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Exposure to Manganese from Agricultural Pesticide Use and Neurodevelopment in Young Children

By

Robert Bruce Gunier

A dissertation submitted in partial satisfaction of the

requirements for the degree of

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in

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in the

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of the

University of California, Berkeley

Committee in charge:

Professor Brenda Eskenazi, Co-Chair
Professor Michael Jerrett, Co-Chair
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ABSTRACT

Exposure to Manganese from Agricultural Pesticide Use and Neurodevelopment in Young Children

By

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Doctor of Philosophy in Environmental Health Sciences

University of California, Berkeley

Professor Brenda Eskenazi, Co-Chair

Professor Michael Jerrett, Co-Chair

Using data from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, this dissertation shows that agricultural use of fungicides that contain manganese (Mn) results in higher levels of Mn in children's homes and teeth, and that higher Mn levels in children's teeth are associated with a modest deficit in neurodevelopment at 6-months of age. In Chapter 2, predictors of Mn concentrations and loadings in house dust samples are evaluated. The fungicides maneb and mancozeb are approximately 21% Mn by weight and more than 150,000 kg are applied each year to crops in the Salinas Valley, California. It is not clear whether agricultural use of these fungicides increases Mn levels in homes. In this study, predictors of Mn levels in house dust samples are evaluated. House dust samples were collected from 378 residences enrolled in the CHAMACOS study with a second sample collected nine months later from 90 residences. House dust samples were analyzed for Mn using inductively coupled plasma optical emission spectroscopy. Information from interviews, home inspections, and pesticide use reporting data was used to identify potential predictors of Mn dust concentrations and loadings. Linear mixed-effects models were used to identify significant predictors. Mn was detectable in dust samples from all homes. The median Mn concentration was 171 µg/g and median Mn loading was 1,908 $\mu g/m^2$ at first visit. In multivariable models, Mn dust concentrations and loadings increased with the number of farmworkers in the home and the amount of agricultural Mn fungicides applied within three kilometers of the residence. Dust concentrations and loadings were higher in residences located on Antioch Loam soil than other soil types, in homes with poor or average compared to excellent housekeeping practices, and residences located in the southern Salinas Valley compared those located in the town of Salinas or the northern part of the Salinas Valley. In summary, agricultural use of Mn containing fungicides were found to contribute to Mn dust concentrations and loadings in nearby residences and farmworker homes.

Chapter 3 presents an analysis that identifies determinants of Mn in prenatal dentin from children's shed teeth. Mn is an essential nutrient, but over-exposure can be neurotoxic. Over 800,000 kilograms of Mn-containing fungicides are applied each year in California. Manganese

levels in teeth are a promising biomarker of perinatal exposure. Participants in this analysis included 207 children enrolled in the CHAMACOS study, a longitudinal birth cohort study in an agricultural area of California. Mn was measured in teeth using laser-ablation-inductively coupled plasma-mass spectrometry. The purpose of this analysis was to determine environmental and lifestyle factors related to prenatal Mn levels in shed teeth. Storage of farmworkers' shoes in the home, maternal farm work, agricultural use of Mn-containing fungicides within 3 km of the residence, residence built on Antioch Loam soil and Mn dust loading (μg/m^2 of floor area) during pregnancy were associated with higher Mn levels in prenatal dentin ($p<0.05$). Maternal smoking during pregnancy was inversely related to Mn levels in prenatal dentin ($p<0.01$). Multivariable regression models explained 22 – 29% of the variability of Mn in prenatal dentin. These results suggest that Mn measured in prenatal dentin provides retrospective and time specific levels of exposure to the fetus resulting from environmental and occupational sources.

Chapter 4 evaluates the association between Mn in prenatal and postnatal dentin of children's shed teeth and early neurodevelopment. Previous studies have observed associations between Mn exposure and children's neurodevelopment, primarily using concurrent exposure measurements in blood or hair. Prenatal and postnatal Mn exposures have not been evaluated together in a prospective study of neurodevelopment in young children. Mn levels in prenatal and postnatal dentin were measured from children's shed teeth. The relationship between prenatal and postnatal exposure and children's performance at 6, 12 and 24-months of age on the Bayley Scales of Infant Development mental and psychomotor development indices was examined. The possibility of an inverted U-shaped association with neurodevelopment was explored since Mn is an essential nutrient. Potential interactions between Mn exposure and blood lead concentrations were also evaluated as well as effect modification by maternal iron status during pregnancy. An inverse association between postnatal Mn levels in dentin and psychomotor development at 6-months of age was observed with a modest decrease in psychomotor development scores, which followed an inverse U-shape, with the strongest effect observed when comparing the highest tertile of Mn levels in teeth to the middle tertile of Mn levels in teeth (-4.6 points; 95% Confidence Interval: -8.0, -1.3). Among children whose mothers' were iron deficient during pregnancy, prenatal Mn levels in dentin were associated with both mental and psychomotor development at 6-months. No interactions with prenatal or postnatal blood lead concentrations were observed in this cohort. In conclusion, a modest decrease in psychomotor development at 6-months of age was associated with postnatal Mn levels in dentin from a mean score of 96 for the middle tertile compared to 94 at the highest tertile. Iron status during pregnancy appeared to be a potentially important effect modifier of prenatal Mn exposure and neurodevelopment at 6-months of age.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AUC = area under the curve
CHAMACOS = Center for the Health Assessment of Mothers and Children of Salinas
GIS = Geographic information system
GM = geometric mean
GSD = geometric standard deviation
HFE = hemochromatosis
ICC = intraclass correlation coefficient
MDI = Mental Development Index
MMT = methylcyclopentadienyl manganese tricarbonyl
Mn = manganese
Mn_{Pre} = manganese in prenatal dentin
Mn_{Post} = manganese in prenatal dentin
PDI = Psychomotor Development Index
PM = particulate matter
PUR = pesticide use report
TF = transferrin

