3. Comparison of blood Mn concentrations (μg/L) in pregnant women by study site.

Author (year)	Location	Sample collection	n	Median	5th-95th percentile	Range	Matrix
Mora et al. (current study)	Matina County, Costa Rica	I trimester	96	23.2	12.2-35.0	10.8-36.9	Whole blood
		II trimester	328	23.7	13.5-33.4	9.4-56.3	Whole blood
		III trimester	240	25.3	15.6-38.9	8.9-50.6	Whole blood
Bradman et al. (unpublished)	California, US	II trimester	53	14.8	6.7-26.8	4.3-32.8	Whole blood
		Delivery	53	20.3	12.5-34.1	6.7-35.7	Whole blood
Callan et al. (2013)	Western Australia	III trimester	173	6.45	<0.1–27.7	<0.1–50.3	Whole blood
Guan et al. (2013)	Liaoning, China	Delivery	125	50.6	29.9-105.8		Whole blood
Yu et al. (2013)	Shanghai, China	Delivery	1377	2.8			Serum
Vigeh et al. (2013)	Tehran, Iran	I trimester	224	15.2[a]		6.5-36.4	Whole blood
		II trimester	224	15.1[a]		0.1-41.6	Whole blood
		III trimester	224	17.8[a]		0.4-34.7	Whole blood
Ajayi et al. (2012)	Ibadan, Nigeria	I and II trimester	34 (controls)	703.5[a]			Serum
Kopp et al. (2012)	Bochum, Germany	Delivery	50	17.0	8.0-32.3	6.4-38.4	Whole blood
Claus Henn et al. (2011)	Mexico City, Mexico	Delivery	332	17.1	8.7-32.8	4.2-66.2	Whole blood
Hansen et al. (2011)	Northern Norway	II trimester	211	11.3[a]		3.8–37.8	Whole blood
		Delivery	211	16.5[a]		6.6–43.1	Whole blood
Rudge et al. (2011)	Sao Paulo, Brazil	Delivery	155	16.7		7.0-39.7	Whole blood
Xu et al. (2011)	Shanghai, China	Delivery	142	58.7[a]		23.6-187.5	Whole blood
Abdelouahab et al. (2010)	Nancy and Poitiers, France	II trimester	160	10.0	3.0-23.5	3.0-29.0	Whole blood
Lin et al. (2010)	Taipei, Taiwan	Delivery	308	21.3			Whole blood
Afridi et al (2009)	Hyderabad, Pakistan	Delivery	115	46.9[a]			Whole blood
Ljung et al. (2009)	Matlab, Bangladesh	II trimester	408	22.0		10.0-53.0	Whole blood
Röllin et al. (2009)	South Africa	Delivery	96	16.7	8.9-26.3	7.9-63.5	Whole blood
Rudge et al. (2009)	South Africa	Delivery	62	16.8		8.7-63.5	Whole blood
Zota et al. (2009)	Oklahoma, US	Delivery	470	22.0	13.0-41.0		
Wang et al. (2008)	Shanghai, China	Delivery	130	54.3			
Vigeh et al. (2006)	Tehran, Iran	Delivery	365 (controls)	18.5		6.9-39.4	Whole blood
Yazbeck et al. (2006)	Paris, France	Delivery	224	21.4[b]	11.1-40.4		
Takser et al. (2004)	Quebec, Canada	I trimester	40	8.3	5.5-14.5	4.6-25.0	Whole blood
		II trimester	149	9.7	5.9-15.3	3.7-25.3	Whole blood
		III trimester	101	15.5	10.0-25.9	9.2-37.1	Whole blood
Anetor et al. (2003)	Ibadan, Nigeria	I trimester	10	7.4[a]			Serum
		II trimester	15	8.4[a]			Serum
		III trimester	15	9.9[a]			Serum
Takser et al. (2003)	Paris, France	Delivery	222	20.4[b]	11.1-40.4	6.3-151.2	Whole blood
Smargiassi et al. (2002)	Montreal, Canada	Delivery	160	23.0[a]	6.0-52.0		Whole blood
	Paris, France	Delivery	206	23.0[a]	12.0-40.0		Whole blood
Krachler et al (1999)	Maribor, Slovenia	Delivery	29	2.4		0.6-7.2	Serum
Spencer et al (1999)	Queensland, Australia	I trimester	34	7.9	4.9-11.5	3.8-20.1	Whole blood
		II trimester	34	9.2	5.9-13.3	4.9-21.5	Whole blood

		III trimester	34	12.2	7.6-18.7	7.3-23.2	Whole blood
Stoll et al. (1999)	Strasbourg, France	I trimester	340 (controls)	2.0[a]			Whole blood
Tholin et al. (1995)	Orebro, Sweden	I trimester	66	8.5	4.3-19.8		Whole blood
		II trimester	66	10.4	5.4-22.4		Whole blood
		III trimester	66	12.6	7.3-26.4		Whole blood
Sachdeva et al. (1993)	Punjab, India	I trimester	33 (controls)	7.0[a]			Serum
		III trimester	30 (controls)	8.0[a]			Serum
Wilson et al. (1991)	Belfast, Ireland	Delivery	56	4.2[a]			Plasma
Tsuchiya et al. (1984)	Nagoya, Japan	Delivery	102	31.0[a]		1.0-83.0	Whole blood
Hambidge et al. (1974)	Colorado, US	I trimester	20	1.5[a]			Plasma
		III trimester	20	2.0[a]			Plasma

[a] Geometric mean.

[b] Arithmetic mean.

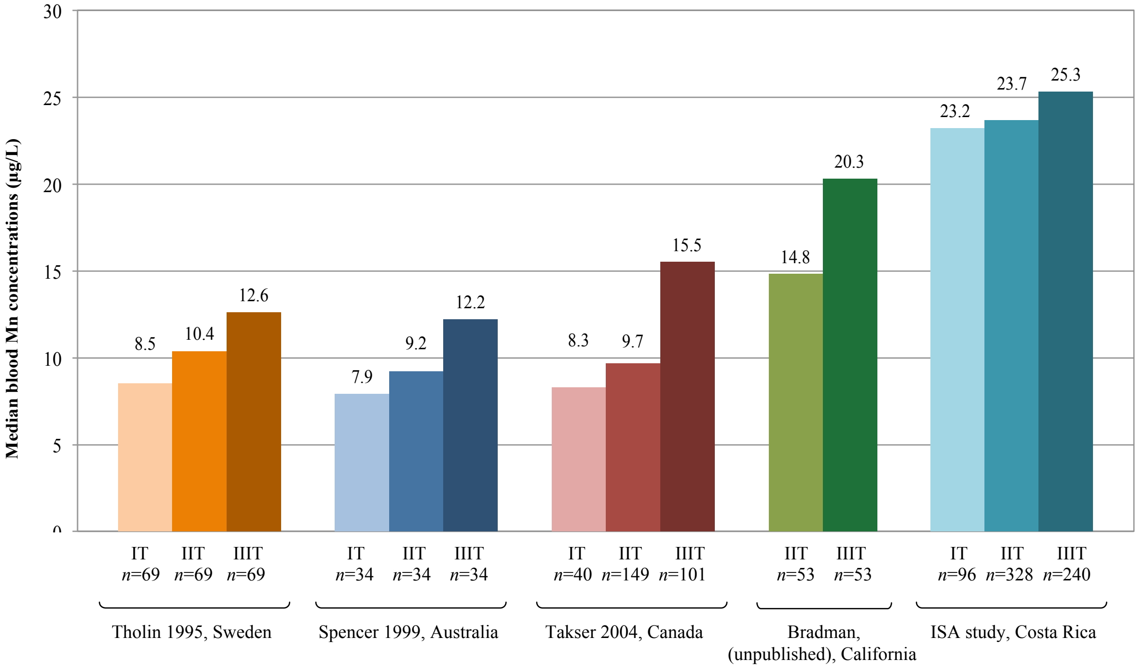

Figure S3. Variability in median blood Mn concentrations (µg/L) during pregnancy by study site. *Abbreviations*: *n*, number of observations; IT, first trimester; IIT, second trimester; IIIT, third trimester.

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Chapter 3:
Maternal blood and hair manganese concentrations, fetal growth, and length of gestation in the ISA cohort in Costa Rica

1. Introduction

Manganese (Mn) is an essential nutrient and an important cofactor in enzymatic reactions involved in bone formation and metabolic regulation in animals and humans (ATDSR 2012). Food is the main source of Mn for the general population, but exposure to Mn can occur through the environment (ATDSR 2012). Environmental sources of Mn exposure include water from wells with high concentrations of natural Mn or contaminated by industrial waste (Bouchard et al. 2007; Bouchard et al. 2011; He et al. 1994; Kondakis et al. 1989), combustion of anti-knock additives in gasoline (Zayed et al. 1999), dust emissions from industrial sources (Menezes-Filho et al. 2011) and Mn mines (Riojas-Rodriguez et al. 2010), and spraying of Mn-containing ethylene bisdithiocarbamate fungicides (Gunier et al. 2013; Mora et al. 2014).

Evidence suggests that Mn can interfere with insulin metabolism at very low or high concentrations (Hiney et al. 2011; Keen et al. 1999) and it can induce cellular free radical damage and mitochondrial dysfunction at high concentrations (HaMai and Bondy 2004; Keen et al. 2000). Several animal studies have found that both Mn deficiency and overexposure during gestation is associated with decreased fetal size and weight (Colomina et al. 1996; Hansen et al. 2006; Sanchez et al. 1993; Treinen et al. 1995). Notably, a small number of studies in pregnant rodents exposed to elevated doses of Mn orally or subcutaneously did not detect any association between dose of Mn and fetal size or weight (Gray and Laskey 1980; Molina et al. 2011; Pappas et al. 1997; Torrente et al. 2002). A recent study found that pups prenatally exposed to Mn in drinking water were born with significantly higher body weights compared to controls (Betharia and Maher 2012).

Few studies have examined the effects of prenatal Mn exposure on birth outcomes in humans (Chen et al. 2014; Eum et al. 2014; Guan et al. 2013; Takser et al. 2004; Vigeh et al. 2008; Zota et al. 2009). Cross-sectional studies in Oklahoma (*n* = 470 term newborns), China (*n* = 172 preterm and term infants), and Korea (*n* = 331 term newborns) have reported nonlinear associations between maternal blood Mn concentrations at delivery (medians = 22, 53.8, and 21.5 µg/L, respectively) and birth weight (Chen et al. 2014; Eum et al. 2014; Zota et al. 2009). Infant birth weight increased linearly with Mn concentrations up to 31 µg/L in the Oklahoma study, 41.8 µg/L in the Chinese study, and 30-35 µg/L in the Korean study. At higher Mn concentrations, a non-significant inverse relationship was observed between maternal Mn and birth weight in all three studies. A smaller study in China (*n* = 125 mother-child pairs) did not observe an association between maternal blood Mn concentrations at delivery (median = 50.6 µg/L) and birth weight, but found significant inverted U-shaped relationships between Mn concentrations, head circumference, and chest circumference (Guan et al. 2013) Additionally, a case-control study of 271 Iranian mothers and their children found that mothers of newborns with intrauterine growth retardation had significantly lower blood Mn concentrations shortly after delivery compared to mothers of newborns with sizes appropriate for gestational age (means = 16.7 vs. 19.1 µg/L, respectively) (Vigeh et al. 2008). To date, only one epidemiological study has been published on the relationship between blood Mn concentrations measured at multiple time points during pregnancy (means in first, second, and third trimesters of gestation = 9.0, 9.9, and 16.3 µg/L, respectively) and birth outcomes (Takser et al. 2004). This study of 149

Canadian mother-child pairs did not find any significant associations between Mn concentrations and newborn growth parameters.

Previous studies have exclusively examined the association between blood Mn concentrations and birth outcomes. In the present study, we measured Mn in maternal hair as well as blood samples collected multiple times over pregnancy and assessed its association with fetal growth and length of gestation in a mother-infant cohort living near banana plantations aerially sprayed with Mn-containing fungicide mancozeb in Costa Rica.

2. Materials and methods

2.1. *Study population*

The Infants' Environmental Health Study (ISA) is a prospective birth cohort study of fetal and early childhood exposure to pesticides and their impact on growth and neurodevelopment in children living near banana plantations in Matina County, Costa Rica. Subject recruitment and procedures for the ISA study have been described elsewhere (Mora et al. 2014; van Wendel de Joode et al. submitted). Briefly, pregnant women were recruited through meetings in local schools, communal groups, advertisements, and friends' referrals between March 2010 and June 2011. Women were eligible to participate in the study if they were < 33 weeks of gestation, ≥ 15 years of age, and living in one of the 40 villages of Matina County located < 5 km of a banana plantation. A total of 451 women were enrolled, and after losses due to miscarriage and stillbirths (n = 21), loss to follow-up (n = 39), and the exclusion of twins (n = 2) and women who did not have delivery medical records available at the time of the postpartum interview (n = 9), information on birth weight and length of gestation was available for 380 singleton liveborn infants. Participants included in this analysis did not differ significantly from the original full cohort on most socio-demographic factors, including maternal education, marital status, parity, family income, and blood and hair Mn concentrations during pregnancy. Written informed consent was obtained from all women and additional informed consent was obtained from the parents or legal guardians of participants under the age of 18. All study activities were approved by the Ethical Committee of the Universidad Nacional in Costa Rica.

2.2. *Data collection*

Women were interviewed one to three times during pregnancy (depending on their gestational age at enrollment) and following delivery. Interviews were conducted using structured questionnaires and occurred at enrollment (median, 19 weeks gestation), at the beginning and in the middle of the third trimester of pregnancy (median, 30 and 33 weeks gestation), and after delivery (median, 7 weeks postpartum). Socio-demographic information, including maternal age, education, marital status, parity, and family income, was collected at the baseline interview. Information on smoking, alcohol consumption, drug use, caffeine consumption, occupational status, medical conditions, medications, pregnancy complications, and obstetric ultrasounds was gathered at each interview. Data completed by hospital/clinic personnel were abstracted from prenatal medical records provided to the study participants. The abstracted data included maternal pre-pregnancy weight, timing of prenatal care initiation, maternal weight and blood pressure levels at each prenatal care visit, and medical conditions recorded in the medical records by the treating physician. Data on hemoglobin concentrations was abstracted for a subset of women (n = 52), and we when grouped them by maternal report of gestational anemia, we observed a significant difference between the two groups ($p_{\text{Mann-Whitney}}$ =

0.03). Pre-pregnancy body mass index (BMI) was calculated as (weight in kilograms)/(height in meters)^2, using maternal pre-pregnancy weight (if available) or weight at the first prenatal care visit (if < 14 weeks gestation) abstracted from medical records, and height measured by the study interviewers. Gestational hypertension was determined using the blood pressure levels abstracted from prenatal medical records (blood pressure at least 140 mm Hg systolic and/or at least 90 mm Hg diastolic in at least two visits at > 20 week of pregnancy in women with previously normal blood pressure), diagnosis abstracted from medical records, and maternal report of hypertensive drug use during pregnancy. Women with gestational diabetes were identified using maternal report and diagnosis abstracted from medical records.

2.3. Fetal growth and length of gestation

Data on infant birth weight, body length, head circumference, and chest circumference were abstracted from delivery medical records provided to the study participants and used as recorded in grams or centimeters. Infant ponderal index, a measure of proportionality of growth, was calculated as (birth weight in grams × 100)/(body length in centimeters)^3. Information on chest circumference was measured by the hospital staff according to standard procedures (without clothes at the level of the nipples and below the inferior angle of the scapulae), but was only available for a subset of newborns (n = 165). Those with data on chest circumference and those missing these data were similar on most socio-demographic characteristics, such as maternal age, maternal education, parity, and family income. However, mothers of infants with chest circumference data had lower hair Mn concentrations during the second trimester, third trimester, and averaged concentrations during pregnancy compared to mothers of infants with no chest circumference data (data not shown).

Length of gestation was determined using the date of the last menstrual period (LMP) reported by women at enrollment, early obstetric ultrasounds (< 14 weeks gestation), and/or estimates abstracted from medical records. If the gestational age estimated using the LMP date and the gestational age estimated using an early ultrasound differed by < 7 days, the LMP estimate was used as the length of gestation; otherwise, the ultrasound estimate was used. If the woman did not have an early ultrasound, was sure about her LMP date, and the LMP and medical record estimates differed by < 14 days, the LMP estimate was used; otherwise, the medical record estimate was used. Women with a gestational age at birth > 44 weeks and 6 days were excluded from the present analyses (n = 4). Gestational age at the time of biological sample collection was calculated using data on length of gestation and the date of sample collection.

2.4. Blood Mn measurements

Blood samples were collected by venipuncture at enrollment or shortly after (median, 19 weeks gestation) and between the second and third trimesters of pregnancy (median, 30 weeks gestation). Whole blood samples were stored at -20°C until its shipment to the University of California, Santa Cruz, where they were analyzed for Mn using high-resolution inductively coupled plasma mass spectrometry (Finnigan XR ICP-MS) (Smith et al. 2007). Details of blood collection, analysis, and quality control procedures are described elsewhere (Mora et al. 2014). Blood Mn concentrations measured in this study were all above the analytical limit of detection (LOD = 0.003 μg/L). Whole blood Mn was measured in 613 samples collected from 375 women. A total of 238 women provided two samples during pregnancy and 137 provided only one.

2.5. *Hair Mn measurements*

Hair samples (~ 20-30 strands) were collected from the occipital region, within 2 mm from the scalp, at enrollment (median, 19 weeks gestation) and between the second and third trimesters of pregnancy (median, 30 weeks gestation). Samples were stored in plastic bags at room temperature and shipped in batches to the Federal University of Bahia, Brazil. The one-centimeter closest to the scalp from each hair sample was cleaned as described elsewhere (Menezes-Filho et al. 2009) and analyzed for Mn using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). Only three hair samples analyzed in this study had Mn concentrations below the limit of detection (LOD = 0.01 µg/g) and their values were set at LOD/2. Mn was measured in 708 hair samples collected from 380 study participants; 328 women provided two hair samples and 52 provided only one.

2.6. *Statistical analysis*

To account for the well-known increase in blood Mn concentrations over pregnancy (Mora et al. 2014; Hambridge and Droegemueller 1974; Spencer 1999; Tholin et al. 1995) and the decrease in hair Mn that we observed in previous analyses (Mora et al. 2014), we grouped blood and hair Mn concentrations by trimester of gestation at the time of sample collection and then averaged the concentrations for each trimester. We also averaged the Mn concentrations across the repeated samples collected for each woman throughout pregnancy. Blood Mn concentrations were normally distributed, whereas hair Mn concentrations were skewed to the left. Thus, we transformed hair Mn concentrations to the $\log_{10}$ scale to normalize the residuals.

Several factors were identified *a priori* as potential confounders of the association of Mn with fetal growth or length of gestation on the basis of directed acyclic graphs (Greenland et al. 1999). These confounders included maternal age, timing of prenatal care initiation, and caffeine consumption during pregnancy (mg per day) as continuous variables, and maternal marital status, maternal country of birth, maternal education, parity, pre-pregnancy BMI, maternal occupation during pregnancy, family income, alcohol consumption (< 1 vs. ≥ 1 drink per week), smoking, and drug use during pregnancy (any vs. none), caffeinated tea intake during pregnancy, iron intake during pregnancy, history of miscarriage, vaginal bleeding, gestational diabetes, and infant sex as categorical variables (categorized as shown in Table 1). Covariates were included in the regression models if they were associated with blood or hair Mn and any of the birth outcomes in bivariate analyses at a p-value < 0.10. Missing covariate values for parity ($n = 12$), history of abortion ($n = 12$), maternal height ($n = 14$), and caffeinated tea consumption during pregnancy ($n = 2$) were imputed at random based on observed probability distributions (< 5% missing) (Lubin et al. 2004). For women missing weight ($n = 32$), this variable was predicted from a regression model that included maternal age, history of abortion, parity, maternal education, maternal height, and timing of prenatal care initiation as predictor variables. Because birth weight in preterm newborns may reflect growth restriction and/or prematurity (Hernandez-Diaz et al. 2008; Wilcox 2006), we restricted our analyses on exposure to Mn and birth weight, birth length, ponderal index, head circumference, and chest circumference to term newborns (≥ 37 weeks).

We ran statistical analyses for blood and hair Mn separately. First, linear regression models were used to examine linear associations between blood and hair Mn and birth weight, length, ponderal index, head circumference, chest circumference, and length of gestation (as continuous variables). We then assessed potential nonlinear associations between blood and hair Mn and the birth outcomes of interest by examining penalized splines for Mn using generalized additive models (Hastie and Tibshirani 1990). We examined the linearity of these associations using a likelihood ratio test that compared models with smoothed and linear terms. If the associations were linear, blood or hair Mn was used as a continuous term in the adjusted linear regression models. If a potentially nonlinear association between blood or hair Mn concentrations and any of the birth outcomes was identified, we created indicator variables for tertiles of each Mn biomarker distribution and included them in the adjusted regression models for blood and hair Mn.

We examined differences in the associations of mean blood and mean hair Mn concentrations with birth outcomes for boys and girls, and women living above and below the poverty line, using cross-product terms. We also examined effect measure modification by gestational anemia because evidence suggests that iron deficiency may result in increased absorption and retention of Mn in various organs (Chandra and Shukla 1976; Finley 1999; Garcia et al. 2007; Mena et al. 1969; Thompson et al. 2006; Yokoi et al. 1991). We tested for effect modification using mean blood and hair Mn concentrations and not Mn concentrations by trimesters of pregnancy because of sample size limitations, and used a p-value < 0.20 as the cut-off point for significance.

We conducted several sensitivity analyses to assess the robustness of our results. First, we reran models after excluding outliers with studentized residuals (residuals divided by the model standard error) greater than three standard units. Second, we reran the analyses restricted to the subset of women with data available on all covariates (n = 329 and 310 mother-child pairs for blood and hair Mn, respectively) to evaluate the appropriateness of using imputation for missing data. Third, we included gestational hypertension as a covariate in the adjusted regression models to examine whether it might be a confounder or mediator of the association between Mn exposure and birth outcomes (Lee and Kim 2011; Taneja and Mandal 2007; Vigeh et al. 2013). Fourth, we reran the analyses using blood and hair Mn concentrations by halves of pregnancy (< 20 and ≥ 20 weeks gestation) to assess whether the associations with the birth outcomes of interest would change by increasing power. Finally, we examined the associations of maternal blood and hair Mn concentrations with head circumference/birth weight, head circumference/body length, and head circumference/chest circumference ratios.

Statistical analyses were conducted using Stata version 11.2 (StataCorp, College Station, TX).

3. Results

Most of the women in the study were young (median age = 22 years), born in Costa Rica (81%), married or living as married (74%), and multiparous (64%) (Table 1). About half of them completed primary school (49%) and were overweight or obese before pregnancy (48%). More than half of the women were living below the poverty line (59%). Few women smoked (4%), consumed alcohol (3%, data not shown), or used illegal drugs (2%, data not shown) during pregnancy; and more than two-thirds began prenatal care in the first trimester (80%, data not shown). Approximately 3% of women had gestational hypertension, 4% had gestational diabetes, and 40% reported having been diagnosed with gestational anemia at any point during pregnancy. Mean (± SD) birth weight was 3,235 ± 410 g; mean body (crown-heel) length was 49.9 ± 2.2 cm; mean ponderal index was 2.6 ± 0.3 g/cm^3; mean head circumference was 33.8 ± 1.4 cm; mean chest circumference was 33.0 ± 1.7 cm; and mean length of gestation was 39.5 ± 1.8 weeks. A total of 12 infants (3%) weighed < 2,500 g at birth and 24 infants (6%) were preterm. Smaller size infants were born to women who were born outside of Costa Rica, primiparous, lived below the poverty line, reported taking iron during pregnancy, did not have gestational diabetes, and had a vaginal bleeding in the first trimester of pregnancy. Boys and girls had similar birth weights and body lengths, but girls had slightly smaller head circumferences and longer gestational durations.

The distribution of maternal blood and hair Mn concentrations by trimester and average during pregnancy are shown in Table 2. Mean (± SD) blood Mn concentration during pregnancy was 24.5 ± 6.1 µg/L and geometric mean (geometric SD) hair Mn concentration was 1.9 (2.8) µg/g. Blood Mn concentrations increased significantly between the second and third trimesters of pregnancy (means = 23.8 and 26.5 µg/g, respectively), whereas hair Mn concentrations showed a small decrease between the second and third trimesters (geometric means (GM) = 1.8 and 1.7 µg/g, respectively). Maternal blood and hair Mn concentrations were not correlated ($r_s = -0.06$, $p = 0.22$, $n = 375$).

Table S2 and Table 3 present the unadjusted and adjusted multivariable models for the associations of maternal blood and hair Mn concentrations with measures of fetal growth and length of gestation, respectively. After adjusting for covariates, a one-unit (µg/L) increase in maternal blood Mn concentrations during the third trimester was associated with a 9.3-g (95% CI: 0.8, 17.8) linear increase in birth weight, whereas a ten-fold increase in hair Mn concentrations during the second trimester was associated with a linear increase of 0.65 cm (95% CI: 0.18, 1.12) in chest circumference. We observed nonlinear associations of maternal blood Mn concentrations during the second trimester with birth weight and body length in the adjusted generalized additive models ($p_{\text{GAM}} = 0.04$ and 0.02, respectively), but the adjusted linear regression models fitted with indicator variables for each tertile of maternal blood Mn concentrations did not show any statistically significant association. We observed a nonlinear association between maternal blood Mn concentrations during the second trimester and length of gestation ($p_{\text{GAM}} = 0.04$) in the adjusted analyses. When we included maternal blood Mn during the second trimester ranked in tertiles in multivariable linear regression models, women with blood Mn concentrations in tertile 2 during the second trimester had a very similar length of gestation (β = 0.50 days; 95% CI: -3.38, 4.39) compared to women with blood Mn concentrations in tertile 1, but women with Mn concentrations in tertile 3 had slightly shorter gestations (β = -3.50 days; 95% CI: -7.52, 0.54) compared to women with Mn concentrations in tertile 1 during the same trimester. Differences between tertiles of blood Mn during the second trimester did not reach statistical significance.

We did not observe significant differences between boys and girls in the associations of mean maternal blood or hair Mn concentrations with birth outcomes (Table S3). However, although we did not observe differences in blood or hair Mn concentrations in women who reported having gestational anemia and women who did not, we found evidence of effect modification by gestational anemia for the association between mean maternal hair Mn concentrations during pregnancy and chest circumference (Table S4). Each 10-fold increase in mean maternal hair Mn concentrations during pregnancy was associated with a 1.32 cm (95% CI: 0.54, 2.09) increase in chest circumference among infants whose mothers did not have gestational anemia, but only a 0.24 cm (95% CI: -0.57, 1.05; $p_{\text{INT}} = 0.09$) increase among infants of mothers who had gestational anemia (Figure S1).

We observed effect modification by family income for some of the associations between blood and hair Mn concentrations and birth outcomes of interest (Table S5). Mean maternal blood Mn concentrations during pregnancy were associated with decreased chest circumference in infants whose mothers were living below the poverty line (β = -0.06 cm; 95% CI: -0.12, -0.01), but not in infants whose mothers were living above poverty (β = 0.02 cm; 95% CI: -0.06, 0.09; $p_{\mathrm{INT}} = 0.08$). In addition, we found a positive association between mean maternal hair Mn concentrations and length of gestation in infants born to women living below the poverty line (β = 4.11 days; 95% CI: 0.47, 7.75) and a negative association in those born to women living above the poverty line (β = -3.17 days; 95% CI: -7.93, 1.58; $p_{\mathrm{INT}} = 0.03$).

Sensitivity analyses were conducted excluding outliers and excluding women with missing covariates. Findings were consistent with those from the main multivariate linear regression and generalized additive models, except for the associations of maternal blood Mn concentrations in the second and third trimester with birth weight and body length that were no longer statistically significant. When gestational hypertension was included in the models, results were not appreciably altered. Finally, when we assessed the associations of maternal blood and hair Mn concentrations with head circumference/birth weight, head circumference/body length, and head circumference/chest circumference ratios, results were consistent with those observed with the individual measures of fetal growth.

4. Discussion

In this prospective cohort study, we found a linear and positive association between hair Mn concentrations during the second trimester and chest circumference for all study participants combined. We also observed that higher mean maternal hair Mn concentrations during pregnancy were associated with increased chest circumference in infants born to mothers with no gestational anemia. Conversely, higher mean blood Mn concentrations in mothers during pregnancy were associated with decreased chest circumference in infants born to mothers living below the poverty line. Lastly, higher mean maternal hair Mn concentrations during pregnancy were also associated with increased length of gestation in mothers living below the poverty line. We did not find significant linear or nonlinear associations of maternal Mn concentrations with lowered birth weight or head circumference, as reported in previous studies (Chen et al. 2014; Guan et al. 2013; Zota et al. 2009).

This study adds to the limited and somewhat conflicting data on the effects of prenatal Mn exposure on fetal growth and length of gestation (Table S1) (Chen et al. 2014; Eum et al. 2014; Guan et al. 2013; Takser et al. 2004; Vigeh et al. 2008; Zota et al. 2009; Osada et al. 2002; Xu et al. 2011; Yu and Cao 2013). Numerous epidemiological studies have examined the association between maternal blood Mn concentrations during pregnancy and birth weight, but only half of them have found significant associations (Chen et al. 2014; Eum et al. 2014; Vigeh et al. 2008; Zota et al. 2009). Cross-sectional studies in Oklahoma, China, and Korea, with different concentrations of Mn in maternal blood at delivery (medians = 22, 53.8, and 21.5 μg/L, respectively), reported inverted U-shaped relationships between Mn concentrations and birth weight (Chen et al. 2014; Eum et al. 2014; Zota et al. 2009). Maternal blood Mn concentrations in the third trimester and averaged over pregnancy in our study were similar to those observed at delivery in the Oklahoma and Korean study (Eum et al. 2014; Zota et al. 2009), but we did not observe linear or nonlinear associations of blood Mn concentrations with birth weight that would remain statistically significant after excluding outliers. Similarly, the only other prospective cohort study that has examined the relationship of maternal Mn concentrations at different time points during pregnancy (median for third trimester = 15.5 μg/L) with fetal growth and length of gestation, besides ours, did not observe any linear or nonlinear associations between blood Mn concentrations and birth weight (Takser et al. 2004). Inconsistencies between the cross-sectional studies that reported inverted U-shaped associations between maternal Mn concentrations and birth weight and our study may arise from differences in the study population, sample size, time of biological sampling (at delivery versus third trimester or averaged over pregnancy), and exposure pathways.

Consistent with previous studies (Guan et al. 2013; Takser et al. 2004; Yu and Cao 2013), we did not find associations of maternal blood Mn concentrations with body length and ponderal index. We did not observe an association between blood Mn concentrations during pregnancy and head circumference either. However, a study of 125 Chinese mother-child pairs found an inverted U-shaped relationship of maternal Mn concentrations with head circumference in their unadjusted analyses (Guan et al. 2013). This Chinese study reported maternal blood Mn concentrations at delivery that were much higher than the Mn concentrations observed in our study during the third trimester (medians = 50.6 versus 26.4 μg/L, respectively). This cross-sectional study determined that the main sources of Mn exposure of their study population were living near major transportation routes and exposure to harmful occupational factors (i.e., industrial dust, decorating materials, chemical reagents, and gasoline). Disparities between the Chinese study and ours may be due to differences in exposure levels and sources of Mn exposure, mainly because it remains unclear how different sources and pathways of exposure to Mn translate into Mn concentrations in various biological media (e.g., blood, hair, teeth) (Smith et al. 2007).

To our knowledge, this is the first epidemiological study to assess the relation of hair Mn concentrations during pregnancy with fetal growth and length of gestation. We did not observe associations between maternal hair Mn concentrations and birth weight, body length, ponderal index, or head circumference. Nevertheless, we found a positive linear association between hair Mn concentrations during the second trimester and chest circumference. The only other study that has looked at chest circumference, the study of 125 Chinese mother-child pairs mentioned above, reported that chest circumference increased with maternal blood Mn concentrations at delivery up to 55.7 μg/L and then decreased slightly after Mn concentrations exceeded this point (Guan et al. 2013); we had few women with blood Mn concentrations as high as this value and this may have affected our ability to observe a decrease in chest circumference. More importantly, the Chinese study did not measure Mn concentrations in maternal hair samples, and given that blood measurements reflect body Mn dynamics in adults (not specific to pregnant women) on the order of days or weeks whereas hair is thought to integrate circulating Mn concentrations over longer periods of time (Smith et al. 2007), it is not appropriate to compare their findings with ours. It is also important to mention that, in our study, data on chest circumference was only available for a subset of infants and that mothers of those infants had significantly lower hair Mn concentrations compared to mothers of infants for who did not have chest circumference data. We compared the subset of mother-child pairs with chest circumference data with the rest of the study population and did not find any significant differences in their socio-demographic characteristics, which suggests that the possibility of selection bias may be small. However, it is possible that the relationship between hair Mn concentrations and chest circumference may have been different if the entire population had been included in the analyses.

Evidence suggests that iron deficiency results in increased uptake and distribution of Mn in animals and humans (Smith et al. 2007; Finley 1999; Mena et al. 1969; Heilig et al. 2005; Thompson et al. 2007). We did not observe differences in blood or hair Mn concentrations in women who reported having gestational anemia at any time point during pregnancy and women who reported not having it. However, we did find a positive association between mean maternal hair Mn concentrations during pregnancy and chest circumference in women without gestational anemia, but not in women with anemia. We hypothesize that the hair Mn concentrations observed in pregnant women in our study (with and without anemia) may be concentrations that are required for normal fetal growth and this is why we observed a positive association with chest circumference. Half of the published studies have adjusted for iron status or hemoglobin concentrations during pregnancy in their analyses of Mn exposure and fetal growth, but none has examined effect modification by anemia. Although some researchers have suggested that chest circumference may be especially sensitive to the environmental factors during late pregnancy (Silventoinen et al. 2012), few studies have been published on the importance of infant chest circumference as an indicator of health outcomes (e.g., it has been referred to as indicator of nutritional status and a screening tool for childhood obesity) (Akaboshi et al. 2012; Nichols 1996; Sundaram et al. 1995). In the absence of an association of blood and hair Mn concentrations with fetal growth in our study, it remains unclear what the clinical significance of an increased chest circumference may be.

We found that among pregnant women living below the poverty line, higher mean blood Mn concentrations were associated with decreased infant chest circumference but higher mean maternal hair Mn concentrations were associated with increased length of gestation. We hypothesize that differences between mother-child pairs living above and below the poverty line may be due to unmeasured socio-demographic and environmental factors. None of the studies published to date has examined the effect of family income on the relation of prenatal Mn exposure and fetal growth or length of gestation. However, some studies have examined socio-economic status as a modifier of the association between lead and children's neurodevelopment (Bellinger 2008). These studies have suggested that greater exposures to environmental pollutants, nutritional deficiencies, and increased stress or reduced resources for coping with stress could potentially exacerbate the toxicity of contaminants and explain the higher prevalence of poorer health outcomes among children living in poverty (Verwer et al. 2007; Gallo and Matthews 2003; Hougaard and Hansen 2007; Kordas et al. 2007; Naess et al. 2007). Such assumptions could potentially explain the negative association between mean maternal blood Mn concentrations during pregnancy and chest circumference (assuming the chest circumference is an indicator of good health), but not the positive association between mean maternal hair Mn concentrations and length of gestation.

This study has several limitations. To date, there is no consensus on which is the best biomarker to assess human exposure to Mn (Smith et al. 2007; Eastman et al. 2013) and how reliable the available biomarkers are for assessing prenatal exposure to the fetus. Blood and hair Mn concentrations measured in this study may not be the best surrogates for maternal Mn load or fetal exposure. In addition, unmeasured factors related to the study population, such as nutritional and genetic characteristics, and measurement error in explanatory variables may have biased our results. In this study, the effect estimates for the associations of blood and hair Mn concentrations with fetal growth and length of gestation by trimester of pregnancy were imprecise because of the small number of observations. Lastly, we examined the effects of one specific environmental toxicant, but our study population is exposed to multiple pesticides that could also be associated with fetal growth and length of gestation. Further studies will characterize exposure to multiple pollutants over the course of pregnancy. Despite its limitations, this study has the benefits of a strong prospective design, with blood and hair Mn concentrations measured at different time points during pregnancy, and data on birth outcomes collected by hospital/clinic staff blinded to the Mn exposure status.

In summary, the results of our study failed to replicate the nonlinear associations between maternal blood Mn concentrations and measures of fetal growth that previous studies have observed. However, we did find a significant linear association of mean maternal blood concentrations during pregnancy with decreased chest circumference among infants whose mothers lived below the poverty line. We also observed positive linear associations of mean hair Mn concentrations during pregnancy with chest circumference and gestational duration among infants whose mother lived below the poverty line and/or in mothers without gestational anemia. Given the complexity of Mn, an essential element that is also an environmental pollutant, and the inconsistencies between previous epidemiological studies, further studies are needed before a clear profile can be determined.

5. Tables and figures

Table 1. Distribution of maternal and infant characteristics for the study population (n = 380).

	***n* (%)**
Maternal characteristics	
Age (years)	
< 18	68 (17.9)
18-24	177 (46.6)
25-29	65 (17.1)
30-34	37 (9.7)
≥ 35	33 (8.7)
Education	
≤ 6th grade	194 (51.0)
> 6th grade	186 (49.0)
Marital status	
Married or living as married	283 (74.5)
Unmarried	97 (25.5)
Country of birth	
Costa Rica	309 (81.3)
Other Central American countries	71 (18.7)
Family income[a]	
Above poverty line	145 (41.4)
Below poverty line	205 (58.6)
Parity	
0	136 (35.8)
≥ 1	244 (64.2)
History of miscarriage	
No	314 (82.6)
Yes	66 (17.4)
Pre-pregnancy BMI (kg/m^2)	
Underweight (< 18.5)	12 (3.2)
Normal (18.5-24.9)	186 (49.0)
Overweight (25.0-29.9)	110 (28.9)
Obese (≥ 30)	72 (18.9)
Smoking during pregnancy	
No	363 (95.5)
Yes	17 (4.5)
Caffeinated tea consumption during pregnancy	
No	301 (79.2)
Yes	79 (20.8)
Iron intake during pregnancy	
No	30 (7.9)
Yes	350 (92.1)

Gestational hypertension[a]	
No	365 (96.3)
Yes	14 (3.4)
Gestational diabetes	
No	365 (96.0)
Yes	15 (4.0)
Gestational anemia	
No	227 (59.7)
Yes	153 (40.3)
Vaginal bleeding during the 1st trimester[a]	
No	338 (96.0)
Yes	14 (4.0)
Work status during pregnancy	
Did not work or worked at home	282 (74.2)
Agricultural work	33 (8.7)
Other work (non-agricultural)	65 (17.1)
Infant characteristics	
Sex	
Male	193 (50.8)
Female	187 (49.2)
Low birth weight	
No	368 (96.8)
Yes	12 (3.2)
Preterm birth	
No	356 (93.7)
Yes	24 (6.3)

Abbreviations: *n*, number of mother-child pairs; BMI, body mass index.

[a] Missing values: family income (*m* = 30, 7.9%), gestational hypertension (*m* = 1, 0.3%), and vaginal bleeding during the 1st trimester (*m* = 28, 7.4%).

Table 2. Distribution of blood and hair manganese concentrations in the study population.

Biomarkers	***n***	**Mean (SD)**	**GM (GSD)**	**Min**	**Percentile**			**Max**
					25th	**50th**	**75th**	
Blood Mn (μg/L)								
By trimesters of pregnancy								
1st trimester	94	22.7 (6.4)	21.8 (1.3)	10.8	18.0	22.5	26.9	36.9
2nd trimester	276	23.8 (6.1)	23.0 (1.3)	9.4	19.8	24.0	27.7	56.3
3rd trimester	203	26.5 (6.6)	25.7 (1.3)	13.2	21.4	26.4	30.3	50.6
Mean during pregnancy[a]	375	24.5 (6.1)	23.7 (1.3)	10.7	20.4	24.0	28.0	50.6
Hair Mn (μg/g)								
By trimesters of pregnancy								
1st trimester	107	3.6 (6.4)	1.8 (3.0)	0.1	0.9	1.6	3.8	53.3
2nd trimester	309	3.4 (4.8)	1.8 (3.1)	0.05	0.9	1.7	3.8	36.0
3rd trimester	229	3.6 (6.2)	1.7 (3.2)	0.2	0.7	1.4	3.3	52.6
Mean during pregnancy[a]	380	3.5 (5.3)	1.9 (2.8)	0.1	0.9	1.8	4.0	53.3

Abbreviations: SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

[a] In the women for whom only one Mn measurement was available, the single measurement was used in lieu of the average.