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CHAPTER 1

Background on manganese health effects, sources and biomarkers of exposure

Health effects of manganese

Studying the health effects of manganese (Mn) is complex because it is both an essential nutrient and a neurotoxicant, depending on the dose (ATSDR, 2008). Manganism is a syndrome observed in smelter workers, miners and welders caused by high inhalation exposure to Mn. Early signs include behavior changes and slowed movements (Martin, 2006). Residential proximity to agricultural applications of maneb, a Mn-containing fungicide, has been associated with Parkinson's disease (Costello, 2009; Wang, 2011). Parkinson's disease is a movement disorder associated with degeneration of dopaminergic neurons (Twelves, 2003). Exposure to maneb produces dopaminergic neurodegeneration in mice, possibly by increasing oxidative stress and disrupting mitochondrial function (Zhang, 2003). Mn is a potent dopamine oxidant, and the mechanism of Mn neurotoxicity, as related to cognitive function and attention, also appears to involve dopamine receptor damage or loss (Graham, 1984; Pal, 1999). In rat studies, early life exposure to Mn alters dopaminergic systems that control behavior and cognitive function and leads to lasting functional impacts long after Mn exposure levels return to normal (McDougall, 2008; Kern, 2010; Kern, 2011).

Epidemiological studies of environmental exposure to Mn, primarily through contaminated drinking water, have found associations with decreased neuromotor function and psychomotor development, poorer intellectual function and learning disabilities, as well as hyperactive behavior in children 6 – 13 years of age (Mergler, 1999; Takser, 2003; Wasserman, 2006; Ericson, 2007; Bouchard, 2011a). Recent studies of children exposed to airborne Mn from living near a Mn mining operation or living downwind of a ferro-manganese plant observed associations between higher Mn levels in hair and poorer cognitive performance (Riojas-Rodriguez, 2010; Menezes-Filho, 2011). An inverted U-shaped association was observed between 12-month blood Mn and mental development scores measured concurrently in a birth cohort from Mexico City, consistent with Mn being an essential nutrient at low doses and a neurotoxicant at high doses (Claus Henn, 2010).

Recent evidence suggests that Mn levels may modify the effects of lead exposure on neurodevelopment. A cross-sectional study of environmental exposure in South Korea observed additive interaction and effect modification between lead and Mn on the intelligence of school-aged children (Kim, 2009). A longitudinal analysis of the Mexico City birth cohort found significant interactions between blood lead and blood Mn concentrations and mental and psychomotor development scores measured up to five times between 12 and 36 months (Claus Henn, 2012).

Exposure to manganese

For most people, diet is the primary source of Mn. As an essential nutrient, Mn ingestion is well regulated in adults by homeostatic mechanisms (ATSDR, 2008). Mn also occurs naturally in drinking water. In the United States, about 6% of domestic drinking water wells are above the drinking water advisory level of 300 µg/L (Ljung, 2007). Ingestion of Mn in dust and soil is also important, especially for young children, who spend more time close to the ground and have more frequent hand to mouth activity (Xue, 2010). However, exposure by inhalation is the most important route for neurotoxicity because inhalation bypasses homeostatic mechanisms, and inhaled Mn can be taken up by the olfactory nerve and reach the brain directly (Teeguarden, 2007; Nong, 2009). Pregnancy and the first year of life are potentially important periods of exposure. Mn crosses the placenta during pregnancy. Young children absorb greater amounts of Mn due to rapid bone mineralization and may not excrete it as well as adults (Yoon, 2009; Santamaria, 2010).

In the Salinas Valley of California, agricultural use of maneb and mancozeb, fungicides containing approximately 21% Mn by weight, exceeds 350,000 pounds per year. Over 90% of use is on lettuce (CDPR, 2011). Lettuce cultivation creates a significant amount of dust, mostly from leveling (or "planing") soil after harvesting, with an estimated 12.5 pounds of particulate matter less than 10 microns (PM_{10}) emitted per acre each year (Gaffney, 2003). Wind can disperse dust containing Mn from treated fields into nearby residences, resulting in higher levels of Mn in air and house dust. House dust provides a potentially good long term marker of exposure to Mn since the environmental persistence of Mn is essentially infinite. Mn is naturally present in soil and typically detectable in house dust samples. Geometric mean concentrations range from 54 to 658 μg/g, depending on the presence of Mn emission sources such as metal processing plants or the use of Mn as a gasoline additive (Rasmussen, 2001; Chattopadhyay, 2003; Turner, 2006; Kuo, 2010). Dust loadings of Mn (the mass per unit floor area) could be a good indicator of inhalation exposure, reflecting fugitive dust emissions from nearby agricultural fields.

Manganese in house dust

House dust is considered a good environmental medium for assessing long term exposure in the home because pesticides and other chemicals persist indoors, where they are protected from degradation by sunlight, moisture, and microorganisms (Lioy, 2002; Roberts, 2009). As mentioned above, children ingest more dirt and dust than adults by bodyweight (Butte, 2002; Xue, 2010). Concentrations of metals, pesticides, nicotine and persistent organic pollutants in dust have been used as indicators of exposure in previous epidemiological studies (Rudel, 2001; Colt, 2005; Ward, 2009; Whitehead, 2010). For lead, dust loading (the mass per unit floor area) was more predictive of children's blood lead levels than dust concentration (the mass of lead per unit mass of dust) (Lanphear, 1995). Agricultural pesticide concentrations in house dust have been shown to be higher in residences closer to treated fields and in farm homes (Simcox, 1995; Lu, 2000; Fenske, 2002; Curwin, 2005; Obendorf, 2006; Ward, 2006; Quiros-Alcala, 2011). Previous studies that used a geographic information system (GIS) to summarize pesticide use observed significantly higher concentrations of pesticides in dust in homes near fields than in homes far from fields. However, the models explained only 4 – 28% of the variability in pesticide concentrations (Harnly, 2009; Gunier, 2011). Previous studies have focused on more volatile pesticides not Mn or other metals, have used only a single dust measurement from each home, and did not incorporate wind direction in the models.

Biomarkers of manganese

Blood has been the most commonly used biomarker of exposure for Mn, but it only reflects recent exposures (e.g., 10 – 30 days before sampling) (ATSDR, 2008). Urinary Mn levels have not been found to be very informative since only a small proportion of Mn is excreted in urine (Smith, 2007). Hair Mn levels have problems related to separating exogenous from internal exposures, even after adjusting for darker hair absorbing Mn more efficiently than light hair (Sturaro, 1994; ATSDR, 2008).