Table 3. Adjusted linear regression and generalized additive models for fetal growth and length of gestation, per one-unit increase in blood Mn (μg/L) and 10-fold increase in hair Mn (μg/g) concentrations.[a]

Exposure	**Birth weight (g)**		**Body length (cm)**		**Ponderal index (g/cm^3)**		**Head circumference (cm)**		**Chest circumference (cm)**		**Length of gestation (days)**	
	n	**β (95% CI)**	***n***	**β (95% CI)**	***n***	**β (95% CI)**	***n***	**β (95% CI)**	***n***	**β (95% CI)**	***n***	**β (95% CI)**
Blood Mn[b, c]												
By trimesters												
Trimester 1	88	2.9 (-8.4,14.2)	87	0.03 (-0.03,0.09)	87	0.00 (-0.01,0.01)	86	0.02 (-0.02,0.06)	56	0.04 (-0.01,0.09)	94	-0.19 (-0.65,0.27)
Trimester 2	258	2.0 (-5.8,9.8)$^{\#}$	255	-0.02 (-0.06,0.03)$^{\#}$	255	0.01 (0.00,0.01)	253	-0.01 (-0.04,0.02)	128	-0.02 (-0.06,0.03)	276	-0.04 (-0.31,0.23)**
Trimester 3	194	9.3 (0.8,17.8)**	190	0.03 (-0.02,0.08)	190	0.00 (0.00,0.01)	187	0.02 (-0.01,0.05)	79	0.01 (-0.04,0.07)	203	-0.01 (-0.23,0.22)
Mean during pregnancy	351	3.6 (-3.2,10.3)	347	0.00 (-0.04,0.04)	347	0.00 (0.00,0.01)	343	-0.01 (-0.03,0.02)	153	-0.02 (-0.06,0.02)	375	-0.08 (-0.29,0.14)
Hair Mn[d, e]												
By trimesters												
Trimester 1	95	36.9 (-109.5,183.2)	94	-0.13 (-0.89,0.63)	94	0.08 (-0.03,0.18)	93	0.17 (-0.41,0.75)	60	0.26 (-0.50,1.02)	101	2.45 (-2.99,7.88)
Trimester 2	274	67.7 (-20.9,156.4)	270	0.11 (-0.40,0.62)	270	0.04 (-0.03,0.11)	268	-0.02 (-0.33,0.30)	133	0.65 (0.18,1.12)**	290	-0.01 (-2.82,2.80)
Trimester 3	202	34.7 (-73.6,143.0)	198	-0.01 (-0.67,0.64)	198	0.04 (-0.05,0.13)	196	-0.11 (-0.51,0.28)	82	0.65 (-0.09,1.39)*	215	-0.62 (-3.77,2.54)
Mean during pregnancy	331	58.8 (-29.9,147.4)	326	-0.02 (-0.51,0.48)	326	0.06 (-0.01,0.13)	323	-0.02 (-0.34,0.30)	154	0.70 (0.22,1.18)**	352	0.69 (-2.09,3.46)

Abbreviations: CI, confidence interval.

[a] Models for birth weight, body length, ponderal index, head circumference, and chest circumference include only term births.

[b] Results are for one-unit increase in blood Mn concentrations.

[c] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, gestational diabetes, caffeinated tea intake during pregnancy, and timing of prenatal care initiation.

[d] Results are for 10-fold increase in hair Mn concentrations.

[e] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, infant sex, maternal country of birth, vaginal bleeding in the first trimester of gestation (*m* = 28, 7.4%), caffeinated tea intake during pregnancy, iron intake during pregnancy, and maternal age.

*$p_{Linear} < 0.10$, **$p_{Linear} < 0.05$, $^{\#}p_{GAM} < 0.05$

6. Supporting information

Table S1. Comparison of published studies on maternal Mn concentrations during pregnancy and birth outcomes.

Author (year)	**Country**	***n***	**Biological matrix**	**Sample collection**	**Results**					
					Birth weight	**Body length**	**Ponderal index**	**Head circumference**	**Chest circumference**	**Length of gestation**
Mora et al. (current study)[a]	Costa Rica	380	Whole blood	Multiple time points during pregnancy	No association	No association	No association	No association	↓ infants born to mothers living below poverty line	No association
			Hair	Multiple time points during pregnancy	No association	No association	No association	No association	↑ infants born to mothers with no gestational anemia	↑ infants born to mothers living below poverty line
Eum et al. (2014)	Korea	331	Whole blood	Delivery	∩ association ↑ up to 30-35 µg/L and then NS ↓					
Chen et al. (2014)	China	172	Whole blood	Delivery	∩ association ↑ up to 41.8 µg/L and then NS ↓					
Guan et al. (2013)	China	125	Whole blood	Delivery	No association	No association		∩ association ↑ up to 60.6 µg/L and then ↓	∩ association ↑ up to 55.7 µg/L and then ↓	
Yu et al. (2013)	China	1,377	Serum	Delivery	No association	No association	No association			
Xu et al. (2011)	China	142	Whole blood	Delivery	No association					No association
Zota et al. (2009)[a]	United States	470	Whole blood	Delivery	∩ association ↑ up to 30 µg/L and then NS ↓					
Vigeh et al. (2008)	Iran	271	Whole blood	Delivery	↑					
Takser et al. (2004)	Canada	149	Whole blood	Multiple time points during pregnancy	No association	No association				No association
Osada et al. (2002)	Japan	51	Serum	Delivery	NS ↓					

Abbreviations and symbols: NS, not statistically significant; ∩, inverse U-shaped; ↑, increased; ↓, decreased.

[a] Models for birth weight, body length, ponderal index, head circumference, and/or chest circumference include only term births.

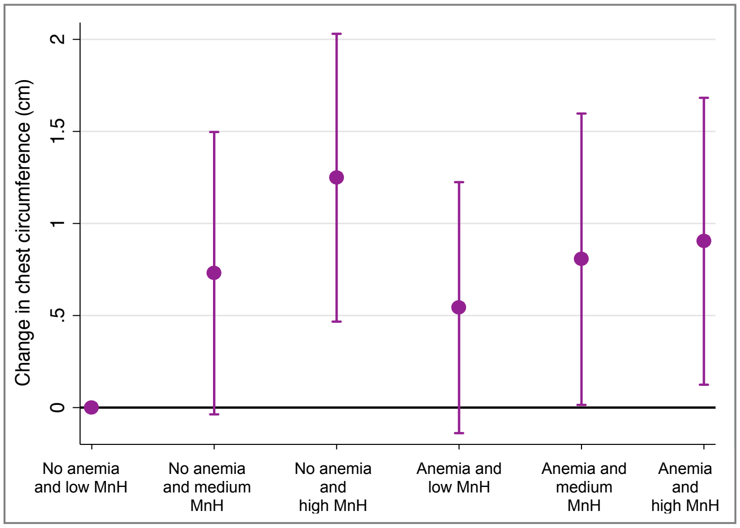

Figure S1. Adjusted regression coefficients and 95% confidence intervals for the association between tertiles of maternal mean hair Mn concentrations during pregnancy and chest circumference by gestational anemia. Regression model included only term births and was adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, gestational diabetes, caffeinated tea intake during pregnancy, timing of prenatal care initiation, gestational age, and gestational age squared. Hair Mn concentrations among women with no gestational anemia: low = 0.14-1.14 (*n* = 72), medium = 1.17-2.80 (*n* = 78), high = 2.96-53.34 μg/g (*n* = 77). Hair Mn concentrations among women with gestational anemia: low = 0.32-1.12 (*n* = 55), medium = 1.19-2.83 (*n* = 49), high = 2.95-26.88 μg/g (*n* = 49).

Table S2. Unadjusted linear regression and generalized additive models for fetal growth and length of gestation, per one-unit increase in blood Mn (μg/L) and 10-fold increase in hair Mn (μg/g) concentrations.[a]

Exposure	Birth weight (g)		Body length (cm)		Ponderal index (g/cm^3)		Head circumference (cm)		Chest circumference (cm)		Length of gestation (days)	
	n	**β (95% CI)**	*n*	**β (95% CI)**	*n*	**β (95% CI)**	*n*	**β (95% CI)**	*n*	**β (95% CI)**	*n*	**β (95% CI)**
Blood Mn[b, c]												
By trimesters												
Trimester 1	88	-1.0 (-12.6,10.5)	87	0.02 (-0.04,0.08)	87	0.00 (-0.01,0.00)	86	0.01 (-0.04,0.05)	56	0.01 (-0.04,0.06)	94	-0.15 (-0.58,0.27)
Trimester 2	258	1.9 (-5.7,9.5) #	255	-0.02 (-0.06,0.03) #	255	0.01 (0.00,0.01)	253	-0.01 (-0.04,0.02)	128	-0.03 (-0.07,0.02)	276	-0.06 (-0.32,0.20)
Trimester 3	194	7.8 (-0.6,16.3) *	190	0.02 (-0.03,0.07)	190	0.00 (0.00,0.01)	187	0.01 (-0.02,0.04)	79	0.01 (-0.04,0.07)	203	0.02 (-0.21,0.24)
Mean during pregnancy	351	3.6 (-3.2,10.3)	347	0.00 (-0.04,0.04)	347	0.00 (0.00,0.01)	343	-0.01 (-0.03,0.02)	153	-0.02 (-0.06,0.02)	375	-0.09 (-0.30,0.13)
Hair Mn[d, e]												
By trimesters												
Trimester 1	100	48.1 (-99.7,196.0)	99	-0.15 (-0.88,0.59)	99	0.08 (-0.01,0.18) *	98	0.05 (-0.49,0.59)	61	0.31 (-0.35,0.96)	107	2.32 (-3.01,7.64)
Trimester 2	290	48.6 (-38.3,135.6)	286	0.14 (-0.36,0.64)	286	0.02 (-0.05,0.09)	284	-0.04 (-0.35,0.26)	135	0.63 (0.18,1.08) **	309	-0.41 (-3.30,2.47)
Trimester 3	216	9.8 (-92.0,111.6)	212	-0.05 (-0.65,0.55)	212	0.03 (-0.06,0.11)	209	-0.21 (-0.58,0.17)	83	0.53 (-0.06,1.11) *	229	-0.40 (-3.31,2.52)
Mean during pregnancy	356	44.0 (-42.1,130.0)	351	-0.01 (-0.49,0.47)	351	0.05 (-0.02,0.11)	347	-0.07 (-0.39,0.24)	156	0.65 (0.19,1.11) **	380	0.26 (-2.54,3.06)

Abbreviations: CI, confidence interval.

[a] Models for birth weight, body length, ponderal index, head circumference, and chest circumference include only term births.

[b] Results are for one-unit increase in blood Mn concentrations.

[c] Results are for 10-fold increase in hair Mn concentrations.

*$p_{Linear} < 0.10$, **$p_{Linear} < 0.05$, #$p_{GAM} < 0.05$

Table S3. Adjusted linear regression and generalized additive models for fetal growth and length of gestation, per one-unit increase in blood Mn (μg/L) and 10-fold increase in hair Mn (μg/g) concentrations stratified by infant sex.[a]

Outcome	**Mean blood Mn (μg/L)**[b,c]			**Mean hair Mn (μg/g)**[d,e]		
	n	**β (95% CI)**	p_{INT}	***n***	**β (95% CI)**	p_{INT}
Birth weight (g)						
Boys	172	5.8 (-4.3,16.0)	0.42	164	70.9 (-65.0,206.8)	0.78
Girls	179	1.3 (-8.2,10.8)		167	27.7 (-98.4,153.8)	
Body length (cm)						
Boys	170	0.03 (-0.03,0.08)	**0.10**	161	0.17 (-0.54,0.87)	0.48
Girls	177	-0.04 (-0.10,0.02)		165	-0.40 (-1.18,0.37)	
Ponderal index (g/cm^3)						
Boys	170	0.00 (-0.01,0.01)	0.21	161	0.03 (-0.06,0.12)	0.51
Girls	177	0.01 (0.00,0.02) *		165	0.10 (-0.01,0.22) *	
Head circumference (cm)						
Boys	169	0.00 (-0.03,0.04)	0.44	160	0.01 (-0.47,0.49)	0.63
Girls	174	-0.01 (-0.05,0.02)		163	-0.11 (-0.57,0.36)	
Chest circumference (cm)						
Boys	75	-0.04 (-0.10,0.03)	0.44	76	0.41 (-0.42,1.24)	0.31
Girls	78	0.01 (-0.05,0.07)		78	0.85 (0.19,1.51) **	
Length of gestation (days)						
Boys	190	-0.07 (-0.40,0.26)	0.93	180	2.08 (-2.10,6.26)	0.28
Girls	185	-0.06 (-0.35,0.23)		172	-0.67 (-4.42,3.07)	

Abbreviations: CI, confidence interval.

[a] Models for birth weight, body length, ponderal index, head circumference, and chest circumference include only term births.

[b] Results are for each one-unit increase in blood Mn concentrations.

[c] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, gestational diabetes, caffeinated tea intake during pregnancy, and timing of prenatal care initiation.

[d] Results are for each 10-fold increase in hair Mn concentrations.

[e] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, maternal country of birth, vaginal bleeding in the first trimester of gestation (m = 28, 7.4%), caffeinated tea intake during pregnancy, iron intake during pregnancy, and maternal age.

*$p_{Linear} < 0.10$, **$p_{Linear} < 0.05$, $^{\#}p_{GAM} < 0.05$

Table S4. Adjusted linear regression and generalized additive models for fetal growth and length of gestation, per one-unit increase in blood Mn (µg/L) and 10-fold increase in hair Mn (µg/g) concentrations stratified by gestational anemia.[a]

Outcome	**Mean blood Mn (µg/L)[b,c]**			**Mean hair Mn (µg/g)[d,e]**		
	n	**β (95% CI)**	**p_{INT}**	***n***	**β (95% CI)**	**p_{INT}**
Birth weight (g)						
No gestational anemia	205	6.3 (-2.1,14.8)	0.35	195	44.2 (-77.4,165.7)	0.97
Gestational anemia	146	-0.4 (-11.6,10.8)[#]		136	25.2 (-114.1,164.5)	
Body length (cm)						
No gestational anemia	204	0.00 (-0.05,0.05)	1.00	193	-0.22 (-0.91,0.47)	0.98
Gestational anemia	143	0.00 (-0.06,0.06)[#]		133	-0.13 (-0.91,0.64)	
Ponderal index (g/cm^3)						
No gestational anemia	204	0.01 (0.00,0.01)	0.31	193	0.09 (-0.01,0.19) *	0.70
Gestational anemia	143	0.00 (-0.01,0.01)		133	0.03 (-0.07,0.12)[#]	
Head circumference (cm)						
No gestational anemia	202	0.00 (-0.03,0.03)	0.22	191	0.10 (-0.32,0.52)	0.50
Gestational anemia	141	-0.02 (-0.07,0.02)		132	-0.28 (-0.81,0.24)	
Chest circumference (cm)						
No gestational anemia	82	-0.02 (-0.08,0.03)	0.80	83	1.32 (0.54,2.09) **	**0.09**
Gestational anemia	71	-0.03 (-0.09,0.04)		71	0.24 (-0.57,1.05)	
Length of gestation (days)						
No gestational anemia	223	-0.05 (-0.32,0.22)	0.65	213	0.51 (-3.41,4.43)	0.77
Gestational anemia	152	-0.14 (-0.51,0.24)		139	1.03 (-2.97,5.04)	

Abbreviations: CI, confidence interval.

[a] Models for birth weight, body length, ponderal index, head circumference, and chest circumference include only term births.

[b] Results are for each one-unit increase in blood Mn concentrations.

[c] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, gestational diabetes, caffeinated tea intake during pregnancy, and timing of prenatal care initiation.

[d] Results are for each 10-fold increase in hair Mn concentrations.

[e] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, infant sex, maternal country of birth, vaginal bleeding in the first trimester of gestation (m = 28, 7.4%), caffeinated tea intake during pregnancy, iron intake during pregnancy, and maternal age.

*$p_{Linear} < 0.10$, **$p_{Linear} < 0.05$, [#]$p_{GAM} < 0.05$

Table S5. Adjusted linear regression and generalized additive models for fetal growth and length of gestation, per one-unit increase in blood Mn (μg/L) and 10-fold increase in hair Mn (μg/g) concentrations stratified by family income.[a]

Outcome	**Mean blood Mn (μg/L)[b,c]**			**Mean hair Mn (μg/g)[d,e]**		
	n	**β (95% CI)**	p_{INT}	***n***	**β (95% CI)**	p_{INT}
Birth weight (g)						
Above poverty line	133	12.2 (-0.7,25.1) *	**0.10**	125	82.3 (-92.5,257.1)	0.54
Below poverty line	190	0.8 (-7.6,9.2)		177	18.9 (-97.9,135.8)	
Body length (cm)						
Above poverty line	131	0.03 (-0.04,0.09)	0.40	123	-0.02 (-0.87,0.82)	0.83
Below poverty line	188	-0.01 (-0.07,0.04)		174	-0.09 (-0.84,0.66)	
Ponderal index (g/cm^3)						
Above poverty line	131	0.01 (0.00,0.01) *	0.38	123	0.08 (-0.02,0.18)	0.46
Below poverty line	188	0.00 (0.00,0.01)		174	0.04 (-0.06,0.14)	
Head circumference (cm)						
Above poverty line	130	0.01 (-0.03,0.06)	0.31	122	0.04 (-0.50,0.58)	0.44
Below poverty line	186	-0.02 (-0.05,0.02)		173	-0.23 (-0.69,0.24)	
Chest circumference (cm)						
Above poverty line	57	0.02 (-0.06,0.09)	**0.08**	56	0.88 (-0.21,1.97)	0.56
Below poverty line	83	-0.06 (-0.12,-0.01) **		84	0.62 (-0.08,1.32) *	
Length of gestation (days)						
Above poverty line	145	-0.07 (-0.48,0.34)	0.78	134	-3.17 (-7.93,1.58)	**0.03**
Below poverty line	201	-0.14 (-0.40,0.12)		188	4.11 (0.47,7.75) **	

Abbreviations: CI, confidence interval.

[a] Models for birth weight, body length, ponderal index, head circumference, and chest circumference include only term births.

[b] Results are for each one-unit increase in blood Mn concentrations.

[c] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, gestational diabetes, caffeinated tea intake during pregnancy, and timing of prenatal care initiation.

[d] Results are for each 10-fold increase in hair Mn concentrations.

[e] Adjusted for maternal education, parity, smoking during pregnancy, pre-pregnancy body mass index, length of gestation, length of gestation squared, infant sex, maternal country of birth, vaginal bleeding in the first trimester of gestation (m = 28, 7.4%), caffeinated tea intake during pregnancy, iron intake during pregnancy, and maternal age.

*$p_{\text{Linear}} < 0.10$, **$p_{\text{Linear}} < 0.05$, $^{\#}p_{\text{GAM}} < 0.05$

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Chapter 4:
Prenatal and postnatal manganese exposure and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort

1. Introduction

Manganese (Mn) is an essential element involved in important enzymatic reactions (Aschner 2000; Gwiazda et al. 2002), but in excess it can be a potent neurotoxicant. Food is the main source of Mn for the general population (ATDSR 2012), but environmental exposure to Mn can occur drinking water with high concentrations of natural Mn or contaminated by industrial waste (Bouchard et al. 2007; Bouchard et al. 2011a; He et al. 1994; Kondakis et al. 1989), and inhalation of emissions from combustion of anti-knock additives in gasoline (Zayed et al. 1999), Mn mining operations (Riojas-Rodriguez et al. 2010), ferromanganese production facilities (Haynes et al. 2010; Menezes-Filho et al. 2009), and spraying of Mn-containing fungicides (Gunier et al. 2013; Mora et al. 2014). Absorption and distribution of ingested Mn are closely regulated through homeostatic mechanisms (Papavasiliou et al. 1966; Roth 2006). Nevertheless, inhaled Mn can directly enter the systemic circulation through the lungs (Vitarella et al. 2000) and access the brain directly through the olfactory bulb (Dorman et al. 2002; Elder et al. 2006; Leavens et al. 2007), bypassing biliary excretion mechanisms.

Children and infants may be particularly susceptible to the neurotoxic effects of Mn exposure as their Mn homeostatic mechanisms are poorly developed (Aschner 2000; Ljung and Vahter 2007; Yoon et al. 2009) and Mn can enter their developing brains by crossing the blood-brain barrier (Aschner 2000; Aschner and Dorman 2006). Multiple studies have reported associations between exposure to Mn and cognitive deficits or behavioral problems in children. Higher *in utero* Mn concentrations measured in blood and teeth have been associated with attention problems (Ericson et al. 2007; Takser et al. 2003), behavioral disinhibition (Ericson et al. 2007), impaired non-verbal memory (Takser et al. 2003), and poor cognitive and language development (Lin et al. 2013) in toddlers and preschoolers, and with externalizing behavior and attention problems (Ericson et al. 2007) in school-aged children. Early postnatal Mn exposure has also been associated with poor language development in toddler boys (Rink et al. 2014), and behavioral problems in school-aged boys and girls (Ericson et al. 2007). Notably, one study of children aged 12-36 months observed an inverted U-shaped relationship between child blood Mn concentrations at 12 months and concurrent mental development (Claus Henn et al. 2010). Studies of school-aged children and adolescents (6-14 year olds) have linked elevated Mn concentrations in drinking water, blood, and hair samples with oppositional behavior and hyperactivity (Bouchard et al. 2007), decreased Full-Scale, Performance and Verbal IQ (Bouchard et al. 2011a; Riojas-Rodriguez et al. 2010; Kim et al. 2009; Menezes-Filho et al. 2011; Wasserman et al. 2006), and poor memory (He et al. 1994; Torres-Agustin et al. 2013), motor coordination (He et al. 1994; Hernandez-Bonilla et al. 2011; Lucchini et al. 2012), and visuoperceptive speed (He et al. 1994; Zhang et al. 1995). Few studies have assessed exposure to Mn both prenatally and postnatally.