Mn levels in teeth have been proposed as a reliable long-term biomarker of dose because teeth provide a longitudinal record similar to tree rings (Ericson, 2001). Like calcium and lead, Mn has a 2+ charge and becomes incorporated into teeth. Higher levels of lead in tooth dentine have been correlated with higher blood lead levels in children (Arora, 2005). In a pilot study of Mn in teeth, Mn ranged from 0.1 to 2.0 µg/g of tooth and the highest Mn concentrations were found in the prenatally formed dentine (Arora, 2011). Advantages of using teeth as biomarkers are that sample collection is non-invasive, storage stability is excellent and the neonatal line in primary teeth that distinguishes between prenatal and postnatal tooth development offers the possibility to assess exposure retrospectively for prenatal and early postnatal periods (Arora, 2011). The only health study that measured Mn levels in the enamel of shed teeth from children found significant correlations between Mn levels in prenatal tooth enamel and behavioral disinhibition in the children including attention deficit and hyperactivity (Ericson, 2007). Measurements in enamel cannot be readily linked to developmental timing of exposure because, unlike dentin, initial deposits of enamel matrix are not completely mineralized immediately but rather more slowly and diffusely during maturation. Measurements of Mn levels in dentin could provide more specific time points during the perinatal period using knowledge of tooth mineralization.

Research needs

Children living in agricultural communities have higher intake of pesticides based on urinary metabolite levels, likely due to greater residential non-dietary exposures from inhalation and ingestion of house dust from local agricultural uses and farm worker take-home exposures (Lu, 2000; McKone, 2007). Metabolites of organophosphates in urine of children have been associated with their poorer performance on neurobehavioral tests (Rohlman, 2005; Jurewicz, 2008). Higher maternal organophosphate metabolite levels during pregnancy have also been related to lower neurodevelopment scores in children (Engel, 2011; Rauh, 2011). In the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, elevated maternal organophosphate metabolite levels have been associated with lower mental development scores at 2 years of age, attention deficits at 5 years of age and decreased intellectual development at 7 years of age indicating the potential influence of prenatal exposures on neurodevelopment in children later in life (Eskenazi, 2007; Marks, 2010; Bouchard, 2011b).

Statement of research question

Manganese is neurotoxic at higher exposure levels. In several community studies, higher estimated exposure to Mn has been associated with decreased intellectual function and increased hyperactive behavior in children. Previous epidemiological studies observed associations between residential proximity to agricultural use of the Mn containing fungicide maneb and Parkinson's disease. However, the relationship between residential proximity to agricultural Mn use and exposure has not been well characterized using environmental or biological samples. The current methods for estimating pesticide levels in residences near treated fields could be improved by incorporating information on wind direction. Mn exposure assessment has also been limited by the lack of a well characterized biomarker of body burden.

The objectives of this dissertation are to characterize Mn transport from agricultural fields to the home environment using house dust samples, estimate children's exposure to Mn from treated fields using Mn levels in shed deciduous teeth, and to determine the relationship between prenatal Mn levels in teeth and early neurodevelopment. The central hypothesis of this proposal is that children living downwind and near fields treated with Mn containing pesticides will have higher levels of Mn in their environment and bodies than children with little or no Mn pesticide use nearby. Children with higher Mn body burden are likely to be at an increased risk for compromised neurodevelopment. The specific aims are to:

Specific Aim 1: Identify predictors of manganese levels in house dust. A geographic information system (GIS) will be used to estimate Mn emissions from agricultural pesticide use, vehicle traffic and industrial emissions near each residence. Wind direction will be used to better account for environmental transport of Mn from these sources. Mixed-effects models will be used to identify determinants of Mn dust concentrations (µg/g) and loadings ($\mu g/m^2$) in house dust samples (n=468) with repeated measures in a subset of homes (n=90).

Specific Aim 2: Quantify the contribution of agricultural pesticide use to manganese levels in shed deciduous teeth. Mn levels in children's shed deciduous teeth will be compared to GIS estimates of Mn emissions developed in Aim 1. Food frequency questionnaire and drinking water monitoring data will be used to adjust for dietary intake of Mn. Regression models will be used to determine the contribution of agricultural Mn pesticide use to Mn levels in children's teeth (n=207) during the prenatal period.

Specific Aim 3: Determine the relationship between manganese levels in shed teeth and neurodevelopment in young children. The association between mental and psychomotor development index scores measured at 6, 12 and 24 months of age and perinatal Mn levels in shed teeth will be evaluated in CHAMACOS children (n=198) living in an agricultural area. Interactions between blood lead concentrations and Mn levels in teeth will be explored. Effect modification of prenatal Mn levels in teeth and neurodevelopment by maternal iron status during pregnancy will be also evaluated.

Significance

This study will help characterize the distance of residences from treated fields where environmental Mn concentrations are elevated; evaluate Mn levels in shed teeth as a biomarker of internal dose; help identify critical exposure periods for Mn, and evaluate interactions between Mn and lead exposure with early neurodevelopment in children. This study offers an excellent opportunity to combine environmental and biological measurements to conduct a more comprehensive exposure assessment and will help inform researchers and regulatory agencies about indoor environmental concentrations of Mn, teeth as a biomarker of Mn body burden and neurodevelopmental risks to children associated with non-dietary ingestion and inhalation of Mn from pesticides used in agriculture.

Epidemiological studies have observed associations between residential proximity to reported use of agricultural pesticides and fetal death (Bell et al. 2001), neural tube defects (Rull, 2006), autism (Roberts, 2007), Parkinson's disease (Costello, 2009; Ritz, 2009; Wang, 2011) and childhood cancer (Carozza, 2008). However, a major limitation of these studies is the lack of validation and uncertainty of the exposure estimates. House dust samples provide a long-term indicator of chemical exposures in the home and an opportunity to evaluate the accuracy of GIS exposure estimates. Children of farm workers are at an increased risk of exposure to agricultural pesticides due to take-home transport and residential location (Lu, 2002; Lambert, 2005; McKone, 2007). The USEPA is seeking additional information on exposures and risks to children from non-dietary ingestion and inhalation to complete a more thorough assessment of risks to children from pesticides used in agriculture (USEPA, 2010). If Mn levels in teeth are associated with exposure, this would provide a non-invasive method to study exposure to several metals and possibly other compounds of interest.

California has the largest population, agricultural revenue and pesticide use in the United States. Population growth in California has expanded the interface between agricultural and suburban land and increased the population potentially exposed to agricultural pesticide drift. This study would utilize data from a cohort that was designed to assess the effects of pesticide exposure on neurodevelopment in children living in an intensely agricultural area. Salinas is "the salad bowl of the United States". More than 70% of the lettuce produced in the United States comes from the Salinas Valley in California (Boriss, 2005). As a result, the Salinas Valley is also an area of intensive agricultural pesticide use, especially for Mn based fungicides. The results from this study will help inform researchers and regulators regarding the amount of Mn containing fungicides that get inside children's bodies in surrounding communities (intake fraction). Children's intellectual function has significant societal costs related to education, health care and lost productivity (Landrigan, 2002; Weiss, 2006).

Innovation

The proposed study combines the availability of repeated measures of Mn concentrations in house dust and Mn levels in shed teeth from children living in an agricultural community with extensive interview data that can be used to evaluate GIS exposure methods based on proximity to reported agricultural Mn use. The prospective CHAMACOS cohort study, centered in the Salinas Valley, with extensive assessments of neurodevelopment in children presents a unique opportunity to study Mn exposure from agricultural pesticide use and sensitive health outcomes measured in a potentially highly exposed and more susceptible population of children.

In this study, longitudinal measures of Mn concentrations in house dust will be used to better characterize the relationship between exposure and proximity to reported agricultural pesticide use. Land-use regression methods like these have not been widely applied to agricultural pesticide studies. Dust concentrations will be used to empirically determine the best buffer distance from a residence, time period prior to sample collection and method for incorporating wind data. No previous health studies have utilized environmental or biological samples to complement pesticide use data, and few exposure studies have attempted to incorporate wind direction into the exposure model. Multi-level mixed effects models will be used to accommodate the temporal correlation of Mn levels in dust within residences over time and spatial correlation within communities. The use of Mn levels in teeth will be evaluated as a biomarker of exposure to environmental sources of Mn. This study will be the first to evaluate both prenatal and postnatal levels of Mn and early neurodevelopment. Improved methods for assessing community exposure to agricultural pesticides will be developed that could be used in future health studies of asthma, respiratory function, birth defects and other health outcomes.

CHAPTER 2

Determinants of manganese levels in house dust samples from the CHAMACOS cohort

2.1. INTRODUCTION

Manganese (Mn) is an essential nutrient but is neurotoxic at high exposure levels (ATSDR 2008). In children, higher exposure to Mn has been associated with poorer mental development (Claus Henn et al. 2010), lower full-scale and verbal intelligence quotient (Menezes-Filho et al. 2011), lower verbal and performance intelligence quotient (Riojas-Rodriguez et al. 2010), poorer attention and non-verbal memory (Takser et al. 2003), increased externalizing behavior problems (Ericson et al. 2007), and more oppositional and hyperactive behaviors (Bouchard et al. 2011). An interaction between Mn and lead resulting in poorer overall, cognitive and language development has also been observed (Lin et al. 2013). Water contaminated with Mn is one potential exposure pathway (Bouchard et al. 2011; Wasserman et al. 2006). Exposure may also occur, however, through airborne Mn released from Mn mining operations (Riojas-Rodriguez et al. 2010), ferro-manganese production facilities (Haynes et al. 2010; Menezes-Filho et al. 2009) or agricultural use of Mn containing fungicides (Gunier et al. 2013; Takser et al, 2004).

House dust serves as a reservoir for persistent compounds including metals (Whitehead et al. 2013). House dust is an important exposure pathway for young children because they spend more time close to the ground and have greater hand to mouth activity (Roberts et al. 2009). Lead dust loading, the amount of lead per area of floor space, has been shown to be a significant predictor of blood lead levels in children (Lanphear et al. 1995). Previous studies have demonstrated that levels of pesticides are higher in house dust of homes with farmworkers (Curl et al. 2002; Harnly et al. 2009) and residences located near agricultural pesticide applications (Fenske et al. 2002; Gunier et al. 2011; Harnly et al. 2009; Lu et al. 2000; Ward et al. 2006).

Maneb and mancozeb are fungicides that contain approximately 21% Mn by weight (FAO 1979). A study conducted in Quebec, Canada, found that self-reported proximity to agricultural pesticide applications was related to higher blood Mn (Takser et al. 2004). Agricultural use of these Mn fungicides exceeds 150,000 kg per year in the Salinas Valley of California with more than 90% used on lettuce (CDPR 2011). In a previous analysis, we found higher levels of Mn in shed teeth of children from the CHAMACOS study who lived within 3 km of agricultural applications of Mn fungicides, whose mothers were farmworkers, and among those with higher Mn dust loading in their homes (Gunier et al. 2013).

In the present study, we evaluated potential predictors of Mn concentrations and loadings in house dust from the homes of CHAMACOS participants, including agricultural work, proximity to agricultural use of Mn fungicides, soil type at the residence, and the amount of bare soil near the homes.

2.2. METHODS

Study population

Between September 1999 and November 2000, the Center for Health Assessment of Mothers and Children of Salinas or the CHAMACOS study enrolled 601 pregnant women from health clinics in the agricultural Salinas Valley primarily serving low-income families. Participants were eligible if they spoke English or Spanish and qualified for state funding of well-pregnancy care (within 200% of the Federal poverty level). A total of 513 participated in a prenatal home visit, of which 385 participated in a visit when the child reached six months of age. Participants were included in this analysis if there was adequate house dust sample available from the prenatal or six month home visit to measure Mn concentrations (n=378). For 90 homes, samples were analyzed from both prenatal and six month visits (postnatal) to assess Mn levels within homes over time. Written informed consent was obtained from all participants and all research was approved by the University of California, Berkeley Committee for the Protection of Human Subjects prior to commencement of the study.