Blood Mn has been typically used as a biomarker of exposure to Mn in occupational and population-based studies (Takser et al. 2003; Mergler et al. 1999), while studies in environmentally-exposed children have also measured Mn concentrations in hair (Bouchard et al. 2007; Bouchard et al. 2011a; Riojas-Rodriguez et al. 2010; Menezes-Filho et al. 2011; Eastman et al. 2013; Wright et al. 2006), in the exposure medium (e.g., water) (Bouchard et al. 2011a;

Wasserman et al. 2006; Khan et al. 2012), or in teeth (Ericson et al. 2007; Arora et al. 2012). Studies on Mn toxicokinetics suggest that biomarkers of exposure such as blood may best reflect recent exposures (i.e., days), while teeth may integrate longer-term exposures (e.g., months or longer) (Ericson et al. 2007; Arora et al. 2012; Arora et al. 2011; Smith et al. 2007). Deciduous teeth incorporate Mn in an incremental pattern and dentine, unlike deciduous enamel, can provide reliable information on the developmental timing of exposures to Mn that occur between the second trimester of pregnancy (13-16 weeks gestation, when incisors start forming) and 10-11 months after birth (when molars stop developing) (Arora et al. 2012).

In this study, we measured prenatal and postnatal dentine Mn levels in children's deciduous teeth, and examined the relationship of Mn concentrations with attention, cognition, memory, and motor development in 7-, 9-, and 10.5-year-old children living in an agricultural community in California where large amounts of Mn-containing fungicides are applied.

2. Methods

2.1. *Study population*

The Center for the Health Assessment of Mother and Children of Salinas (CHAMACOS) is a cohort study examining the health effects of prenatal and postnatal exposure to pesticides and other environmental exposures in Mexican-American children living in the Salinas Valley, California. Common crops in this is agricultural region include lettuce, strawberries, tomatoes, and broccoli. About 110,000 kg of Mn-containing fungicides, mancozeb and maneb (20% Mn by weight) (FAO 1980), were used in the Monterey County in 2012 (CDPR 2014), but almost 160,000 kg were applied in 1999-2000, when study participants were pregnant (CDPR 2001).

Participant enrollment procedures for the initial CHAMACOS cohort (CHAM1) have been described elsewhere (Eskenazi et al. 2004; Eskenazi et al. 2006). Briefly, eligible pregnant women (≥ 18 years old, < 20 weeks of gestation, Spanish- or English-speaking, qualified for low-income health insurance, and planning to deliver at the county hospital) were recruited in community clinics between October 1999 and October 2000. Six hundred and one pregnant women were enrolled and 526 of them delivered live-born singletons (referred to henceforth as CHAM1). A total of 339 of these children were assessed at age 7, 326 at age 9, and 320 at age 10.5 years.

A second cohort of 300 9 year-olds (CHAM2) was recruited between September 2009 and August 2011. Children were eligible to participate if their mother, when pregnant, was ≥ 18 years old, Spanish- or English-speaking, qualified for low-income health insurance, and received prenatal care at any low-income provider in the Salinas Valley. Children had to be born between February 2000 and August 2002 to approximately match the birth dates of CHAM1 children. A total of 308 and 295 of CHAM2 children completed the neurobehavioral assessment at ages 9 and 10.5 years, respectively.

CHAM1 children, but not CHAM2, were administered a neurobehavioral test battery at age 7 ($n = 339$). CHAM1 and CHAM2 children completed identical neurobehavioral assessments at ages 9 ($n = 634$) and 10.5 ($n = 615$). Assessments were conducted by bilingual psychometricians who were trained and supervised by a pediatric neuropsychologist. Subtests were administered in the dominant language of the child, as ascertained via direct assessment.

Teeth were collected and analyzed for 227 CHAM1 children and 69 CHAM2 children. For this study, we excluded 39 children who provided a shed molar instead of an incisor, seven children with a medical condition that would affect the neurobehavioral assessment (deafness, autism, Down syndrome, cerebral palsy/hydrocephalus, and seizures), and three children missing all neurobehavioral assessments. Children included in these analyses ($n = 247$) did not differ significantly from the original CHAM1 and CHAM2 children on most attributes, including maternal marital status, poverty category at age 9, and child's birth weight. However, children included in these analyses were breast-fed longer (mean breastfeeding duration, 9.7 vs. 7.6 months, $p < 0.01$), and had older mothers (mean age, 26.8 vs. 25.6 years, $p < 0.01$) with poorer cognitive ability (mean PPVT score at 9 year-visit = 88.9 vs. 93.2 points, $p < 0.01$) than CHAM1 and CHAM2 children together.

All study activities were approved by the University of California at Berkeley Committee for the Protection of Human Subjects, and written informed consent was obtained from all participants at enrollment. Child assent was obtained at 7, 9, and 10.5 years of age.

2.2. Maternal interviews

CHAM1 mothers were interviewed twice during pregnancy (median, 13 and 26 weeks gestation), shortly after delivery, and when children were 6 months, and 1, 2, 3.5, 5, 7, 9, and 10.5 years old. CHAM2 mothers were interviewed when their children were 9 and 10.5 years old. Interviews were conducted in English or Spanish by trained bilingual interviewers. CHAM1 mothers were administered the Peabody Picture Vocabulary Test (PPVT) of verbal intelligence (Dunn and Dunn 1981) at the 6-month and 9-year visits. They also completed the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977) at the 7- and 9-year visits, and the Infant-toddler HOME (Home Observation for Measurement of the Environment) inventory short form (Caldwell and Bradley 1984) at the 7-, 9-, and 10.5-year visits. CHAM2 mothers completed the PPVT and CES-D at the 9-year visit (enrollment) and the HOME inventory short form at the 9- and 10.5-year visits. Additional information, such as birth weight, and gestational duration, was abstracted from prenatal and delivery medical records for CHAM1 and CHAM2 mother-child pairs. Data on maternal hematocrit to hemoglobin ratio during pregnancy was abstracted for CHAM1 children only.

2.3. Attention

Mothers and teachers of CHAM1 children were administered Parent and Teacher Rating Scales of the Behavior Assessment System for Children, 2nd edition (BASC-2) (Reynolds and Kamphaus 2004) and the Conners' Attention Deficit Hyperactivity Disorder (ADHD)/Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) Scales (CADS) (Conners 2001) when children were 7 years old. CHAM1 and CHAM2 mothers were administered the CADS at the 9-year visit and the BASC-2 at the 10.5-year visit. CADS scores for four subscales (Conners ADHD index, and DSM-IV-based Inattentive, Hyperactive/Impulsive, and Total ADHD) and BASC-2 scores for two subscales (Hyperactivity and Attention Problems) and two composite scales (internalizing and externalizing problems) were standardized to a nonclinical population (age-standardized *T*-scores, mean = 50 ± 10), with higher values indicating more frequent problem behaviors.

At 9 years of age, CHAM1 and CHAM2 children completed the Conners' Continuous Performance Test II, Version 5 (CPT-II) (Conners 2002), a computerized test that assesses accuracy and impulse control. Scores for errors of commission and errors of omission were analyzed as continuous, sex- and age-standardized *T*-scores (mean = 50 ± 10). A continuous ADHD Confidence Index score, indicating the probability of children being correctly classified as having clinical ADHD, was also examined.

At the 10.5-year visit, CHAM1 and CHAM2 children were administered the BASC-2 Self-Report of Personality, Child Version (Reynolds and Kamphaus 2004). Scores for two subscales (hyperactivity and attention problems) were compared to national norms to generate age-standardized *T*-scores (mean = 50 ± 10), with higher scores indicating more frequent behavioral problems.

2.4. Cognition

CHAM1 children were administered the Wechsler Intelligence Scale for Children, 4th edition (WISC-IV) (Wechsler 2003) at the 7-year study visit. At 10.5 years of age CHAM1 and CHAM2 children were administered the WISC-IV. Scores for four domains were calculated at both time points: Verbal Comprehension, Perceptual Reasoning, Working Memory, and Processing Speed. A Full-Scale intelligence quotient (IQ) was also calculated (mean = 100 ± 15 for the Full Scale IQ and other subdomains). WISC-IV scores were analyzed as continuous variables.

2.5. Memory

At the 9-year visit, CHAM1 and CHAM2 children completed a test of visuospatial memory, the NEPSY-II Memory for Designs (Korkman et al. 2007). We calculated continuous scaled scores (mean = 10 ± 3) for immediate and delayed memory using normative values for the corresponding chronological age.

At age 10.5 years, children's verbal learning and memory abilities were assessed using the Children's Auditory Verbal Learning Test, 2nd edition (CAVLT-2) (Talley 1997). We analyzed five subscales as continuous standardized scores (mean = 100 ± 15): learning curve, immediate recall, delayed recall, recognition accuracy, immediate memory span, and level of learning. We also standardized CAVLT-2 scores within our study population (*z*-scores, mean = 0 ± 1) because the Spanish version of the test has not been validated in Spanish-speaking populations (Torres-Agustin et al. 2013).

2.6. Motor functioning

CHAM1 children were administered finger-tapping (Reitan Neuropsychology Laboratory, Tucson, AZ) and pegboard (Wide Range Assessment of Visual Motor Ability, WRAVMA) (Adams and Sheslow 1995) tests at age 7 to assess fine motor dexterity. Finger-tap scores were standardized within our study population (*z*-scores, mean = 0 ± 1), but pegboard scores were age-standardized to a mean of 100 (SD = 15).

At ages 9 and 10.5 years, CHAM1 and CHAM2 children were administered parts of the Luria Nebraska Motor Battery (Golden et al. 1980). We selected for analysis 7 subtests that have shown to be sensitive to Mn exposure (Lucchini et al. 2012) or have been used in other studies examining the neurodevelopmental effects of Mn in children (Hernandez-Bonilla, Mora AM, personal communication) including: dominant hand clench, non-dominant hand clench, alternative hand clench, finger-thumb touching with dominant hand, finger-thumb touching with non-dominant hand, alternative hand tapping twice with dominant hand and once with non-dominant hand, and alternative hand tapping twice with non-dominant hand and once with dominant hand. The sum of the scores of the 5 subtests administered by Lucchini et al. (2012)

and the sum of all 7 subtests were standardized within our study population (*z*-scores, mean = 0 ± 1).

2.7. Tooth Mn measurements

Teeth collection started at the 7-year visit for CHAM1 children and at the 9-year visit for the CHAM2 children. Participants were asked to mail or bring to the study visits the child's shed teeth. Detailed methods for measuring Mn in teeth has been described elsewhere (Arora et al. 2012; Arora et al. 2011). Briefly, incisors were sectioned in a vertical plane, cleaned in an ultrasonic bath of Milli-Q water, and dried in an oven at 60°C for 24 h. Then the neonatal line, a histological feature used to demarcate prenatally and postnatally formed regions of enamel and dentine, was identified using light microscopy. Mn levels and spatial distribution in prenatal and postnatal mantle dentine were determined with laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) using the neonatal line as a reference point. Because multiple measurements were taken in prenatal and postnatal dentine, we calculated the area under the curve (AUC) to estimate cumulative Mn exposure in prenatal (from 3 months of gestation to birth) and postnatal (from birth to approximately 2.5 months of age) periods. Mn levels were normalized to ^{43}Ca to adjust for variations in mineralization. Coefficients of variation for five teeth measured on three different days ranged from 4.5% to 9.5% indicating good reproducibility of ^{55}Mn:^{43}Ca dentine measurements.

2.8. Other environmental toxicants

We examined the potential confounding and effect modification effects of known or suspected neurotoxicants, including organophosphorous (OP) pesticides, lead, and polybrominated diphenyl ether flame retardants (PBDEs), within CHAM1 children. Maternal exposure to OP pesticides during pregnancy, indicated by urinary dialkyl phosphate (DAP) metabolite concentrations, was measured at approximately 13 and 26 weeks of gestation using an isotope dilution gas chromatography-tandem mass spectrometry method (Bradman et al. 2005). Lead was quantified in maternal blood samples collected at approximately 26 weeks of gestation using graphite furnace atomic absorption spectrophotometry. PBDEs were measured in maternal blood samples at approximately 26 weeks of gestation using high-resolution gas chromatography/high-resolution mass spectrometry with isotope dilution quantification (Sjodin et al. 2004). PBDEs concentrations were expressed on a serum lipid basis. Total lipids were quantified by measuring triglycerides and total cholesterol in serum (Phillips et al. 1989).

2.9. Data analysis

Prenatal dentine Mn levels were normally distributed, whereas postnatal dentine Mn levels were skewed to the left. Thus, we transformed postnatal dentine Mn levels to the $\log_2$ scale to normalize the residuals. We examined the association between teeth Mn levels and neurodevelopment using multivariable linear regression models. We also examined potential non-linear associations using generalized additive models with a three-degrees-of-freedom cubic spline function. If a potentially nonlinear association between dentine Mn levels and any of the neurodevelopmental outcomes was identified ($p_{\mathrm{GAM}} < 0.05$), we created indicator variables for tertiles of each Mn biomarker distribution and included them in the adjusted regression models. We used generalized estimating equation (GEE) models to examine relationships of prenatal and postnatal dentine Mn levels with repeated-outcome measurements (i.e., outcomes that were examined in two of the three neurobehavioral assessments).

We built separate models for attention, cognition, memory, and motor outcomes, and used the same covariates in the model for all outcomes within a category. Main covariates of interest were selected using directed acyclic graphs and based on statistical considerations if covariates were associated with the exposure in the bivariate analyses ($p < 0.20$). We retained the following variables as covariates for all analyses: maternal education (three categories), intelligence (PPVT score, continuous), years in the US (continuous), depression at time of assessment (dichotomous: < 16 vs. ≥ 16 points in CES-D); child's sex, age at assessment or at maternal interview (continuous), and language of the assessment or maternal language at interview (two categories); psychometrician (two or three categories); HOME z-score at time of assessment (continuous); household income at time of assessment (three categories), number of children in the home at time of assessment (continuous); and housing density at time of assessment (continuous). Missing values (< 5%) for covariates were imputed by randomly selecting a value from the dataset (Lubin et al. 2004).

We conducted several sensitivity analyses to assess the robustness of our results. First, we reran models after excluding outliers defined as studentized residuals (residuals divided by the model standard error) greater than three standard units. Second, we reran the analyses excluding the CHAM2 children to assess whether differences between CHAM1 and CHAM2 influenced the associations of prenatal and postnatal dentine Mn with neurodevelopmental outcomes. Third, we fitted the adjusted regression models excluding preterm ($n = 21$) and other low birth weight ($n = 2$) children given that these variables may mediate the associations between Mn exposure and neurodevelopmental outcomes. We also reran the regression models including birth weight as a covariate. Fourth, we fitted the adjusted regression models for postnatal dentine Mn including prenatal dentine Mn as a confounder for participants with both measurements. Lastly, in the subset of CHAM1 children for whom we had measured levels during pregnancy, we examined the confounding effect of potential neurotoxicants (i.e., DAPs and PBDEs) by adding them individually to the prenatal dentine Mn final models (Bouchard et al. 2011b; Eskenazi et al. 2013; Marks et al. 2010).

We evaluated effect modification of the associations of prenatal and postnatal dentine Mn with neurodevelopmental outcomes by child sex in CHAM1 and CHAM2 children combined. Because there is a well-known inverse association between iron stores and Mn absorption and also evidence of synergism between lead and manganese, we examined effect modification by gestational anemia (maternal hemoglobin levels during pregnancy < 11 vs. ≥ 11 g/dL) and prenatal lead exposure (maternal blood lead levels during pregnancy < 2 vs. ≥ 2 µg/dL) in the subset of CHAM1 children for whom these measurements were available.

Main effects were considered significant with $p < 0.05$ based on two-tailed tests, and interactions were considered significant if $p < 0.10$. All analyses were conducted using STATA version 12.1 (StataCorp, College Station, TX).

3. Results

Most women in the present study were young (mean age = 26.8 ± 5.1 years), multiparous (66.8%), Spanish speaking (~89.6%), did not complete high school (75.7%), and had a family income below the U.S. poverty threshold (~71.0%, Table 1). Many women reported sufficient symptoms at the 7- and 9-year follow-up visits to qualify as depressed on the CES-D scale (21.8% and 26.6%, respectively). The mean (± SD) prenatal dentine Mn level was 0.50 ± 0.18

$^{55}Mn:^{43}Ca$ AUC x 10^4 and the median was 0.49 $^{55}Mn:^{43}Ca$ AUC; during the early postnatal period, these values were 0.19 ± 0.21 and 0.14 $^{55}Mn:^{43}Ca$ AUC x 10^4 (Table S1). Prenatal and postnatal dentine Mn levels were moderately correlated ($r_s = 0.49$, $p < 0.001$, $n = 243$). Maternal intelligence, parity, gestational anemia, low birth weight, prematurity, and family income were not associated with the child's prenatal or postnatal dentine Mn levels (Table 1). However, higher prenatal and postnatal dentine Mn levels were observed in children of mothers aged 25-34 years, poorly educated, and who had lived for a shorter time in the U.S. Prenatal Mn levels were also higher among women who reported smoking during pregnancy.

3.1. Attention

In general, prenatal and postnatal dentine Mn levels were not associated with measures of attention at 7, 9, or 10.5 years (Table 2). We observed evidence of a nonlinear association between prenatal Mn levels and maternally reported scores for internalizing problems on BASC-2 at age 7, but tertile categorization showed no clear trend (data not shown). However, we found a borderline significant linear association between prenatal Mn levels and maternally reported BASC-2 internalizing problems at age 7 among children born to mothers with higher lead exposure during pregnancy (β for a one-unit increase in Mn levels = 33.1, 95% confidence interval (CI): -2.6, 68.8), but not among children born to mothers with lower lead exposure (β = -3.0, 95% CI: -12.6, 6.6; $p_{INT} = 0.01$; Table S3). Additionally, prenatal Mn levels were positively associated with errors of commission scores on the CPT-II at age 9 among children whose mothers had gestational anemia (β for a one-unit increase in Mn levels = 93.3, 95% CI: 18.7, 167.9), but not among children whose mothers had a normal iron status during pregnancy (β = -8.0, 95% CI: -17.7, 1.7; $p_{INT} = 0.08$; Table S5). Prenatal dentine Mn levels were also associated with more frequent maternal reports of externalizing and attention problems on BASC-2 at age 10.5 years among boys (β = 11.3, 95% CI: 1.6, 20.9; and β = 14.8, 95% CI: 1.8, 27.7; respectively) than among girls (β = -0.9, 95% CI: -7.7, 5.9; $p_{INT} = 0.04$; and β = -6.4, 95% CI: -16.2, 3.4; $p_{INT} < 0.01$; respectively; Table S7).

No associations were observed between postnatal dentine Mn levels and attention-related outcomes at ages 9 and 10.5 years in the combined or stratified analyses (Table 2). Multivariable GEE analyses did not show any statistically significant associations of prenatal or postnatal dentine Mn levels with CADS and BASC-2 scores at 7, 9, and/or 10.5 years of age (Table 4).

3.2. Cognition

Prenatal and postnatal dentine Mn levels were either positively or not significantly associated with cognitive outcomes at ages 7, 9, or 10.5 in the analyses of all study participants combined (Table 3). We observed evidence of a nonlinear association between prenatal dentine Mn levels and WISC-IV Perceptual Reasoning IQ scores at age 7, but no clear trend when we categorized Mn levels in tertiles (data not shown). Nevertheless, we found that higher prenatal dentine Mn levels were linearly associated with better scores on Full-Scale IQ at age 7 (β for a one-unit increase in Mn levels = 60.9, 95% CI: 2.5, 119.3; $p_{INT} = 0.07$) and Perceptual Reasoning IQ at age 10.5 (β = 92.9, 95% CI: 24.5, 161.3; $p_{INT} = 0.03$) in children born to mothers with gestational anemia (Table S6).

Postnatal dentine Mn levels were positively and linearly associated with Working Memory IQ scores at 10.5 years of age (β for a 2-fold increase in Mn levels = 1.6, 95% CI: 0, 3.2), but not any other measures of IQ, in the combined analyses (Table 3). In stratified analyses, postnatal dentine Mn levels were positively associated with Processing Speed IQ scores at age 7 among children whose mothers had gestational anemia (β = 6.2, 95% CI: 0.5, 11.9) but not among children whose mothers had a normal iron status during pregnancy (β = 0.9, 95% CI: -2.0, 3.7; p_{INT} = 0.02; Table S6). Similarly, we observed that higher postnatal dentine Mn levels were associated with better scores on Perceptual Reasoning IQ at ages 7 (β = 5.0, 95% CI: 1.5, 8.6) and 10.5 years (β = 5.8, 95% CI: 2.3, 9.3), and better Full-Scale IQ scores at 10.5 years (β = 2.8, 95% CI: 0.3, 5.3) among boys, but not among girls (p_{INT} = 0.02; p_{INT} < 0.01; and p_{INT} = 0.02; respectively; Table S8). We did not observe evidence of effect modification of the associations of prenatal and postnatal dentine Mn levels with cognitive outcomes by prenatal lead exposure (Table S4).