Dust sample collection

House dust samples were collected using a High Volume Small Surface Sampler (HVS3, Envirometrics, Inc., Seattle, WA), which allows for the determination of dust loading in grams per square meter of floor area in order to better characterize Mn available for contact by children (Roberts et al. 2009). Details of the dust sample collection methods are provided elsewhere (Harnly et al. 2009); briefly dust samples were collected primarily from a one square meter area of floors. Dust samples were collected at mean of 18 (SD=6) weeks gestation (prenatal) and when the child was 7 (SD=1) months old (postnatal). If an adequate amount of dust could not be collected from the floor, furniture in the room was sampled using an attachment (n=7). Field interviewers collected information on recent cleaning and made an assessment of the overall quality of housekeeping (poor, average or excellent), recorded the type of surface sampled (floor, carpet or furniture) and measured the area of floor sampled in square meters. Samples were collected from the living room (n=403), child's bedroom (n=58) or other room (n=6). Season of dust sample collection was defined as winter (December-February); spring (March-May); summer (June-August); and fall (September-November). Field staff also recorded the latitude and longitude coordinates of the residence using a hand-held global positioning system unit (eTrex, Garmin, Kansas, USA).

Laboratory analysis of dust samples

Dust samples were stored at -80 °C for approximately ten years before shipping them on dry ice for analysis. Samples were sieved to 150 μm and then weighed. For analysis, 500 mg the dust samples was digested in 7.5 N nitric acid overnight and quantified Mn concentrations in using inductively coupled plasma optical emission spectroscopy. Mn standards were used to develop a calibration curve and based on sample blanks the limit of detection was 0.1 μg Mn/g dust. To determine the reproducibility of Mn measurement, 26 dust samples were run in triplicate and the relative standard deviation for Mn among these samples was 2.7%. Mn dust loading ($\mu g/m^2$) was calculated by multiplying the Mn concentration (μg/g) by the dust loading (g/m^2).

Questionnaire data

Participants were interviewed shortly before and at the time of dust sample collection. Information obtained included the number of agricultural workers, maternal age and education, maternal country of birth, parity, household income and number of people supported by this income, housing density (number of persons/room), number of doormats at the home, housing type (house, apartment or condominium), number of pets in the home, whether an air conditioner was present in the home, smoking in the home and self-reported distance to the nearest agricultural field.

Geographic data

Based on the latitude and longitude coordinates, geographic information system (GIS) software (ArcInfo 10, ESRI, Redlands, CA) was used to evaluate possible geographic predictors of Mn levels in house dust, including region within the Salinas Valley (North County, Salinas, South County), population density, and median year built of homes in the resident census tract (U.S. Bureau of Census 2001). To account for variations in soil Mn concentrations, each residence was linked to detailed soil maps to identify the soil type (USDA 2013). The National Elevation Data set at 30 m resolution was used to assign an elevation to each home (USGS 2006).

The California Department of Pesticide Regulation maintains the California Pesticide Use Report (PUR) system (CDPR 2013). Pesticide applicators are required to report the active ingredient, quantity applied, acres treated, crop treated, date and location to one square mile in area (Public Land Survey Section (PLSS)) for all agricultural pesticide applications. Nearby maneb and mancozeb use was calculated for combinations of distance from the residence (buffer radii of 500, 1000 and 3000 meters) and time prior to dust sample collection (4, 8 and 16 weeks). Fungicide use near homes was weighted based on the proportion of each square-mile PLSS that was within each buffer around a residence (Gunier et al. 2011). To account for the potential downwind transport of fungicides from the application site, data on wind direction was obtained from the five closest meteorological stations in the study area. The direction of each PLSS centroid relative to residences was determined and weighted fungicide use in a section by the percentage of time that the wind blew from that direction for each time period. The average wind speed and total precipitation was calculated during the 4, 8 and 16 weeks prior to dust sample collection using data from the nearest weather station.

The proportion of bare soil within 3000 meters of each residence was estimated from Landsat remote-sensing data images collected at three time points during dust sample collection: October 4, 1999, April 29, 2000 and October 22, 2000 (USGS 2013). The soil line index was calculated using the Landsat image produced closest in time prior to the dust sample collection by taking the ratio of Landsat band 4 (near infrared) to band 3 (red); bare soil was identified as areas with a ratio between 0.95 and 1.05 (Dematte et al. 2009). Similarly, data from the only available particulate air pollution monitor in the Salinas Valley was used to determine the average particulate matter $< 10\ \mu m$ (PM_{10}) and particulate matter $< 2.5\ \mu m$ ($PM_{2.5}$) concentrations for the four and eight weeks prior to dust sample collection (CARB 2008). Mn emissions from vehicle traffic were estimated at each residence by calculating the traffic density using previously published methods (Gunier et al. 2003) that involve summing vehicle kilometers traveled for all major roads (CDOT 2003) by the length of the road segments within 1,000 m of the residence.

Statistical analysis

The distribution of Mn dust loading was skewed to the right with a long tail; therefore the geometric mean (GM) Mn dust loadings were used in bivariate analyses. Analysis of variance (ANOVA) was used to evaluate bivariate relationships between categorical predictors and Mn dust concentrations and loadings. Spearman rank correlation coefficients were calculated to evaluate bivariate relationships with continuous predictor variables. Variance components models with random intercepts for each residence were used to determine the intraclass correlation coefficient (ICC) of Mn dust concentrations and natural-logarithm transformed Mn dust loadings to assess the variability of Mn dust levels over time within a home. Linear mixed-effects models with random intercepts were used to identify significant predictors ($p<0.1$) of Mn dust concentrations and natural-log transformed Mn dust loadings and estimate the amount of variability in measured levels explained by the model while accounting for the correlation among repeat samples collected from the same residence (Peretz et al. 2002). Potential explanatory variables were first identified that were associated with Mn dust concentrations or loadings with $p<0.2$ from bivariate analyses. Manual forward selection was then used to derive final multivariate models including all explanatory variables that predicted Mn concentrations or loadings with $p<0.1$.

Generalized additive models (GAM) with a 3-degrees-of-freedom cubic spline were constructed to evaluate the shape of the curves between predictor variables and Mn dust concentrations and loadings in our study population. None of the tests for digression from linearity were significant ($p<0.1$), suggesting that relations did not depart from linearity. Therefore, linear terms were included in multiple linear regression models. Outliers were evaluated using standardized residuals from the multivariate mixed-effects models. There were no outliers from the Mn dust concentration and loading models with standardized residuals less than -3 or greater than 3, therefore results are reported including all dust measurements.

A 10-fold cross-validation was performed to evaluate whether our models were over fitting the data by setting aside 10% of the data and rerunning the models (Shao 1993). To further evaluate non-linear responses and interactions among predictor variables, a machine learning procedure called SuperLearner was used to estimate the prediction model (van der Laan et al. 2007). SuperLearner is a statistical package (Polley and van der Laan 2012) available in the programming language R (version 2.15.2, The R Foundation for Statistical Computing, 2012) that combines a set of candidate prediction algorithms specified by the user to create the best performing algorithm and performs a cross-validation procedure to assess the model fit. The following learners from R packages were used for the prediction model: generalized linear models (glm), generalized additive models (gam), Bayesian generalized linear models (bayesglm), random forest models (randomForest) and Lasso and elastic-net regularized generalized linear models (glmnet).

2.3. Results

Distribution of manganese levels in dust

All house dust samples had detectable levels of Mn. The GM and geometric standard deviation (GSD) of Mn dust concentrations during the prenatal and postnatal visits were 150 (2) and 129 (2) µg/g of dust respectively (Table 2.1). The GM and GSD for Mn dust loadings were 435 (7) and 259 (7) µg/m^2 of floor area sampled at the prenatal and postnatal visits. The maximum Mn dust concentration was 414 µg/g and the maximum Mn dust loading was more than 25,000 µg/m^2. Among the 90 residences with dust samples collected at the prenatal and postnatal visits, both Mn dust concentrations ($p<0.001$) and loadings ($p<0.05$) were significantly higher from the prenatal visit. The intraclass correlation coefficients for residences with repeat samples collected were 0.58 for Mn dust concentration and 0.55 for Mn dust loading.

Bivariate analyses of manganese predictors

Table 2.2 provides the levels of Mn dust concentrations and loadings from the prenatal visit (n=371) for categorical predictor variables. Concentrations and loadings of Mn in house dust were significantly higher in homes with farmworkers, residences located in the southern portion of the Salinas Valley, residences located on Antioch Loam soil and residences with poor housekeeping. There were also significantly higher Mn concentrations and loadings in homes where the mother was born in Mexico or had a 6th grade education or less. The Mn dust loadings were higher in residences without a doormat, in samples collected from carpet compared to bare floor or furniture, and in samples collected in the bedroom versus the living room or kitchen. There was no difference in Mn dust concentration or loading based on maternal age, parity, family income or season of dust sample collection.

Table 2.3 presents the distributions of continuous predictor variables and Spearman correlation coefficients with Mn dust concentration and loading are presented. The Spearman correlation was strongest with the number of farmworkers in the home (r_s=0.24, $p<0.01$). There was a negative correlation between precipitation during the 8 weeks prior to dust sample collection and Mn dust concentration (r_s=-0.13, $p<0.01$) and Mn dust loading (r_s=-0.12, $p<0.05$), and a positive relationship with elevation for both Mn dust concentration and loading ($p<0.01$). Average wind speed during the 16 weeks prior to dust sample collection was correlated with Mn dust concentration (r_s= 0.15, $p<0.01$) and loading (r_s= 0.13, $p<0.01$). The median agricultural Mn fungicide use within 3 km of the residence during the 8 weeks prior to dust sample collection was 372 kg and the correlations with Mn dust concentration (r_s=0.11, p=0.04) and loading (r_s=0.10, p=0.05) were significant when agricultural Mn fungicide use was weighted by the proportion of time the wind blew from the direction of the section where the fungicides were applied. The correlation with Mn dust loading (r_s=0.94) was stronger with total dust loading (g/m^2) than with Mn dust concentration (r_s=0.62). Total dust loading (g/m^2) was also correlated with Mn dust concentration (r_s=0.40).

Multivariate analyses

Significant determinants of Mn dust concentration from multivariable linear mixed-effects models are presented in Table 2.4. Dust Mn concentrations were higher in residences in southern Monterey County (β=35.3 μg/g, 95% CI: 21.0, 49.6) and homes located on Antioch Loam soil (β=21.9 μg/g, 95% CI: 7.7, 36.0). There were no homes in southern Monterey County located on Antioch soil so these predictors are independent. Mn dust concentration was also associated with greater house dust loading (g/m^2) with an increase of 9.8 μg/g dust (95% CI: 6.3, 13.4) per interquartile range increase (8.7 g/m^2) in dust loading. Dust Mn concentrations were higher in homes with poor housekeeping (β=18.0 μg/g, 95% CI: 8.9, 27.1), homes with at least one farmworker living in the residence (β=15.3 μg/g, 95% CI: 2.1, 28.6), and with greater wind weighted agricultural Mn fungicide use within 3 km during the 4 weeks prior to dust sample collection (β=6.1 μg/g, 95% CI: 0.6, 11.5). House dust loading explained the greatest amount of variability in Mn dust concentration (6.4%), followed by residence in the southern Salinas Valley (4.0%), poor or average vs. excellent housekeeping practices (3.6%), residence located on Antioch Loam soil (2.7%) and having a farmworker in the home (1.9%).

The percentage change in Mn dust loading for select predictor variables are shown in Figure 2.1. Residence located in southern Monterey county was associated with an increase of 278.5% (95% CI: 143.0, 488.8) in Mn dust loading and residences located on Antioch Loam soil had 140.8% (95% CI: 60.6, 261.5) higher Mn dust loading. The Mn dust loading levels increased 12.6% (95% CI: 3.4, 22.9) per farmworker living in the home and 26.2% (95% CI: 5.9, 50.4) per interquartile range (37 kg) increase in wind weighted agricultural Mn fungicide use within 3 km of the residence during the four weeks prior to dust sample collection. Residences that did not have a doormat and residences with poor housekeeping practices also had higher Mn dust loading. The cross-validation results showed that the models were not over fit, with the same independent variables significant ($p<0.1$) in each subset of the data, similar regression coefficients (±10%) and overall adjusted R^2 values for Mn dust concentration and loading.