Adjusted GEE analyses of repeated outcome measures at ages 7 and 10.5 years showed that higher postnatal dentine Mn levels were related to better cognitive scores (Table 4). Specifically, a two-fold increase in postnatal dentine Mn levels was associated with an increase of 2.3 (95% CI: 0.2, 4.4) and 1.6 (95% CI: 0, 3.2) points in WISC-IV Perceptual Reasoning and Full-Scale IQ, respectively.

3.3. *Memory*

No linear associations were observed between prenatal dentine Mn levels with memory outcomes in the analyses of all children combined (Table 3). We did find evidence of nonlinear associations of dentine Mn levels during pregnancy with CAVLT-2 Immediate recall and Level of learning scores at 10.5 years of age, but when we categorized Mn levels in tertiles we did not observe a clear dose-response relationship (data not shown).

Postnatal dentine Mn levels were not associated with CAVLT-2 subtests scores but related with better scores on NEPSY-II Memory for Designs Immediate (β for a 2-fold increase in Mn levels = 0.7, 95% CI: 0.1, 1.2) and Delayed memory (β = 0.8, 95% CI: 0.3, 1.3) at age 9 in the combined analyses (Table 3). Sex-stratified analyses revealed that higher postnatal dentine Mn levels were also associated with better scores on CAVLT-2 Delayed recall at age 10.5 among boys (β = 3.9, 95% CI: 0.5, 7.4), but not among girls (β = 0.1, 95% CI: -3.9, 4.0; p_{INT} = 0.09; Table S8).

3.4. *Motor function*

We did not observe linear associations between prenatal dentine Mn levels and motor outcomes at 7, 9, or 10.5 years in the combined analyses (Table 3). However, when we stratified by gestational anemia, we found that dentine Mn levels during pregnancy were related to better performance on the Finger tapping test for both dominant and non-dominant hands at age 7 among children whose mothers had anemia (β for a one-unit increase in Mn levels = 5.6, 95% CI: 1.7, 9.5; and β = 3.9, 95% CI: 0.8, 7.1; respectively), but not among children whose mothers had a normal iron status during pregnancy (β = 0.5, 95% CI: -0.4, 1.4; p_{INT} < 0.01; and β = 0.2, 95% CI: -0.8, 1.1; p_{INT} = 0.02; respectively; Table S6). We also observed a positive association between prenatal dentine Mn levels and Finger tapping scores for the dominant hand at 7 years among boys (β = 1.5, 95% CI: 0.2, 2.7; p_{INT} = 0.09; Table S8).

Higher postnatal dentine Mn levels were associated with higher scores in the Finger tapping test for the dominant hand at 7 years (β for a 2-fold increase in Mn levels = 0.2, 95% CI: 0, 0.3) in the combined analyses (Table 3). We also found that postnatal dentine Mn levels were associated with increased scores in the Finger tapping test for the non-dominant hand at age 7 among children born to mothers with higher lead exposure during pregnancy (β = 9.5, 95% CI: -

0.2, 19.3; $p_{\mathrm{INT}} = 0.01$; Table S4) and with increased scores in the 5-item sum of the Luria-Nebraska Motor Scale at 9 years of age among children born to mothers with gestational anemia (β = 0.7, 95% CI: 0.2, 1.3; $p_{\mathrm{INT}} = 0.03$; Table S6).

No associations were observed between dentine Mn levels and motor outcomes at 7, 9, and/or 10.5 years of age in multivariable GEE analyses (Table 4).

3.5. Sensitivity analyses

In general, the point estimates did not change appreciably after removal of the outliers from the final multivariable models. Restricting the analyses to CHAM1 children yielded results similar to those obtained for the entire group. Including potential neurotoxicants (i.e., DAPs, PBDEs, and lead) and birth weight in the adjusted models only marginally altered the results (change in estimates < 10%). However, excluding preterm and other low birth weight children (*n* = 23) from the analyses attenuated the linear associations of postnatal dentine Mn levels with Finger tapping test scores for the dominant hand at age 7 and Working Memory IQ scores at age 10.5 (β for a 2-fold increase in Mn levels = 0.1, 95% CI: -0.1, 0.3; and β = 1.4, 95% CI: -0.3, 3.1; respectively, data not shown). Conversely, the positive linear association between postnatal Mn levels and Full-Scale IQ scores at 10.5 years (β = 1.5, 95% CI: -0.2, 3.3) reached statistical significance after excluding preterm and other low birth weight children from the analyses.

Including prenatal dentine Mn levels in the adjusted models for postnatal Mn levels did not change the point estimates observed in the final models (data not shown), except for the positive association between postnatal Mn levels and Finger tapping test scores for the dominant hand at age 7 that became not statistically significant (β = 0.2, 95% CI: 0, 0.3; data not shown).

4. Discussion

We found that Mn levels in prenatal and postnatal dentine were not adversely associated with several measures of attention, cognition, memory, and motor function of school-age children. In fact, we observed that higher prenatal and postnatal Mn levels in dentine of deciduous teeth were associated with better cognitive and motor function scores. Higher postnatal dentine Mn levels were also associated with improved scores in memory tests. Conversely, we found that higher prenatal dentine Mn levels were associated with poorer attention scores but only among boys and children born to mothers with higher lead exposure during pregnancy or gestational anemia. These associations were all linear, and we observed no threshold. Our results appeared to be independent of the associations of prenatal PBDE and OP pesticide exposure with child neurobehavioral development that have been previously reported in the CHAMACOS cohort (Bouchard et al. 2011b; Eskenazi et al. 2013; Marks et al. 2010).

This is the largest study to date on the potential neurodevelopmental effects of prenatal and early postnatal Mn status in school-age children. Our findings are somewhat similar to those of previous studies of adverse associations between *in utero* and early postnatal exposure to Mn and behavioral outcomes (Ericson et al. 2007; Takser et al. 2003). Consistent with our findings of prenatal Mn levels associated with poorer attention in the stratified analyses, a small study of 27 U.S. children found that higher prenatal Mn levels (~20 weeks gestation) in enamel of deciduous teeth were associated with poorer performance in behavioral disinhibition tests at ages 3 and 4.5 years (including increased errors of commission on a continuous performance test), and more adverse maternal and teacher reports of internalizing and externalizing problems at ages 6-7 (1st grade) and 8-9 years (3rd grade) (Ericson et al. 2007). A positive association between early postnatal enamel Mn levels (~62-64 weeks gestation) and teacher-reported scores of externalizing problems at ages 6-7 and 8-9 years was also reported in this study. A study of 247 French children observed a negative linear relationship between cord blood Mn concentrations and performance in the McCarthy Attention subscale at age 3 years, but not at 6 years, and did not find any differences between boys and girls (Takser et al. 2003).

Although numerous cross-sectional studies of school-age children have reported that higher Mn concentrations were related to poorer cognitive, memory, and motor outcomes (Bouchard et al. 2011a; He et al. 1994; Riojas-Rodriguez et al. 2010; Kim et al. 2009; Menezes-Filho et al. 2011; Wasserman et al. 2006; Torres-Agustin et al. 2013; Hernandez-Bonilla et al. 2011; Lucchini et al. 2012), we observed that both prenatal and postnatal dentine Mn levels were associated with better cognitive, memory, and motor function scores at ages 7, 9, and/or 10.5 years. These inconsistent findings may be due to differences in the study design (cross-sectional vs. prospective), timing of Mn measurements (Mn levels measured at ages 6-14 years vs. prenatal and early postnatal), and/or exposure matrix (Mn levels measured in drinking water, blood, or hair samples vs. in teeth). Notably, the two other prospective studies, besides ours, that have examined the relationship of prenatal and early postnatal exposure to Mn with cognitive outcomes in preschool- and school-age children did not find significant adverse associations (Ericson et al. 2007; Takser et al. 2003). However, one of these studies did show that higher cord blood Mn concentrations were associated with lower non-verbal memory (in boys and girls combined) and hand skill scores (in boys only) at age 3 years (Takser et al. 2003). Interestingly, a study of 448 infants born in Mexico found an inverted U-shaped association between child blood Mn concentrations at 12 months of age and concurrent mental development, but this association was no longer present at 24 months (Claus Henn et al. 2010). We assessed for nonlinearity in our study, but did not find any evidence of biphasic dose-response relationships.

Several epidemiologic studies have shown that exposure to Mn and lead can result in synergistic effects on neurodevelopment (Lin et al. 2013; Kim et al. 2009; Claus Henn et al. 2012). A study of 455 Mexican children observed an interaction between the highest quintile of 12-month blood Mn concentrations and continuous blood lead concentrations on both mental and psychomotor development at ages 12-36 months (Claus Henn et al. 2012). A recent study of 230 children in Taiwan reported significantly lower cognitive, language, and overall development quotients at 2 years of age in the group with the highest cord blood Mn and cord blood lead concentrations (≥ 75th percentile for both metals) (Lin et al. 2013). Prenatal blood lead concentrations (measured at ~ 26 weeks gestation) were relatively low in our study (median = 0.8 µg/dL, range = 0-13.1 µg/dL), but we did find that joint prenatal exposure to Mn and lead was associated with higher internalizing problem scores but better fine motor coordination at age 7 years. We did not find evidence of effect modification of the associations of prenatal and postnatal dentine Mn levels with cognitive abilities by prenatal lead exposure. Similarly, a study of 299 Italian adolescents aged 11-14 years did not observe interactions of blood lead concentrations with blood, hair, air, nor soil Mn concentrations in relation to cognitive function, measured using the Wechsler Intelligence Scale for Children (Lucchini et al. 2012).

It is well known that there is an inverse association between body iron status and Mn absorption. Animal studies have shown that iron deficiency increases Mn absorption and retention in various organs (Flanagan et al. 1980; Garcia et al. 2007; Kim et al. 2012; Thompson et al. 2006; Yokoi et al. 1991), and human studies have reported inverse associations of blood

Mn concentrations with serum ferritin concentrations and genetic variants in iron metabolism genes (Claus Henn et al. 2011; Finley 1999; Mena et al. 1969; Park et al. 2013; Smith et al. 2013). Consistent with these findings, we found evidence suggesting that gestational anemia may potentiate some of the positive or negative neurodevelopmental effects of Mn. For instance, children whose mothers had gestational anemia showed a stronger positive association of prenatal dentine Mn levels with Perceptual Reasoning and Full-Scale IQ at age 7 and a stronger negative association between prenatal dentine Mn levels and CPT-II errors of commission scores at age 9 compared to children whose mothers did not have anemia. We did not observe significant independent associations between gestational anemia and neurodevelopmental outcomes (data not shown).

Previous studies have reported stronger negative associations of Mn concentrations with attention-related, cognitive, memory, and motor outcomes for girls than for boys (Riojas-Rodriguez et al. 2010; Torres-Agustin et al. 2013; Hernandez-Bonilla et al. 2011; Bouchard et al. 2011b; Menezes-Filho et al. 2013). Our study did not show negative associations between prenatal or postnatal dentine Mn levels and these outcomes for girls, but we did find some positive and significant associations for boys. Biological differences in response to Mn may explain differences between boys and girls. Animal studies have shown that Mn accumulation across body tissues (Dorman et al. 2004) and changes in striatal morphology (Madison et al. 2011) differ between male and female rodents. Further animal and epidemiologic studies are needed to elucidate possible biological differences between males and females.

This study has several limitations. First, the small sample size, further reduced in the stratified analyses (i.e., prenatal lead exposure and gestational anemia), limited our statistical power. Second, Mn levels were only measured in dentine of deciduous teeth, so we were unable to compare our levels directly to other measures such as in hair or blood reported in other study populations. Third, we conducted multiple comparisons and cannot rule out the possibility that some associations were due to chance. Fourth, residual confounding in the relationships between exposure to Mn and neurodevelopmental outcomes could exist. However, we did control for important factors such as maternal cognitive abilities, indicators of socioeconomic status (i.e., household income, housing density, and maternal education), child stimulation, and exposure to other environmental agents. Despite its limitations, the present study also has considerable strengths including its longitudinal design, use of comprehensive neurodevelopmental assessments at different ages, and information on a wide variety of potential confounders. In addition, we measured Mn levels in dentine of deciduous teeth, a novel matrix that, unlike enamel, can be directly linked to the developmental timing of exposure.

Overall, we found that Mn exposure related to agricultural pesticide use, as measured in deciduous teeth, was not consistently associated with poorer neurodevelopmental outcomes in the CHAMACOS children. Additional research is needed to understand the relationship between Mn levels in dentine of deciduous teeth and other biological matrices (i.e., blood and hair), the shape of the dose-response relationships between Mn exposure and neurodevelopment in children, and the impact various routes of exposure have on this dose-response curve.

5. Tables and figures

Table 1. Study population characteristics and children's prenatal and postnatal dentine Mn levels (^{55}Mn:^{43}Ca AUC x 10^4).

Characteristic	**Prenatal Mn**		**Postnatal Mn**	
	***n* (%)**[a]	**Mean (95%CI)**	***n* (%)**[b]	**GM (95%CI)**
All participants	247 (100.0)	0.50 (0.47,0.52)	243 (100.0)	0.15 (0.14,0.17)
Maternal characteristics				
Age				
18-24	91 (36.8)	0.46 (0.42,0.49) **	90 (37.0)	0.14 (0.13,0.16) *
25-29	90 (36.4)	0.52 (0.48,0.57)	87 (35.8)	0.16 (0.14,0.18)
30-34	44 (17.8)	0.53 (0.47,0.60)	44 (18.1)	0.18 (0.15,0.22)
35-45	22 (9.0)	0.46 (0.40,0.51)	22 (9.1)	0.13 (0.10,0.17)
Education				
≤ 6th grade	112 (45.3)	0.54 (0.50,0.58) **	109 (44.9)	0.17 (0.15,0.19) *
7th-12th grade	75 (30.4)	0.49 (0.45,0.53)	75 (30.9)	0.14 (0.12,0.15)
Completed high school	60 (24.3)	0.42 (0.38,0.45)	59 (24.2)	0.15 (0.13,0.17)
Intelligence (PPVT score)[c]				
≤ 74	47 (19.0)	0.47 (0.42,0.51)	47 (19.3)	0.16 (0.14,0.18)
75-99	82 (33.2)	0.51 (0.46,0.56)	79 (32.5)	0.16 (0.14,0.18)
≥ 100	118 (47.8)	0.49 (0.46,0.52)	117 (48.2)	0.15 (0.14,0.17)
Years in US				
≤ 1	53 (21.5)	0.51 (0.46,0.56) **	53 (22.3)	0.16 (0.13,0.20) *
2-5	67 (27.1)	0.52 (0.47,0.58)	65 (26.7)	0.17 (0.14,0.19)
6-10	67 (27.1)	0.50 (0.46,0.53)	65 (25.8)	0.15 (0.13,0.17)
≥ 11	39 (15.8)	0.50 (0.44,0.55)	39 (15.8)	0.16 (0.14,0.20)
Entire life	21 (8.5)	0.36 (0.31,0.41)	21 (9.4)	0.11 (0.09,0.14)
Parity				
0	82 (33.2)	0.50 (0.46,0.54)	81 (33.3)	0.15 (0.13,0.17)
≥ 1	165 (66.8)	0.49 (0.46,0.52)	162 (66.7)	0.16 (0.14,0.17)
Smoking during pregnancy				
No	236 (95.6)	0.50 (0.48,0.52) **	232 (95.5)	0.16 (0.14,0.17)
Yes	11 (4.4)	0.36 (0.24,0.47)	11 (4.5)	0.13 (0.09,0.19)
Gestational anemia[d]				
No	124 (80.5)	0.52 (0.48,0.56)	120 (80.0)	0.17 (0.15,0.19)
Yes	30 (19.5)	0.53 (0.47,0.58)	30 (20.0)	0.14 (0.12,0.15)
Maternal depression at 7-year visit[d]				
No	151 (78.2)	0.50 (0.47,0.53)	148 (78.0)	0.16 (0.14,0.17)
Yes	42 (21.8)	0.53 (0.46,0.61)	41 (22.0)	0.17 (0.13,0.21)
Maternal language at 7-year visit interview[d]				
Spanish	174 (90.2)	0.52 (0.50,0.55) **	170 (90.0)	0.16 (0.15,0.18) *
English	19 (9.8)	0.36 (0.30,0.41)	19 (10.0)	0.12 (0.09,0.17)
Maternal depression at 9-year visit[d]				
No	174 (73.4)	0.49 (0.46,0.51)	172 (73.8)	0.15 (0.14,0.16)
Yes	63 (26.6)	0.52 (0.46,0.57)	61 (26.2)	0.17 (0.14,0.20)

Maternal language at 9-year visit interview[d]						
Spanish	211 (89.0)	0.51 (0.49,0.54)	**	211 (89.0)	0.16 (0.15,0.17)	*
English	26 (11.0)	0.37 (0.33,0.42)		26 (11.0)	0.13 (0.10,0.16)	
Maternal language at 10.5-year visit interview[d]						
Spanish	208 (89.7)	0.36 (0.31,0.41)	**	204 (89.5)	0.13 (0.10,0.16)	*
English	24 (10.3)	0.51 (0.48,0.53)		24 (10.5)	0.16 (0.15,0.17)	
Child characteristics						
Child's sex						
Boy	108 (43.7)	0.49 (0.46,0.52)		105 (43.2)	0.15 (0.13,0.17)	
Girl	139 (56.3)	0.50 (0.47,0.53)		138 (56.8)	0.16 (0.15,0.18)	
Birth weight						
< 2,500 grams	13 (5.3)	0.42 (0.31,0.54)		13 (5.4)	0.13 (0.09,0.18)	
≥ 2,500 grams	234 (94.7)	0.50 (0.48,0.52)		230 (94.6)	0.16 (0.14,0.17)	
Preterm birth						
< 37 weeks	21 (8.5)	0.43 (0.35,0.51)		21 (8.6)	0.15 (0.11,0.20)	
≥ 37 weeks	226 (91.5)	0.50 (0.48,0.53)		222 (91.4)	0.16 (0.14,0.17)	
Child’s language of assessment at 7 years[d]						
Spanish	134 (69.4)	0.54 (0.50,0.57)	**	131 (69.3)	0.17 (0.15,0.19)	
English	59 (30.6)	0.44 (0.40,0.49)		58 (30.7)	0.14 (0.12,0.17)	
Child’s language of assessment at 9 years[d]						
Spanish	114 (48.1)	0.53 (0.49,0.57)	**	112 (48.1)	0.17 (0.15,0.19)	**
English	123 (51.9)	0.47 (0.44,0.49)		121 (51.9)	0.14 (0.13,0.16)	
Child’s language of assessment at 10.5 years[d]						
Spanish	156 (67.2)	0.48 (0.45,0.51)		153 (67.1)	0.15 (0.14,0.17)	
English	76 (32.8)	0.52 (0.47,0.56)		75 (32.9)	0.16 (0.14,0.18)	
Household characteristics						
Family income at 7 years[d]						
< Poverty level	136 (70.5)	0.52 (0.49,0.55)		133 (70.4)	0.16 (0.14,0.18)	
Within 200% of poverty level	57 (29.5)	0.48 (0.44,0.51)		56 (29.6)	0.15 (0.14,0.18)	
Family income at 9 years[d]						
< Poverty level	169 (71.3)	0.50 (0.47,0.53)		167 (71.7)	0.16 (0.15,0.18)	
Within 200% of poverty level	68 (28.7)	0.48 (0.44,0.52)		66 (28.3)	0.14 (0.13,0.17)	
Family income at 10.5 years[d]						
< Poverty level	165 (71.1)	0.50 (0.48,0.53)		162 (71.0)	0.16 (0.15,0.18)	
Within 200% of poverty level	67 (28.9)	0.46 (0.43,0.50)		66 (29.0)	0.14 (0.12,0.16)	

Abbreviations: AUC, area under the curve; GM, geometric mean; CI, confidence interval; PPVT, Peabody Picture Vocabulary Test.

[a] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had prenatal dentine Mn levels measured in shed incisors.

[b] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had postnatal dentine Mn levels measured in shed incisors.

[c] Analyzed as continuous variable in multivariable models.

[d] Information was missing for several mother-child pairs with prenatal dentine Mn measurements (*n* = 93 for gestational anemia, *n* = 54 for maternal depression at the 7-year visit, *n* = 54 for maternal language at the 7-year visit interview, *n* = 10 for maternal depression at the 9-year visit, *n* = 10 for maternal language at the 9-year visit interview, *n* = 15 for maternal language at the 10.5-year visit interview, *n* = 54 for child’s language of assessment at 7 years, *n* = 10 for child’s language of assessment at 9 years, *n* = 10 for child’s language of assessment at 10.5 years, *n* = 54 for family income at the 7-year visit, *n* = 10 for family income at the 9-year visit, *n* = 15 for family income at the 10.5-year visit).

*p < 0.10, **p < 0.05; p-values are for one-way ANOVAs (prenatal Mn levels) or Mann-Whitney tests (postnatal Mn levels) across the different categories of each characteristic.

Table 2. Adjusted linear models for attention-related outcome scores in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4).

Outcomes	**Prenatal Mn**		**Postnatal Mn**	
	n	**β (95% CI)**	***n***	**β (95% CI)**
7-year assessment				
CADS - Maternal report (T-scores)[a]				
ADHD Index	198	-0.9 (-6.9,5.0)	190	0.6 (-0.6,1.8)
Inattentive subscale	198	-3.8 (-9.5,1.9)	190	0.1 (-1.1,1.2)
Hyperactive/Impulsive subscale	198	-1.7 (-7.5,4.1)	190	0.1 (-1.0,1.3)
BASC-2 - Maternal report (T-scores)[a]				
Internalizing problems	193	3.1 (-4.0,10.2)[#]	185	0.9 (-0.4,2.3)
Externalizing problems	193	0.7 (-5.5,6.9)	185	0.8 (-0.4,2.0)
Hyperactivity	193	3.5 (-2.6,9.6)	185	1.1 (-0.1,2.3)
Attention problems	193	1.6 (-7.0,10.2)	185	0.0 (-1.7,1.7)
CADS - Teacher report (T-scores)[b]				
ADHD Index	170	-1.4 (-12.2,9.3)	164	0.2 (-1.9,2.4)
Inattentive subscale	173	2.8 (-5.2,10.8)	167	0.7 (-0.9,2.3)
Hyperactive/Impulsive subscale	173	-3.2 (-12.4,6.1)	167	0.0 (-1.8,1.9)
BASC-2 - Teacher report (T-scores)[b]				
Internalizing problems	173	-8.9 (-19.4,1.7)	167	-1.3 (-3.4,0.8)
Externalizing problems	173	-1.0 (-9.2,7.2)	167	0.3 (-1.4,1.9)
Hyperactivity	173	-3.2 (-12.1,5.6)	167	0.1 (-1.7,1.8)
Attention problems	173	-1.4 (-8.4,5.6)	167	0.2 (-1.2,1.6)
9-year assessment				
CADS - Maternal report (T-scores)[a]				
ADHD Index	242	0.2 (-6.6,6.9)	234	-0.3 (-1.7,1.1)
Inattentive subscale	241	0.7 (-5.9,7.3)	233	-0.3 (-1.7,1.1)
Hyperactive/Impulsive subscale	241	4.0 (-3.7,11.8)	233	-0.4 (-2.0,1.2)
CPT-II (T-scores)[c]				
Errors of omission	237	-3.7 (-16.5,9.2)	229	-0.7 (-3.4,2.1)
Errors of commission	237	-5.8 (-13.0,1.3)	229	0.3 (-1.3,1.8)
ADHD Confidence index	237	-6.5 (-22.9,10.0)	229	-0.5 (-4.0,3.0)
10.5-year assessment				
BASC-2 - Maternal report (T-scores)[a]				
Internalizing problems	231	3.5 (-2.9,9.9)	223	-0.3 (-1.7,1.0)
Externalizing problems	226	2.2 (-3.2,7.5)	219	0.2 (-1.0,1.4)
Hyperactivity	231	1.3 (-4.1,6.7)	223	-0.2 (-1.4,0.9)
Attention problems	231	-1.0 (-8.8,6.8)	223	-0.3 (-1.9,1.4)
BASC-2 - Self-report (T-scores)[c]				
Hyperactivity	227	-2.9 (-10.0,4.1)	219	-0.5 (-2.0,1.0)
Attention problems	224	-3.9 (-11.2,3.4)	216	-0.4 (-1.9,1.1)

Abbreviations: CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; CPT-II, Continuous Performance Test 2nd edition.

[a] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at maternal interview, language of maternal interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment..

[b] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at interview, HOME z-score at time of

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assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[c] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

$^{**}p_{\text{Linear}} < 0.05$, $^{\#}p_{\text{GAM}} < 0.05$

Table 3. Adjusted linear models for cognition, memory, and motor outcomes in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^{4}).

Outcomes	**Prenatal Mn**		**Postnatal Mn**	
	n	**β (95% CI)**	***n***	**β (95% CI)**
Cognition				
7-year assessment				
WISC-IV Full-Scale IQ (scaled scores)	175	2.2 (-9.6,14.0)	167	0.9 (-1.4,3.2)
Verbal Comprehension IQ	193	2.5 (-8.3,13.2)	185	0.3 (-1.9,2.4)
Perceptual Reasoning IQ	193	7.7 (-5.8,21.3)	185	1.7 (-1.0,4.4)
Working Memory IQ	176	1.2 (-10.2,12.5)	168	1.0 (-1.3,3.2)
Processing Speed IQ	176	4.6 (-6.2,15.4)	168	2.0 (-0.1,4.1)
10.5-year assessment				
WISC-IV Full-Scale IQ (scaled scores)	230	1.6 (-6.2,9.5)	222	1.4 (-0.2,3.1)
Verbal Comprehension IQ	232	-3.2 (-11.2,4.9)	224	0.2 (-1.5,1.8)
Perceptual Reasoning IQ	232	7.2 (-3.8,18.2)$^{\#}$	224	2.5 (0.2,4.8) **
Working Memory IQ	232	3.2 (-4.5,10.8)	224	1.6 (0,3.2)
Processing Speed IQ	232	-1.0 (-10.0,8.0)	224	0.2 (-1.7,2.1)
Memory				
9-year assessment				
NEPSY-II Memory for Designs (scaled scores)				
Immediate Total	184	1.5 (-1.2,4.3)	176	0.7 (0.1,1.2) **
Delayed Total	185	2.1 (-0.6,4.9)	177	0.8 (0.3,1.3) **
10.5-year assessment				
CAVLT-2 (z-scores)				
Immediate recall	232	-0.7 (-13.4,12.0)$^{\#}$	224	1.1 (-1.5,3.8)
Delayed recall	232	9.0 (-3.2,21.2)	224	2.0 (-0.4,4.5)
Recognition accuracy	232	0.6 (-1.6,2.8)	224	0.3 (-0.2,0.8)
Immediate memory span	232	-4.4 (-16.2,7.3)	224	-0.3 (-2.8,2.1)
Level of learning	232	6.6 (-4.5,17.7)$^{\#}$	224	1.9 (-0.4,4.2)
Motor function				
7-year assessment				
WRAVMA Pegboard (scaled scores)				
Dominant hand	193	6.0 (-8.1,20.1)	185	-0.2 (-3.0,2.6)
Non-dominant hand	193	4.5 (-10.0,18.9)	185	-0.5 (-3.3,2.3)
Finger Tap (z-scores)				
Dominant hand	193	0.6 (-0.2,1.4)	185	0.2 (0,0.3) **
Non-dominant hand	193	0.2 (-0.6,1.0)	185	0.1 (0,0.3)
9-year assessment				
Luria-Nebraska Motor Scale (z-scores)				
All items	226	0.0 (-0.6,0.7)	218	0.0 (-0.1,0.1)
5-item sum	226	0.0 (-0.7,0.6)	218	0.0 (-0.2,0.1)
10.5-year assessment				
Luria-Nebraska Motor Scale (z-scores)				
All items	232	0.2 (-0.2,0.7)	224	0.0 (-0.1,0.1)
5-item sum	232	0.5 (-0.2,1.2)	224	0.1 (-0.1,0.2)

Abbreviations: CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Ability.

Models adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

$^{**}p_{Linear} < 0.05$, $^{\#}p_{GAM} < 0.05$

Table 4. Generalized estimating equation models for attention-related, cognition, and motor outcomes in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4).

Outcomes	Prenatal Mn			Postnatal Mn		
	n	*k*	β (95% CI)	*n*	*k*	β (95% CI)
Attention-related outcomes						
CADS - Maternal report (T-scores)[a, b]						
ADHD Index	440	250	0.3 (-5.0,5.6)	424	242	0.0 (-1.1,1.1)
Inattentive subscale	439	250	-0.6 (-5.6,4.5)	423	242	-0.2 (-1.2,0.9)
Hyperactive/Impulsive subscale	439	250	1.8 (-3.8,7.5)	423	242	-0.2 (-1.4,0.9)
BASC-2 - Maternal report (T-scores)[a, c]						
Internalizing problems	424	244	4.1 (-1.5,9.7)	408	236	0.3 (-0.8,1.5)
Externalizing problems	419	243	1.5 (-3.2,6.2)	404	235	0.4 (-0.6,1.3)
Hyperactivity	424	244	2.0 (-2.5,6.6)	408	236	0.3 (-0.6,1.3)
Attention problems	424	244	0.9 (-5.7,7.4)	408	236	-0.2 (-1.5,1.2)
Cognitive outcomes						
WISC-IV Full-Scale IQ (Scaled scores)[d, c]	407	242	2.9 (-4.9,10.7)	391	234	1.6 (0.0,3.2)
Verbal Comprehension IQ	425	244	0.8 (-6.6,8.2)	409	236	0.6 (-1.0,2.1)
Perceptual Reasoning IQ	425	244	7.5 (-2.5,17.5)	409	236	2.3 (0.2,4.4)**
Working Memory IQ	408	242	2.1 (-5.2,9.4)	392	234	1.4 (-0.1,2.9)
Processing Speed IQ	408	242	0.9 (-6.7,8.5)	392	234	0.9 (-0.7,2.4)
Motor outcomes						
Luria-Nebraska Motor Scale (z-scores)[d, e]						
All items	458	238	0.1 (-0.3,0.6)	442	230	0.0 (-0.1,0.1)
5-item sum	458	238	0.2 (-0.3,0.8)	442	230	0.0 (-0.1,0.1)

Abbreviations: *n*, number of observations; *k*, number of children; CI, confidence interval; CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient.

[a] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at maternal interview, language of maternal interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[b] Outcomes measured at 7 and 9 years.

[c] Outcomes measured at 7 and 10.5 years.

[d] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician at assessment.

[e] Outcomes measured at 9 and 10.5 years.

**$p_{Linear} < 0.05$

6. Supporting information

Table S1. Distribution of Mn levels in teeth dentine (^{55}Mn:^{43}Ca AUC x 10^4), CHAMACOS Study, Salinas, California.

Biomarkers	***n***	**Mean ± SD**	**GM (GSD)**	**Min**	**Percentile**			**Max**
					25th	**50th**	**75th**	
Prenatal Mn	247	0.50 ± 0.18	0.46 (1.48)	0.07	0.38	0.49	0.57	1.34
Postnatal Mn	243	0.19 ± 0.21	0.15 (1.82)	0.00	0.11	0.14	0.20	2.50

Abbreviations: AUC, area under the curve; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation.

Table S2. Means and standard deviations for behavioral, cognitive, memory, and motor outcomes in children at 7, 9, and 10.5 years.

Outcome	***n***[a]	**Mean ± SD**
7-year assessment		
Behavioral outcomes		
CADS - Maternal Report (T-scores)		
ADHD Index	198	49.5 ± 7.7
Inattentive subscale	198	48.6 ± 7.4
Hyperactive/Impulsive subscale	198	51.0 ± 7.8
BASC-2 - Maternal Report (T-scores)		
Internalizing problems	193	48.5 ± 9.7
Externalizing problems	193	43.9 ± 8.2
Hyperactivity	193	44.9 ± 8.1
Attention problems	193	49.4 ± 10.6
CADS - Teacher Report (T-scores)		
ADHD Index	170	53.4 ± 11.6
Inattentive subscale	173	48.3 ± 8.9
Hyperactive/Impulsive subscale	173	52.0 ± 10.1
BASC-2 - Teacher Report (T-scores)		
Internalizing problems	173	50.2 ± 11.9
Externalizing problems	173	48.6 ± 9.2
Hyperactivity	173	49.0 ± 10.0
Attention problems	173	51.0 ± 7.8
Cognitive Outcomes		
WISC-IV Full-Scale IQ (scaled scores)	175	104.1 ± 14.1
Verbal Comprehension IQ	193	106.8 ± 16.7
Perceptual Reasoning IQ	193	102.1 ± 16.8
Working Memory IQ	176	93.4 ± 13.0
Processing Speed IQ	176	109.0 ± 12.8
Motor Outcomes		
WRAVMA Pegboard (scaled scores)		
Dominant hand	193	120.4 ± 17.0
Non-dominant hand	193	122.6 ± 17.5
Finger Tap (raw scores)[b]		
Dominant hand	193	32.4 ± 6.2
Non-dominant hand	193	28.6 ± 5.7
9-year assessment		
Behavioral Outcomes		
CADS - Maternal Report (T-scores)		
ADHD Index	242	51.1 ± 9.3
Inattentive subscale	241	49.7 ± 9.0
Hyperactive/Impulsive subscale	241	53.5 ± 10.5
CPT-II (T-scores)		
Errors of omission	237	58.1 ± 16.8
Errors of commission	237	49.7 ± 9.3
ADHD Confidence index	237	52.4 ± 22.1

Memory Outcomes		
NEPSY-II Memory for Designs (scaled scores)		
Immediate Total	184	8.8 ± 3.3
Delayed Total	185	9.5 ± 3.2
Motor Outcomes		
Luria-Nebraska Motor Scale (raw scores)[b]		
All items	226	54.8 ± 9.0
5-item sum	226	41.6 ± 7.5
10.5-year assessment		
Behavioral outcomes		
BASC-2 - Maternal Report (T-scores)		
Internalizing problems	231	48.2 ± 8.7
Externalizing problems	226	45.7 ± 7.3
Hyperactivity	231	46.2 ± 7.5
Attention problems	231	48.6 ± 10.6
BASC-2 - Self-report (sex-standardized T-scores)		
Hyperactivity	227	46.8 ± 9.1
Attention problems	224	48.8 ± 9.1
Cognitive Outcomes		
WISC-IV Full-Scale IQ (scaled scores)	230	90.7 ± 10.5
Verbal Comprehension IQ	232	84.8 ± 11.5
Perceptual Reasoning IQ	232	93.2 ± 14.3
Working Memory IQ	232	97.0 ± 10.3
Processing Speed IQ	232	99.2 ± 12.1
Memory Outcomes		
CAVLT-2 (Standardized scores)		
Immediate recall	232	98.9 ± 16.6
Delayed recall	232	96.2 ± 16.0
Recognition accuracy	232	29.1 ± 2.9
Immediate memory span	232	89.5 ± 15.2
Level of learning	232	96.7 ± 14.7
Motor Outcomes		
Luria-Nebraska Motor Scale (raw scores)[b]		
All items	232	48.3 ± 8.9
5-item sum	232	35.1 ± 7.2

Abbreviations: SD, standard deviation; CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; IQ, intellectual quotient; WRAVMA, Wide Range Assessment of Visual Motor Ability; CPT-II, continuous performance test 2nd edition; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition.

[a] Children who completed the 7-, 9-, or 10.5-year neurobehavioral assessment and had dentine Mn levels measured in shed incisors.

[b] For statistical analysis, these scores were converted to z-scores for the CHAMACOS population.

Table S3. Adjusted linear models for attention-related outcome scores in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by prenatal lead exposure (maternal blood lead levels during pregnancy < 2 vs. ≥ 2 µg/dL).

Outcome	Prenatal Mn					Postnatal Mn				
	Lower lead exposure		Higher lead exposure		p_{INT}	Lower lead exposure		Higher lead exposure		p_{INT}
	n	β (95% CI)	*n*	β (95% CI)		*n*	β (95% CI)	*n*	β (95% CI)	
7-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	139	-0.8 (-8.8,7.2)	31	6.9 (-30.7,44.5)	0.78	135	1.0 (-0.6,2.5)	30	3.7 (-1.6,9.0)	0.43
Inattentive subscale	139	-4.9 (-12.8,3.0)	31	0.4 (-34.2,34.9)	0.59	135	0.3 (-1.2,1.8)	30	1.8 (-3.4,6.9)	0.50
Hyperactive/Impulsive subscale	139	-0.4 (-8.7,8.0)	31	1.0 (-24.7,26.6)	0.94	135	0.6 (-1.0,2.1)	30	1.5 (-2.2,5.2)	0.53
BASC-2 - Maternal report (T-scores)[a]										
Internalizing problems	136	-3.0 (-12.6,6.6)	29	33.1 (-2.6,68.8)	**0.01**	132	0.8 (-1.0,2.6)	28	2.1 (-3.0,7.2)	0.16
Externalizing problems	136	2.5 (-5.7,10.8)	29	17.3 (-18.1,52.6)	0.56	132	1.2 (-0.3,2.7)	28	4.0 (-0.3,8.3)	0.13
Hyperactivity	136	4.0 (-4.4,12.3)	29	24.7 (-16.2,65.6)	0.53	132	1.6 (0.1,3.2) **	28	4.6 (-0.5,9.7)	0.26
Attention problems	136	4.9 (-6.7,16.4)	29	-44.3 (-86.5,-2.0) **	0.39	132	0.8 (-1.4,3.0)	28	1.3 (-5.1,7.7)	0.77
CADS - Teacher report (T-scores)[b]										
ADHD Index	118	1.5 (-11.8,14.7)	26	-28.2 (-105.1,48.7)	0.88	115	0.2 (-2.4,2.9)	25	-1.1 (-10.7,8.6)	0.32
Inattentive subscale	120	5.6 (-3.8,15)	27	-29.9 (-79.5,19.7)	0.70	117	1.1 (-0.8,3.0)	26	-1.1 (-7.4,5.2)	0.31
Hyperactive/Impulsive subscale	120	-1.4 (-12.8,10.1)	27	-49.0 (-124.8,26.9)	0.93	117	-0.2 (-2.4,2.1)	26	-1.7 (-11.5,8.1)	0.76
BASC-2 - Teacher report (T-scores)[b]										
Internalizing problems	120	-7.4 (-20.9,6.0)	27	-51.2 (-116.2,13.7)	0.41	117	-1.6 (-4.3,1.1)	26	-1.0 (-9.2,7.2)	0.66
Externalizing problems	120	3.1 (-7.3,13.6)	27	-30.3 (-79.5,18.8)	0.96	117	0.4 (-1.7,2.5)	26	-0.6 (-6.9,5.7)	0.90
Hyperactivity	120	-0.3 (-11.9,11.3)	27	-43.2 (-95.3,9.0)	0.96	117	-0.2 (-2.5,2.2)	26	0.8 (-6.3,7.8)	0.91
Attention problems	120	0.1 (-8.4,8.6)	27	-16.0 (-63.9,31.9)	0.88	117	0.1 (-1.6,1.9)	26	-0.4 (-6.2,5.5)	0.50
9-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	136	-6.1 (-17.1,4.9)	30	2.3 (-16.5,21.2)	0.27	132	-0.1 (-2.1,1.9)	29	1.3 (-2.5,5.0)	0.71
Inattentive subscale	135	-8.4 (-19.3,2.5)	30	3.6 (-12.8,20.1)	0.13	131	-0.5 (-2.5,1.5)	29	1.9 (-1.5,5.3)	0.33
Hyperactive/Impulsive subscale	135	1.8 (-11,14.5)	30	10.4 (-16.1,36.8)	0.42	131	-0.9 (-3.1,1.3)	29	3.9 (-1.6,9.3)	**0.05**
CPT-II (T-scores)[c]										
Errors of omission	131	-10.0 (-32.7,12.7)	30	-18.1 (-56.0,19.7)	0.41	127	-1.2 (-5.4,3.0)	29	-4.3 (-12.4,3.7)	0.32
Errors of commission	131	-2.2 (-14.3,9.9)	30	-3.7 (-25.5,18.1)	0.61	127	0.5 (-1.7,2.7)	29	0.5 (-3.9,4.8)	0.38
ADHD Confidence index	131	-17.3 (-44.1,9.5)	30	-9.7 (-62.6,43.2)	0.67	127	-0.3 (-5.3,4.6)	29	-1.4 (-12.8,10)	0.41

10.5-year assessment										
BASC-2 - Maternal report (T-scores)[a]										
Internalizing problems	130	-1.2 (-10.7,8.3)	28	-1.7 (-26.7,23.3)	0.25	126	-0.1 (-1.8,1.7)	27	-1.0 (-4.9,2.8)	0.82
Externalizing problems	128	5.6 (-2.5,13.8)	27	-5.6 (-24.5,13.3)	0.54	125	0.5 (-1.0,1.9)	26	0.7 (-2.3,3.6)	0.99
Hyperactivity	130	5.5 (-2.3,13.3)	28	1.5 (-22.9,25.9)	0.42	126	0.4 (-1.0,1.8)	27	2.0 (-1.6,5.6)	0.86
Attention problems	130	1.7 (-10.6,14.0)	28	-9.0 (-40.2,22.1)	0.46	126	1.2 (-1.0,3.5)	27	-1.6 (-6.4,3.3)	**0.07**
BASC-2 - Self-report (T-scores)[c]										
Hyperactivity	129	-5.3 (-16.3,5.8)	26	-10.9 (-59.8,38.0)	0.73	125	-0.2 (-2.2,1.9)	25	-0.1 (-5.6,5.4)	0.72
Attention problems	125	-0.7 (-11.6,10.2)	27	12.5 (-32.3,57.4)	0.49	121	0.5 (-1.5,2.5)	26	0.6 (-6.2,7.5)	0.36

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; CPT-II, Continuous Performance Test 2nd edition.

[a] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at maternal interview, language of maternal interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment..

[b] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[c] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear} < 0.05$

Table S4. Adjusted linear models for cognition, memory, and motor outcomes in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by prenatal lead exposure (maternal blood lead levels during pregnancy < 2 vs. ≥ 2 μg/dL).