Figure 2.2 shows the location of residences where dust samples were collected and estimated Mn dust loading levels from a co-kriging model with linear drift using data on agricultural Mn fungicide use summarized for each PLSS Section centroid. The highest estimated Mn dust loading levels were in the southern Salinas Valley and the lowest levels were in the northwestern county. There was significant spatial autocorrelation of Mn dust loading with Moran's I=0.11 ($p=0.04$), but no spatial autocorrelation for Mn dust concentrations or the residuals from the multivariate mixed effects models for Mn dust concentration or loading. The mean squared errors for the models to predict Mn dust concentrations and loadings were similar using SuperLearner and linear regression for dust samples from the prenatal visit, suggesting that there were not strong interactions or other non-linear relationships that were missed using linear regression models.

2.4. Discussion

Farmworkers living in the home and agricultural use of Mn fungicides within 3 km of the residence were associated with higher Mn concentrations and loadings in house dust. These findings suggest that agricultural use of fungicides-containing Mn is a source of Mn in homes. In a previous analysis, an association was observed between levels of Mn in prenatal dentin of shed teeth from children and Mn dust loading from prenatal dust samples, indicating that higher levels of Mn in the home increases exposure to pregnant women and infants (Gunier et al. 2013). These results add to the existing evidence that household proximity to agricultural pesticide applications and parental occupational take-home increases levels of pesticide constituents in the home (Curl et al. 2002; Fenske et al. 2002; Gunier et al. 2011; Harnly et al. 2009; Ward et al. 2006). Maneb, the primary Mn containing fungicide used in the study area during the study period from 1999 - 2001, was not reregistered for sale in the U.S. after 2011 (USEPA 2011). Exposure to Mn from agricultural fungicides, however, will continue because maneb is still being used internationally and mancozeb, another Mn-containing fungicide, continues to be applied in the U.S. on crops.

Residences located in the southern Salinas Valley had higher Mn dust concentrations and loadings. The predominant wind direction in our study area is from the Pacific Ocean to the west and then down the Salinas Valley to the south, likely resulting in PM accumulating in air downwind of agricultural sources and higher background levels of Mn in southern Salinas Valley air than in the northern Salinas Valley air, which is upwind of agricultural fields. Higher Mn dust concentrations and loadings were observed in residences located on Antioch Loam soil than residences located on other soil types. All soil contains naturally occurring Mn and Mn levels vary by soil type, but little information is available on differences in Mn concentrations by soil type (ATSDR 2008). Homes with greater dust loading had a higher average Mn dust concentration which suggests that the additional dust entering homes is enriched in Mn from soil or agricultural use of Mn fungicides. A strong, inverse linear relationship was found between Mn dust loading levels in residences and more frequent or effective housekeeping practices and the number of doormats used at the home. The dust sample collection methods also affected Mn dust loading including whether samples were collected from carpet or bare floors, and from the child's bedroom or the living room suggesting that the type of surface sample and location of dust sample collection can influence the concentration and loading of Mn in house dust. Single Mn dust measurements appear to be a reasonable long-term indicator of indoor levels with intraclass correlation coefficients among repeat dust samples collected nine months apart of 0.6 for both concentrations and loadings, indicating moderate correlation of Mn levels within a home over time. Although moderately correlated, Mn levels decreased in the present study from the prenatal to postnatal visit, possibly due to improved housekeeping practices after the birth of the child.

Table 5 summarizes median Mn dust concentrations measure in this study and previously published studies. In the present study, the median Mn dust concentration from samples collected during the prenatal home visit (178 µg/g) was higher than previous studies with no known source of Mn (Callan et al. 2013; Chattopadhyay et al. 2003; Kurt-Kurakos et al. 2012; Zota et al. 2011) except for a small study conducted in the United Kingdom (Turner and Simmonds 2006). Zota et al. observed a higher median Mn dust concentration (156 µg/g) among thirteen residences located 500 m or less from agricultural fields than residences located farther from fields, although specific information on pesticide use was not available and the median Mn concentration was lower than our study (Zota et al. 2011). Concentrations of Mn in house dust were higher than the present study in other locations with potential Mn sources including residences located in a district with heavy industry and vehicle traffic in Hong Kong, China (Tong and Lam 2000) and in Ottawa, Canada where methylcyclopentadienyl manganese tricarbonyl (MMT) was added to gasoline (Rasmussen eta al 2001). The results from these studies provide evidence that nearby Mn emission sources increase Mn dust concentrations inside homes which is likely to lead to higher exposure from non-dietary ingestion, especially among young children, and may indicate higher exposure to Mn from inhalation as well.

The median Mn dust loading in our study was 488 µg/m^2, higher than levels reported (50 µg/m^2) in the only other study that measured Mn dust loading (Zota et al. 2011). The relatively higher Mn loadings in our study reflect the dusty conditions in this agricultural community with at least one farmworker living in most of the homes (79%). A stronger correlation was observed in this study between Mn loading and total dust loading ($r_s = 0.94$) than between Mn loading and Mn dust concentration ($r_s = 0.62$). In a Canadian study of other metals in dust, stronger correlations were also observed between metal loading and dust loading ($r_s = 0.79 – 0.92$) than between metal loading and metal concentration ($r_s = 0.17 – 0.58$), indicating that dust loading is the primary factor driving metal loadings in homes (Rasmussen et al. 2013).