	Prenatal Mn					**Postnatal Mn**				
Outcome	**Lower lead exposure**		**Higher lead exposure**		p_{INT}	**Lower lead exposure**		**Higher lead exposure**		p_{INT}
	n	**β (95% CI)**	***n***	**β (95% CI)**		***n***	**β (95% CI)**	***n***	**β (95% CI)**	
Cognition										
7-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	129	-6.2 (-21.1,8.6)	29	0.3 (-83.0,83.6)	0.28	125	-1.0 (-3.9,1.8)	28	3.5 (-6.3,13.3)	**0.03**
Verbal Comprehension IQ	136	-7.8 (-21.7,6.1)	29	-12.0 (-98.4,74.4)	0.30	132	-1.0 (-3.7,1.6)	28	-0.3 (-10.7,10.1)	0.15
Perceptual Reasoning IQ	136	-0.3 (-18.5,18.0)	29	-10.0 (-98.8,78.9)	0.93	132	0.0 (-3.5,3.5)	28	2.9 (-7.8,13.7)	0.32
Working Memory IQ	130	-10.1 (-24.8,4.7)	29	15.9 (-45.1,76.9)	**0.07**	126	-0.5 (-3.4,2.3)	28	3.9 (-3.1,10.9)	**0.06**
Processing Speed IQ	130	1.4 (-11.5,14.3)	29	24.7 (-63.4,112.7)	0.31	126	0.6 (-1.8,3.1)	28	5.4 (-3.2,14.0)	**0.05**
10.5-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	129	-3.7 (-16.0,8.6)	28	-22.1 (-87.9,43.7)	0.82	125	-0.3 (-2.5,1.9)	27	2.6 (-7.0,12.2)	0.46
Verbal Comprehension IQ	131	-10.9 (-23.3,1.5)	28	-13.6 (-71.4,44.1)	0.98	127	-0.5 (-2.8,1.7)	27	0.9 (-7.2,9.0)	0.67
Perceptual Reasoning IQ	131	7.2 (-10.7,25.1)	28	-41.0 (-115.8,33.7)	0.34	127	0.7 (-2.4,3.8)	27	1.5 (-9.9,13.0)	0.70
Working Memory IQ	131	-3.2 (-15.8,9.4)	28	-18.9 (-70.0,32.3)	0.63	127	0.4 (-1.9,2.7)	27	3.4 (-3.7,10.6)	0.16
Processing Speed IQ	131	-2.8 (-16.8,11.3)	28	13.4 (-61.9,88.6)	0.76	127	-1.5 (-4.0,1.0)	27	2.5 (-7.7,12.8)	0.22
Memory										
9-year assessment										
NEPSY-II Memory for Designs (scaled scores)										
Immediate Total	128	1.7 (-2.1,5.6)	30	-2.4 (-9.0,4.2)	0.59	124	0.6 (-0.1,1.3)	29	0.5 (-1.0,1.9)	0.95
Delayed Total	130	1.3 (-2.4,4.9)	29	0.8 (-8.2,9.8)	0.87	126	0.5 (-0.2,1.2)	28	1.4 (-0.1,3.0)	0.32
10.5-year assessment										
CAVLT-2 (z-scores)										
Immediate recall	131	-2.3 (-22.8,18.2)	28	-41.7 (-120.8,37.4)	0.78	127	0.1 (-3.7,3.8)	27	0.9 (-11.4,13.2)	0.71
Delayed recall	131	4.1 (-14.6,22.8)	28	-34.2 (-107.8,39.4)	0.87	127	0.9 (-2.3,4.1)	27	0.8 (-10.4,12)	0.53
Recognition accuracy	131	1.0 (-1.7,3.7)	28	-3.8 (-12.9,5.2)	0.96	127	0.3 (-0.2,0.8)	27	0.0 (-1.3,1.3)	0.85
Immediate memory span	131	-18.0 (-36.4,0.4)	28	32.9 (-24.9,90.7)	**0.06**	127	-1.8 (-5.1,1.6)	27	1.0 (-8.1,10.1)	0.16
Level of learning	131	-3.5 (-19.8,12.7)	28	-44.8 (-119.6,29.9)	0.20	127	0.3 (-2.7,3.2)	27	-2.9 (-14.5,8.8)	0.58
Motor function										
7-year assessment										
WRAVMA Pegboard (scaled scores)										
Dominant hand	136	3.3 (-14.6,21.2)	29	59.4 (-13.4,132.1)	0.33	132	-1.9 (-5.3,1.4)	28	7.6 (-1.1,16.3)	**0.02**
Non-dominant hand	136	-1.3 (-19.7,17.1)	29	72.2 (-12.3,156.6)	0.21	132	-2.5 (-5.9,0.9)	28	9.5 (-0.2,19.3)	**0.01**

Finger Tap (z-scores)									
Dominant hand	136	0.1 (-0.9,1.2)	29	1.8 (-4.4,8.0)	0.35	132	0.1 (-0.1,0.3)	28	0.6 (-0.1,1.2)	**0.01**
Non-dominant hand	136	-0.2 (-1.3,0.8)	29	2.2 (-2.9,7.3)	0.42	132	0.1 (-0.1,0.3)	28	0.3 (-0.2,0.9)	0.10
9-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	128	-0.5 (-1.5,0.6)	30	1.4 (-0.5,3.3)	0.37	124	-0.1 (-0.3,0.1)	29	0.2 (-0.2,0.6)	0.55
5-item sum	128	-0.6 (-1.7,0.4)	30	1.4 (-0.7,3.5)	0.22	124	-0.1 (-0.3,0.1)	29	0.2 (-0.3,0.6)	0.54
10.5-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	131	0.2 (-0.5,1.0)	28	0.5 (-3.0,3.9)	0.66	127	0.1 (-0.1,0.2)	27	0.1 (-0.4,0.6)	0.65
5-item sum	131	0.1 (-1.0,1.2)	28	0.8 (-5.4,7.0)	0.90	127	0.0 (-0.2,0.2)	27	0.1 (-0.8,1.0)	0.87

Abbreviations: AUC, area under the curve; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Ability.

Models adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear} < 0.05$

Table S5. Adjusted linear models for attention-related outcome scores in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by gestational anemia (maternal hemoglobin levels < 11 vs. ≥ 11 g/dL).

Outcome	Prenatal Mn					Postnatal Mn				
	No gestational anemia		Gestational anemia		p_{INT}	No gestational anemia		Gestational anemia		p_{INT}
	n	**β (95% CI)**	***n***	**β (95% CI)**		***n***	**β (95% CI)**	***n***	**β (95% CI)**	
7-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	123	-1.7 (-9.9,6.5)	30	-11.2 (-26.4,4.1)	0.88	117	0.9 (-1.0,2.8)	30	0.0 (-1.9,1.8)	0.69
Inattentive subscale	123	-3.8 (-11.3,3.7)	30	-9.6 (-26.8,7.5)	0.75	117	0.4 (-1.4,2.1)	30	0.5 (-1.5,2.5)	0.96
Hyperactive/Impulsive subscale	123	-2.3 (-9.5,4.9)	30	-1.6 (-23.3,20.1)	0.82	117	0.2 (-1.4,1.8)	30	-1.7 (-3.8,0.5)	0.37
BASC-2 - Maternal report (T-scores)[a]										
Internalizing problems	121	5.0 (-3.9,13.8)	30	-10.8 (-39.1,17.5)	0.24	115	1.8 (-0.1,3.8)	30	0.1 (-3.1,3.3)	0.19
Externalizing problems	121	-1.3 (-9.3,6.6)	30	2.5 (-17.7,22.6)	0.98	115	1.0 (-0.7,2.8)	30	0.5 (-1.8,2.7)	0.71
Hyperactivity	121	3.6 (-4.0,11.2)	30	-4.1 (-27.3,19.2)	0.32	115	1.7 (0.1,3.4) **	30	0.0 (-2.6,2.6)	0.18
Attention problems	121	0.9 (-10.1,11.9)	30	9.3 (-13.5,32.0)	0.96	115	1.0 (-1.5,3.4)	30	-0.1 (-2.7,2.5)	0.42
CADS - Teacher report (T-scores)[b]										
ADHD Index	104	-2.0 (-15.3,11.4)	26	-16.6 (-72.5,39.2)	0.87	100	-0.2 (-3.2,2.8)	26	1.0 (-5.2,7.1)	0.85
Inattentive subscale	105	1.8 (-8.5,12.1)	27	-4.6 (-42.4,33.3)	0.97	101	0.9 (-1.4,3.3)	27	0.0 (-4.0,4.1)	0.63
Hyperactive/Impulsive subscale	105	-5.4 (-16.4,5.7)	27	-2.3 (-58.7,54.1)	0.95	101	-1.2 (-3.7,1.3)	27	0.8 (-5.2,6.8)	0.40
BASC-2 - Teacher report (T-scores)[b]										
Internalizing problems	105	-10.2 (-24.1,3.6)	27	-29.6 (-97.0,37.9)	0.84	101	-2.4 (-5.5,0.8)	27	-1.3 (-8.8,6.2)	0.53
Externalizing problems	105	-4.5 (-14.6,5.6)	27	-3.3 (-53.8,47.2)	0.51	101	-1.3 (-3.5,1.0)	27	1.0 (-4.4,6.3)	0.25
Hyperactivity	105	-6.9 (-17.4,3.6)	27	-0.6 (-62.8,61.6)	0.55	101	-1.3 (-3.7,1.1)	27	1.9 (-4.6,8.4)	0.28
Attention problems	105	-0.9 (-9.5,7.6)	27	-11.1 (-53.9,31.7)	0.81	101	0.4 (-1.5,2.4)	27	0.9 (-3.7,5.5)	0.99
9-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	121	-4.1 (-14.0,5.8)	29	-6.0 (-32.9,20.8)	0.83	115	-0.2 (-2.4,2.1)	29	-0.3 (-3.3,2.7)	0.71
Inattentive subscale	121	-2.4 (-11.7,6.9)	28	-11.0 (-43.0,20.9)	0.68	115	-0.2 (-2.3,1.9)	28	-1.5 (-5.1,2.1)	0.65
Hyperactive/Impulsive subscale	121	1.7 (-8.9,12.4)	28	12.2 (-20.7,45.1)	0.94	115	-0.2 (-2.5,2.1)	28	0.8 (-3.0,4.7)	0.23
CPT-II (T-scores)[c]										
Errors of omission	119	-16.0 (-34.3,2.2)	27	50.4 (-42.3,143.1)	0.16	113	-2.9 (-7.1,1.3)	27	-3.3 (-12.7,6.1)	0.46
Errors of commission	119	-8.0 (-17.7,1.7)	27	93.3 (18.7,167.9) **	**0.08**	113	-1.3 (-3.5,0.9)	27	5.2 (-4.4,14.8)	**0.03**
ADHD Confidence index	119	-22.3 (-44.6,-0.1) **	27	40.0 (-36.7,116.6)	0.19	113	-2.6 (-7.7,2.4)	27	0.8 (-7.3,8.8)	0.56

10.5-year assessment									
BASC-2 - Maternal report (T-scores)[a]									
Internalizing problems	117	2.9 (-5.4,11.2)	25	-3.5 (-54.3,47.3)	0.40	111	0.5 (-1.4,2.3)	25	-1.7 (-7.6,4.2)	**0.03**
Externalizing problems	115	0.4 (-7.2,8.0)	24	19.7 (-14.0,53.4)	0.34	110	-0.2 (-1.9,1.5)	24	3.5 (-0.7,7.6)	0.72
Hyperactivity	117	-0.8 (-8.2,6.7)	25	14.0 (-10.1,38.0)	1.00	111	-0.3 (-1.9,1.4)	25	1.3 (-1.7,4.3)	0.44
Attention problems	117	-2.2 (-13.0,8.6)	25	-24.5 (-69.5,20.5)	0.57	111	0.1 (-2.3,2.6)	25	-2.1 (-7.8,3.6)	0.27
BASC-2 - Self-report (T-scores)[c]									
Hyperactivity	114	-5.6 (-15.0,3.8)	26	25.4 (-20.5,71.3)	0.98	108	-0.6 (-2.7,1.5)	26	2.6 (-2.5,7.7)	0.90
Attention problems	111	-2.1 (-12.4,8.3)	26	0.3 (-25.1,25.8)	0.60	105	0.0 (-2.2,2.2)	26	0.3 (-2.5,3.0)	0.78

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; CPT-II, Continuous Performance Test 2nd edition.

[a] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at maternal interview, language of maternal interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[b] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[c] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear} < 0.05$

Table S6. Adjusted linear models for cognition, memory, and motor outcomes in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by gestational anemia (maternal hemoglobin levels < 11 vs. ≥ 11 g/dL).

Outcome	Prenatal Mn					Postnatal Mn				
	No gestational anemia		Gestational anemia		p_{INT}	No gestational anemia		Gestational anemia		p_{INT}
	n	**β (95% CI)**	*n*	**β (95% CI)**		*n*	**β (95% CI)**	*n*	**β (95% CI)**	
Cognition										
7-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	110	0.4 (-12.8,13.5)	27	60.9 (2.5,119.3) **	**0.07**	104	-0.4 (-3.3,2.5)	27	1.3 (-7.1,9.7)	0.28
Verbal Comprehension IQ	121	3.2 (-9.2,15.7)	30	38.4 (-16.2,93.0)	0.38	115	0.3 (-2.5,3.1)	30	-0.9 (-7.8,6.0)	0.93
Perceptual Reasoning IQ	121	6.2 (-10.4,22.7)	30	53.3 (-2.7,109.3)	**0.09**	115	0.6 (-3.1,4.3)	30	-1.4 (-9.0,6.1)	0.51
Working Memory IQ	110	-4.0 (-16.6,8.6)	27	40.0 (-29.4,109.4)	0.34	104	-0.4 (-3.2,2.4)	27	-0.9 (-9.5,7.7)	0.80
Processing Speed IQ	111	9.2 (-3.4,21.8)	27	55.2 (6.0,104.3) **	0.15	105	0.9 (-2.0,3.7)	27	6.2 (0.5,11.9) **	**0.02**
10.5-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	116	-0.3 (-11.4,10.7)	25	37.9 (-65.6,141.5)	0.28	110	-0.1 (-2.4,2.3)	25	1.4 (-6.9,9.7)	0.45
Verbal Comprehension IQ	117	-8.1 (-18.8,2.6)	26	15.3 (-73.2,103.8)	0.99	111	-0.8 (-3.1,1.5)	26	-4.0 (-13.0,5.0)	0.54
Perceptual Reasoning IQ	117	4.6 (-10.9,20.0)	26	92.9 (24.5,161.3) **	**0.03**	111	-0.3 (-3.6,3.0)	26	5.4 (-6.0,16.8)	**0.09**
Working Memory IQ	117	4.7 (-6.0,15.4)	26	45.0 (-24.5,114.4)	0.66	111	1.0 (-1.3,3.3)	26	4.2 (-3.8,12.2)	0.82
Processing Speed IQ	117	0.0 (-12.2,12.2)	26	49.3 (-26.8,125.3)	0.21	111	0.4 (-2.3,3.1)	26	3.7 (-5.5,12.9)	0.33
Memory										
9-year assessment										
NEPSY-II Memory for Designs (scaled scores)										
Immediate Total	116	1.1 (-2.2,4.4)	27	6.9 (-14.5,28.4)	**0.08**	110	0.4 (-0.3,1.2)	27	1.4 (-0.3,3.1)	0.11
Delayed Total	118	1.6 (-1.7,4.8)	26	12.7 (-53.4,78.8)	0.14	112	0.7 (-0.1,1.4)	26	2.1 (-0.9,5.1)	0.24
10.5-year assessment										
CAVLT-2 (z-scores)										
Immediate recall	117	2.4 (-14.5,19.2)	26	57.4 (-56.3,171.1)	0.66	111	1.0 (-2.7,4.6)	26	3.4 (-10.1,16.9)	0.72
Delayed recall	117	13.1 (-3.8,30.0)	26	95.1 (-44.5,234.6)	0.55	111	2.2 (-1.3,5.7)	26	4.3 (-13.7,22.3)	0.60
Recognition accuracy	117	2.1 (0.2,4.1) **	26	4.2 (-9.7,18.1)	0.64	111	0.5 (0.0,0.9) **	26	-0.4 (-1.9,1.2)	0.25
Immediate memory span	117	-4.1 (-20.6,12.5)	26	-17.1 (-66.5,32.3)	**0.07**	111	0.3 (-3.2,3.8)	26	-1.7 (-7.2,3.7)	0.31
Level of learning	117	8.9 (-6.4,24.2)	26	19.0 (-73.7,111.6)	0.39	111	2.6 (-0.7,5.9)	26	2.2 (-8.0,12.3)	0.59
Motor function										
7-year assessment										
WRAVMA Pegboard (scaled scores)										
Dominant hand	121	9.3 (-6.5,25.1)	30	22.1 (-32.5,76.6)	0.17	115	-0.8 (-4.3,2.7)	30	-1.0 (-7.5,5.6)	0.22
Non-dominant hand	121	5.6 (-11.1,22.3)	30	12.1 (-51.2,75.3)	0.16	115	-1.5 (-5.2,2.1)	30	-1.5 (-8.8,5.9)	0.20

Finger Tap (z-scores)										
Dominant hand	121	0.5 (-0.4,1.4)	30	5.6 (1.7,9.5) **	**0.00**	115	0.2 (0.0,0.4)	30	0.0 (-0.6,0.6)	0.71
Non-dominant hand	121	0.2 (-0.8,1.1)	30	3.9 (0.8,7.1) **	**0.02**	115	0.1 (-0.1,0.3)	30	0.0 (-0.4,0.5)	0.54
9-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	112	-0.2 (-1.1,0.7)	25	-3.2 (-13.3,6.9)	0.93	106	-0.1 (-0.3,0.1)	25	0.7 (0.0,1.3)	**0.04**
5-item sum	112	-0.2 (-1.1,0.7)	25	-1.6 (-12.2,9.0)	0.81	106	-0.1 (-0.3,0.1)	25	0.7 (0.2,1.3) **	**0.03**
10.5-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	117	0.2 (-0.4,0.9)	26	1.4 (-3.1,5.9)	0.22	111	0.0 (-0.1,0.1)	26	-0.1 (-0.6,0.4)	0.47
5-item sum	117	0.3 (-0.7,1.3)	26	1.9 (-3.7,7.4)	0.39	111	0.0 (-0.3,0.2)	26	0.1 (-0.6,0.7)	0.19

Abbreviations: AUC, area under the curve; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Ability.

Models adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear} < 0.05$

Table S7. Adjusted linear models for attention-related outcome scores in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by child sex.

Outcome	Prenatal Mn					Postnatal Mn				
	Boys		Girls		p_{INT}	Boys		Girls		p_{INT}
	n	**β (95% CI)**	***n***	**β (95% CI)**		***n***	**β (95% CI)**	***n***	**β (95% CI)**	
7-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	83	4.8 (-5.8,15.4)	115	-4.1 (-11.9,3.8)	0.12	78	0.1 (-1.8,2.0)	112	1.0 (-0.9,2.8)	0.65
Inattentive subscale	83	0.3 (-10.0,10.6)	115	-6.9 (-14.5,0.6)	0.22	78	-0.1 (-1.9,1.7)	112	0.1 (-1.8,1.9)	1.00
Hyperactive/Impulsive subscale	83	2.4 (-7.7,12.5)	115	-4.1 (-11.8,3.6)	0.33	78	-0.5 (-2.2,1.1)	112	0.6 (-1.3,2.4)	0.39
BASC-2 - Maternal report (T-scores)[a]										
Internalizing problems	83	6.8 (-3.7,17.2)	110	0.6 (-9.5,10.7)	0.44	78	0.8 (-0.9,2.5)	107	0.6 (-1.8,2.9)	0.82
Externalizing problems	83	6.1 (-5.3,17.6)	110	-2.4 (-10.2,5.5)	0.22	78	0.3 (-1.6,2.2)	107	0.6 (-1.2,2.5)	0.98
Hyperactivity	83	4.2 (-7.1,15.5)	110	2.4 (-5.3,10.0)	0.68	78	0.1 (-1.7,2.0)	107	1.4 (-0.4,3.1)	0.51
Attention problems	83	6.8 (-8.2,21.7)	110	-0.3 (-11.8,11.2)	0.32	78	0.2 (-2.5,2.9)	107	0.4 (-2.2,3.0)	0.99
CADS - Teacher report (T-scores)[b]										
ADHD Index	70	4.1 (-12.2,20.4)	100	-4.8 (-20.5,10.9)	0.34	66	1.0 (-1.9,3.9)	98	-1.0 (-4.6,2.7)	0.15
Inattentive subscale	71	5.5 (-9.1,20.0)	102	2.7 (-7.6,13.1)	0.76	67	1.2 (-1.4,3.9)	100	0.5 (-1.9,2.9)	0.39
Hyperactive/Impulsive subscale	71	0.2 (-14.4,14.8)	102	-7.1 (-20.4,6.2)	0.32	67	0.8 (-1.8,3.4)	100	-1.9 (-4.9,1.1)	**0.05**
BASC-2 - Teacher report (T-scores)[b]										
Internalizing problems	71	-9.5 (-24.6,5.6)	102	-8.1 (-24.4,8.2)	0.90	67	-0.1 (-2.9,2.7)	100	-2.7 (-6.4,1.0)	0.27
Externalizing problems	71	1.4 (-15.2,18.0)	102	-4.3 (-13.9,5.4)	0.56	67	0.8 (-2.2,3.8)	100	-1.1 (-3.3,1.1)	0.13
Hyperactivity	71	1.3 (-16.8,19.3)	102	-7.0 (-17.5,3.5)	0.43	67	1.3 (-1.9,4.5)	100	-1.7 (-4.1,0.7)	**0.05**
Attention problems	71	-1.5 (-13.9,10.9)	102	-1.3 (-10.4,7.8)	0.79	67	0.2 (-2.0,2.5)	100	0.1 (-2.0,2.2)	0.48
9-year assessment										
CADS - Maternal report (T-scores)[a]										
ADHD Index	107	8.3 (-1.3,17.9)	135	-2.4 (-12.4,7.5)	**0.08**	102	-0.7 (-2.4,1.1)	132	-0.1 (-2.6,2.4)	0.75
Inattentive subscale	107	6.8 (-2.2,15.7)	134	-1.6 (-11.5,8.3)	0.15	102	-1.0 (-2.6,0.6)	131	0.4 (-2.1,2.9)	0.38
Hyperactive/Impulsive subscale	107	15.8 (3.2,28.4) **	134	-0. (-11.6,9.8)	0.12	102	-1.2 (-3.4,1.0)	131	-0.3 (-2.9,2.4)	0.45
CPT-II (T-scores)[c]										
Errors of omission	104	-10.6 (-31.3,10.2)	133	1.4 (-16.3,19.0)	0.31	99	-1.8 (-5.6,1.9)	130	0.6 (-3.8,5.0)	0.40
Errors of commission	104	-7.9 (-22.0,6.1)	133	-6.8 (-15.4,1.9)	0.61	99	-0.3 (-2.8,2.3)	130	0.5 (-1.7,2.7)	0.88
ADHD Confidence index	104	-23.2 (-51.0,4.5)	133	9.2 (-12.1,30.5)	**0.05**	99	-2.2 (-7.1,2.8)	130	2.4 (-3.0,7.7)	0.20

10.5-year assessment										
BASC-2 - Maternal report (T-scores)[a]										
Internalizing problems	99	15.0 (4.6,25.4) **	132	1.4 (-6.7,9.4)	0.19	94	0.4 (-1.6,2.5)	129	0.4 (-1.7,2.4)	0.44
Externalizing problems	96	11.3 (1.6,20.9) **	130	-0.9 (-7.7,5.9)	**0.04**	91	0.6 (-1.4,2.5)	128	0.1 (-1.6,1.8)	0.70
Hyperactivity	99	6.4 (-4.0,16.8)	132	-0.1 (-6.5,6.4)	0.54	94	0.0 (-1.9,2.0)	129	0.1 (-1.5,1.7)	0.75
Attention problems	99	14.8 (1.8,27.7) **	132	-6.4 (-16.2,3.4)	**0.00**	94	0.4 (-2.2,2.9)	129	-0.3 (-2.7,2.2)	0.65
BASC-2 - Self-report (T-scores)[c]										
Hyperactivity	98	3.1 (-9.5,15.6)	129	-3.8 (-12.7,5.1)	0.29	93	-1.0 (-3.2,1.3)	126	0.0 (-2.2,2.2)	0.66
Attention problems	97	4.1 (-10.1,18.3)	127	-4.8 (-13.4,3.8)	0.23	92	-0.5 (-2.8,1.9)	124	-0.3 (-2.4,1.8)	0.92

Abbreviations: AUC, area under the curve; CADS, Conners' ADHD/ DSM-IV Scales; ADHD, Attention Deficit/Hyperactivity Disorder; BASC-2, Behavior Assessment System for Children 2nd edition; CPT-II, Continuous Performance Test 2nd edition.

[a] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at maternal interview, language of maternal interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment..

[b] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at interview, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment.

[c] Adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear}< 0.05$

Table S8. Adjusted linear models for cognition, memory, and motor outcomes in children at 7, 9, and 10.5 years, per one-unit increase in prenatal dentine Mn and 2-fold increase in postnatal dentine Mn (^{55}Mn:^{43}Ca AUC x 10^4) stratified by child sex.

Outcome	Prenatal Mn					Postnatal Mn				
	Boys		Girls		p_{INT}	Boys		Girls		p_{INT}
	n	**β (95% CI)**	**n**	**β (95% CI)**		**n**	**β (95% CI)**	**n**	**β (95% CI)**	
Cognition										
7-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	95	1.8 (-6.5,10.2)	100	1.0 (-7.8,9.9)	0.75	71	2.7 (-0.3,5.6)	96	-0.1 (-4.0,3.7)	0.29
Verbal Comprehension IQ	100	-3.9 (-11.5,3.8)	95	1.9 (-7.5,11.3)	0.42	78	1.7 (-1.1,4.6)	107	-0.7 (-4.2,2.8)	0.61
Perceptual Reasoning IQ	95	-5.0 (-13.2,3.2)	100	3.5 (-3.5,10.5)	0.76	78	5.0 (1.5,8.6) **	107	-1.1 (-5.4,3.2)	**0.02**
Working Memory IQ	100	0.5 (-0.7,1.7)	95	2.2 (-5.3,9.7)	0.63	71	2.0 (-1.3,5.3)	97	0.3 (-3.2,3.7)	0.71
Processing Speed IQ	95	0.3 (-1.0,1.7)	132	0.6 (-3.5,4.6)	0.79	71	1.1 (-2.2,4.5)	97	2.8 (-0.3,6.0)	0.64
10.5-year assessment										
WISC-IV Full-Scale IQ (scaled scores)	129	2.1 (-4.4,8.5)	232	4.5 (-5.4,14.4)	0.24	93	2.8 (0.3,5.3) **	129	-0.5 (-2.9,2.0)	**0.02**
Verbal Comprehension IQ	230	1.6 (-4.7,7.9)	224	3.2 (-5.4,11.8)	0.27	95	1.0 (-1.6,3.5)	129	-1.1 (-3.5,1.2)	0.14
Perceptual Reasoning IQ	230	2.8 (-4.3,9.9)	224	4.8 (-5.0,14.6)	0.69	95	5.8 (2.3,9.3) **	129	-1.2 (-4.6,2.2)	**0.00**
Working Memory IQ	222	2.3 (-4.0,8.6)	232	5.4 (-0.7,11.5)	0.68	95	2.3 (-0.3,4.9)	129	0.8 (-1.5,3.1)	0.36
Processing Speed IQ	222	3.2 (-4.0,10.3)	232	4.7 (-2.2,11.6)	0.94	95	-0.3 (-3.2,2.6)	129	0.5 (-2.5,3.5)	0.81
Memory										
9-year assessment										
NEPSY-II Memory for Designs (scaled scores)										
Immediate Total	129	1.8 (-5.7,9.2)	129	0.7 (-6.1,7.6)	0.99	73	1.0 (0.2,1.9) **	103	0.5 (-0.4,1.3)	0.35
Delayed Total	132	-1.9 (-8.9,5.1)	132	0.1 (-1.2,1.4)	0.64	73	1.1 (0.2,2.0) **	104	0.8 (0.0,1.5)	0.70
10.5-year assessment										
CAVLT-2 (z-scores)										
Immediate recall	232	1.7 (-4.7,8.2)	224	5.5 (-0.6,11.7)	0.97	95	2.4 (-1.1,5.9)	129	-1.3 (-5.6,3.0)	0.12
Delayed recall	232	4.1 (-3.2,11.4)	224	4.2 (-2.8,11.2)	0.30	95	3.9 (0.5,7.4) **	129	0.1 (-3.9,4.0)	**0.09**
Recognition accuracy	224	1.1 (-5.4,7.6)	232	-4.0 (-11.2,3.3)	0.28	95	0.5 (0.0,1.1)	129	0.0 (-0.7,0.8)	0.38
Immediate memory span	224	5.2 (-2.2,12.6)	232	-5.6 (-13.8,2.6)	0.72	95	-0.5 (-4.5,3.5)	129	-1.0 (-4.7,2.7)	0.96
Level of learning	232	1.6 (-7.1,10.4)	224	-3.0 (-10.4,4.4)	0.82	95	3.2 (0.1,6.4) **	129	0.6 (-3.2,4.3)	0.28
Motor function										
7-year assessment										
WRAVMA Pegboard (scaled scores)										
Dominant hand	83	2.2 (-22.4,26.8)	110	4.1 (-15.1,23.2)	0.49	78	-1.3 (-5.5,2.9)	107	-0.6 (-5.0,3.8)	0.54
Non-dominant hand	83	6.5 (-19.3,32.3)	110	2.1 (-17.5,21.7)	0.82	78	-0.8 (-5.2,3.5)	107	-1.2 (-5.7,3.3)	0.85

Finger Tap (z-scores)										
Dominant hand	83	1.5 (0.2,2.7) **	110	0.2 (-0.9,1.4)	**0.09**	78	0.2 (0.0,0.4) **	107	0.1 (-0.2,0.4)	0.30
Non-dominant hand	83	0.9 (-0.3,2.1)	110	-0.2 (-1.4,0.9)	**0.06**	78	0.2 (0.0,0.4)	107	0.1 (-0.2,0.3)	0.32
9-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	100	0.6 (-0.6,1.8)	73	0.0 (0.0,0.0) **	0.43	95	0.0 (-0.2,0.2)	123	0.0 (-0.2,0.2)	0.94
5-item sum	100	0.5 (-0.7,1.7)	78	2.5 (-1.0,6.0)	**0.00**	95	0.0 (-0.2,0.2)	123	0.0 (-0.2,0.2)	0.86
10.5-year assessment										
Luria-Nebraska Motor Scale (z-scores)										
All items	185	0.3 (-0.7,1.4)	93	-1.1 (-7.1,4.8)	0.43	95	0.0 (-0.1,0.2)	129	-0.1 (-0.2,0.1)	0.12
5-item sum	177	-0.7 (-3.0,1.5)	100	0.5 (-5.3,6.2)	0.65	95	0.2 (-0.1,0.4)	129	-0.1 (-0.3,0.1)	**0.05**

Abbreviations: AUC, area under the curve; CI, confidence interval; WISC-IV, Wechsler Intelligence Scale for Children 4th edition; IQ, intellectual quotient; CAVLT-2, Children's Auditory Verbal Learning Test 2nd edition; WRAVMA, Wide Range Assessment of Visual Motor Ability.

Models adjusted for maternal education, maternal intelligence (PPVT score), years in the US, maternal depression at time of assessment, child's sex, child's age at assessment, language of assessment, HOME z-score at time of assessment, household income at time of assessment, number of children in the home at time of assessment, housing density at time of assessment, and psychometrician (9-year and 10.5-year assessments).

**$p_{Linear} < 0.05$

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Chapter 5:
Summary of findings, conclusions, and future research needs

Mn is an essential nutrient for humans and animals, but adverse health effects can be caused by both deficiency and excess of Mn. Overt Mn deficiency is rare and few studies have examined the health effects of subclinical Mn deficiency. In contrast, the effects of high Mn exposures have been widely studied in occupational settings (Deng et al. 2013; Lucchini et al. 1999; Mergler et al. 1994; Racette et al. 2012; Wang et al. 2012). In recent years, there has been an increasing interest in the effects of Mn on other population subgroups, including pregnant women and children. Mn uptake and distribution are regulated through homeostatic mechanisms in humans, but Mn crosses the placenta through active transport mechanisms (Krachler et al. 1999; Leazer and Klaassen 2003), potentially exposing the susceptible fetus to high Mn concentrations. Postnatally, neonates and infants have increased gastrointestinal absorption of ingested Mn and impaired excretion mechanisms (Aschner 2000; Ljung and Vahter 2007; Yoon et al. 2009), and these characteristics increase their vulnerability to high Mn exposures. In addition, children may also have higher exposures to Mn because they eat, drink and breathe more per unit volume compared to adults and their unique exploratory behaviors (e.g., hand-to-mouth movements) predispose them to environmental exposures to Mn (Aschner et al. 2007; Bearer 1995; Roberts et al. 2009; Weiss 2000).

This dissertation focused on environmental exposures to Mn in pregnant women and children living near agricultural fields treated with Mn-containing fungicides in Costa Rica and California, and the relationship of prenatal and early postnatal Mn exposure with fetal growth, length of gestation, and neurodevelopment in children. More specifically, this dissertation aimed to (1) identify lifestyle, occupational, and environmental factors associated with blood and hair Mn concentrations measured during pregnancy in women living near banana plantations with aerial mancozeb spraying in Costa Rica; (2) determine whether prenatal hair and blood Mn concentrations were associated with fetal growth and length of gestation in this mother-infant cohort living near banana plantations in Costa Rica; and (3) examine the relationship of prenatal and postnatal teeth Mn levels with attention, cognition, memory, and motor development in children aged 7, 9, and 10.5 years living near agricultural fields with ground-level mancozeb and maneb spraying in California.

1. Summary of findings

Despite the fact that ethylene bisdithiocarbamate (EBDC) fungicides mancozeb and maneb contain substantial quantities of Mn (21% by weight), few studies have examined the possible increase in Mn absorption and potential toxicity from spraying (Canossa et al. 1993; Gunier et al. 2013; Takser et al. 2004). In the Infants' Environmental Health Study (ISA), a study of 449 Costa Rican pregnant women living near banana plantations with extensive aerial spraying with mancozeb, blood and hair Mn concentrations were higher than most concentrations reported in pregnant women from other countries (Kopp et al. 2012; Yu and Cao 2013; Claus Henn et al. 2011; Rudge et al. 2009; Abdelouahab et al. 2010; Lin et al. 2010; Ljung et al. 2009; Rollin et al. 2009; Zota et al. 2009) (Takser et al. 2003; Hambridge and Droegemueller 1974), including women living near agricultural fields treated with pesticides (Takser et al. 2004; Bradman et al. unpublished results). Although blood Mn concentrations showed inconsistent associations with occupational and environmental factors, hair Mn concentrations were positively related with several occupational and environmental factors such as occupation before pregnancy, number of household members, nearby aerial spraying on the day of the hair sample collection, and drinking water Mn concentrations, and inversely associated with distance between residences and banana plantations.

Epidemiologic studies on the effects of prenatal Mn exposure on fetal growth and length of gestation are limited and their findings are somewhat conflicting (Takser et al. 2004; Yu and Cao 2013; Zota et al. 2009; Chen et al. 2014; Eum et al. 2014; Guan et al. 2013; Osada et al. 2002; Vigeh et al. 2008; Xu et al. 2011). In the ISA study, Mn concentrations measured in hair samples from pregnant women in their second trimester of gestation were positively and linearly associated with chest circumference. Higher mean maternal hair Mn concentrations during pregnancy were associated with enlarged chest circumferences among infants whose mothers did not have gestational anemia and increased length of gestation in mothers who lived below the poverty line. Notably, higher mean maternal blood Mn concentrations during pregnancy were associated with decreased chest circumference in infants whose mothers lived below the poverty line. The clinical significance of larger chest circumference, in the absence of an association between hair Mn concentrations and other measures of fetal growth in this study, is unknown. No significant linear or nonlinear associations were observed between maternal Mn concentrations and lowered birth weight or head circumference, as reported in previous studies (Zota et al. 2009; Chen et al. 2014; Eum et al. 2014; Guan et al. 2013). Inconsistencies between studies could be due to differences in the study population, sample size, and time of biological sampling (at delivery vs. third trimester or averaged over pregnancy). Disparities between studies could also be due to differences in exposure levels and sources of Mn exposure, largely because it remains unclear how different sources and pathways of exposure to Mn translate into Mn concentrations in various biological media (e.g., blood, hair, teeth) (Smith et al. 2007).

Numerous cross-sectional studies have examined the association between Mn exposure and neurodevelopment in school-age children (Bouchard et al. 2011; He et al. 1994; Hernandez-Bonilla et al. 2011; Kim et al. 2009; Lucchini et al. 2012; Menezes-Filho et al. 2011; Riojas-Rodriguez et al. 2010; Torres-Agustin et al. 2013; Wasserman et al. 2006), but only a few have assessed the neurobehavioral effects of prenatal and early postnatal exposure to Mn (Takser et al. 2003; Ericson et al. 2007; Lin et al. 2013). These studies reported that higher prenatal Mn concentrations, measured in maternal blood collected during pregnancy, cord blood, or children's teeth, were associated with poorer attention (Takser et al. 2003; Ericson et al. 2007), memory (Takser et al. 2003), and cognitive and language development (Lin et al. 2013) in children. In the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, prenatal and postnatal dentine Mn levels measured in children's deciduous teeth were associated with some measures of attention, cognition, memory, and motor function in children aged 7, 9, and 10.5 years. Consistent with previous studies, we found that higher prenatal dentine Mn levels were associated with poorer attention scores but only in boys and children born to mothers with higher lead exposure during pregnancy or gestational anemia. However, higher prenatal and postnatal Mn levels in dentine of deciduous teeth were associated with better cognitive abilities and fine motor coordination. Higher postnatal dentine Mn levels were also associated with better scores in delayed and immediate memory tests. All these associations were linear, and no threshold was identified. The inconsistencies between this study and others could be due to differences in the study design (cross-sectional vs. prospective), timing of Mn measurements (ages 6-14 years vs. prenatal and early postnatal), and/or exposure matrix (Mn levels measured in drinking water, blood, or hair samples vs. in teeth).

2. Conclusions

This dissertation used data from the ISA and CHAMACOS studies, two large mother-child cohorts living near agricultural fields and unique in their exposure to Mn-containing fungicides. The ISA study has the benefits of a strong prospective design, with both blood and hair Mn concentrations measured at different time points during pregnancy, and data on birth outcomes collected by hospital/clinic staff blinded to the Mn exposure status. The CHAMACOS study has numerous strengths including its longitudinal design, use of comprehensive neurodevelopmental assessments at different ages, information on a wide variety of potential confounders, and measurements of Mn levels in dentine of children's deciduous teeth. Both the ISA and the CHAMACOS study are exceptional in that they have collected repeated biological and environmental samples during pregnancy and childhood, but also information on different health outcomes for a large number of pregnant women and their children.

This dissertation is one of the three studies that have examined exposure to Mn in populations living near agricultural fields treated with Mn-containing fungicides (Gunier et al. 2013; Takser et al. 2004), and its findings suggest that pregnant women and children living near banana plantations aerially sprayed with mancozeb in Costa Rica may be environmentally exposed to Mn.

This dissertation is one of the only two studies that have assessed the association of maternal blood Mn concentrations measured repeatedly during pregnancy with fetal growth and length of gestation (Takser et al. 2004), and the first to examine the relationship between maternal hair Mn concentrations during pregnancy and these birth outcomes. This study failed to replicate the nonlinear associations between maternal blood Mn concentrations at delivery and measures of fetal growth reported by previous cross-sectional studies, but its findings were consistent with the other study that measured maternal blood Mn concentrations repeatedly during pregnancy. In addition, maternal hair Mn concentrations during pregnancy were associated with larger chest circumference in infants born to women without gestational anemia and longer gestational durations in women who lived below the poverty line. Maternal blood Mn concentrations during pregnancy were associated with smaller infant chest circumferences.

This dissertation contributes to the growing evidence on the neurodevelopmental effects of prenatal and postnatal Mn levels in school-age children (Ericson et al. 2007). This is the first study to measure Mn levels in dentine of deciduous teeth, a novel matrix that reflects longer-term exposures to Mn compared to other biological matrices such as blood and urine and can be directly linked to the developmental timing of exposure. In this study, prenatal and postnatal Mn dentine levels were associated with poorer attention outcomes, but better cognitive, memory, and motor measures in school-age children. Although prenatal dentine Mn levels in deciduous teeth of CHAMACOS children were associated with agricultural use of Mn fungicides during pregnancy (Gunier et al. 2013), it is possible that the prenatal and early postnatal Mn levels in this study population may not be detrimental to fetal development or not high enough to produce significant, long-term deficits in neurodevelopment.

3. Future research needs

Given the complexity of Mn, an essential element that is also an environmental pollutant, and the inconsistencies between epidemiological studies on its health effects in children, further studies are needed before a clear profile can be determined. Additional research is needed to characterize the relationship between Mn levels in different biological matrices (e.g., blood, hair, and dentine of deciduous teeth), but mainly to understand how accurately the different biomarkers of exposure capture environmental exposures to Mn in different populations, including agricultural communities exposed to Mn-containing fungicides. Further research is also needed to evaluate the effects of simultaneous or sequential exposures to Mn and other toxicants over the course of pregnancy on fetal growth, length of gestation, and neurodevelopment; and to assess the long-term neurodevelopmental effects of *in utero* and postnatal exposure to Mn.

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