There were several limitations of this study. The dust samples were collected from a relatively small geographic area in the Salinas Valley and from a homogeneous population of mostly Mexican-American farmworkers. Only one dust sample was available for most homes with a second sample collected from 25% of the homes within about nine months; therefore longer-term variability could not be evaluated in this study. Because there was only one air monitor available that measured PM concentrations in the study area, only temporal variation in PM and not spatial variation could be accounted for in this study. Air samples were not collected to distinguish between deposition from windblown particles and dust tracked into the homes on shoes. Latitude and longitude coordinates collected using handheld global positioning system units were not compared to address geocoded locations and were not able to evaluate the accuracy of residential locations. As a result, there is likely to be some exposure misclassification in our estimates of proximity to agricultural Mn fungicide use due to measurement error. The identification of bare soil around residences using remote sensing data was challenging due to similarities between urban developed land and bare soil, differences in reflectance between dry and wet bare soil and complex cropping patterns present in the Salinas Valley. There was no spatial autocorrelation for Mn dust concentration among residences and only weak spatial autocorrelation for Mn dust loadings which suggests that differences in Mn levels in the homes were not strongly related to geographic factors. Finally, multivariate models were only able to explain 20-30 % of the variability in Mn concentrations and loadings in dust.

Strengths of the present study include the availability of comprehensive agricultural pesticide use data, a relatively large sample size and a dust sample collection method that allowed for the calculation of Mn dust loading in addition to Mn dust concentration. Extensive interview data was available on potential Mn sources and detailed information on sample collection procedures. An additional strength was the use of GIS methods to estimate proximity to recent agricultural applications of Mn containing fungicides, traffic density, bare soil around the residence and soil type at the residence. Extensive sensitivity analyses were conducted using advanced model selection and GIS techniques that further supported the assumption of linearity in the prediction models. These results provide additional evidence that agricultural pesticide applications result in higher levels of these compounds in nearby homes, especially for pesticides that contain more persistent active ingredients such as Mn (Fenske et al. 2002; Gunier et al. 2011; Harnly et al. 2009; Lu et al. 2000; Ward et al. 2006).

Table 2.1. Distributions of manganese dust concentrations and loadings by home visit for CHAMACOS study cohort.

Variable	N	Mean (SD)	GM (GSD)	Min	25th	50th	75th	Max.
Prenatal Visit Mn Concentration (µg/g)	371	171 (68)	150 (2)	2	134	178	209	414
Postnatal Visit Mn Concentration (µg/g)	97	145 (53)	129 (2)	10	119	148	181	239
Prenatal Visit Mn Loading (µg/m^2)	371	1,908 (3,600)	435 (7)	1	117	488	1,735	25,158
Postnatal Visit Mn Loading (µg/m^2)	97	1,037 (2,070)	259 (7)	1	122	271	710	10,591

Table 2.2. Distributions of manganese dust concentrations and loadings from prenatal home visit (n=371) by demographic and residential characteristics.

Characteristic	N (%)	Mn Concentration (μg/g) Mean (SD)	Mn Loading (μg/m^2) GM (GSD)
Mother's Age			
18 – 24	184 (50%)	172 (69)	434 (8)
25 – 29	125 (34%)	178 (62)	518 (7)
30 – 45	62 (16%)	153 (77)	270 (8)
Mother's birth country			
Mexico	312 (84%)	174 (67)	496 (7)
United States/other	59 (16%)	152 (73)*	262 (7)**
Mother's Education			
≤ 6th grade	156 (42%)	182 (69)	662 (8)
> 6th grade	215 (58%)	162 (66)**	324 (7)**
Parity			
0	142 (38%)	172 (69)	422 (7)
≥ 1	229 (62%)	170 (68)	438 (8)
Family Income			
≤ Poverty line	220 (60%)	173 (68)	518 (8)
> Poverty line	149 (40%)	167 (70)	351 (7)
Housekeeping Quality			
Poor or average	243 (65%)	182 (67)	654 (5)
Excellent	128 (35%)	149 (66)**	184 (9)**
Number of doormats			
0	84 (23%)	184 (66)	665 (7)
1	134 (36%)	163 (64)	446 (6)
2+	153 (41%)	171 (72)	334 (7)*
Surface sampled			
Carpet	323 (87%)	171 (70)	493 (7)
Bare floor/furniture	48 (13%)	169 (53)	245 (9)*
Room sampled			
Bedroom	49 (13%)	178 (56)	812 (9)
Living room/kitchen	322 (87%)	170 (70)	403 (7)*
Antioch Loam Soil			
Yes	59 (16%)	193 (61)	898 (5)
No	312 (84%)	167 (69)**	370 (7)**
Residential Location			
North County	15 (4%)	129 (65)	83 (15)
Salinas	248 (67%)	164 (67)	369 (7)
South County	106 (29%)	194 (65)**	797 (7)**
Farmworker in Home			
Yes	291 (79%)	178 (66)	536 (7)
No	79 (21%)	146 (70)**	198 (8)**

ANOVA * $p<0.05$; ** $p<0.01$

Table 2.3. Distributions of continuous predictor variables and Spearman correlations with manganese dust concentration (µg/g) and loading (µg/m^2) from prenatal visit (n=371).

		Spearman Correlation Coefficient	
Variable	Median (25th – 75th)	Mn Concentration (µg/g)	Mn Loading (µg/m^2)
Housing density (persons/room)	2 (1 – 2)	0.06	0.11*
Number of farmworkers	1 (0 – 3)	0.24**	0.24**
Census tract population density (persons/mi^2)	2,937 (103 – 5,557)	-0.12*	-0.06
Precipitation 4 weeks (mm)	5 (0 – 25)	-0.11*	-0.05
Precipitation 8 weeks (mm)	16 (4 – 51)	-0.13**	-0.12*
Elevation (m)	28 (25 – 56)	0.25**	0.21**
Agricultural Mn Pesticide Use 500 m, 4 weeks (kg)	0 (0 – 2)	0.10*	0.11*
Agricultural Mn Pesticide Use 500 m, 8 weeks (kg)	1 (0 – 5)	0.06	0.05
Agricultural Mn Pesticide Use 1000 m, 4 weeks (kg)	3 (0 – 12)	0.14**	0.11**
Agricultural Mn Pesticide Use 1000 m, 8 weeks (kg)	10 (1 – 26)	0.08	0.05
Agricultural Mn Pesticide Use 3000 m, 4 weeks (kg)	177 (19 – 348)	0.11*	0.08
Agricultural Mn Pesticide Use 3000 m, 8 weeks (kg)	372 (97 – 665)	0.12*	0.09
Agricultural Mn Pesticide Use Wind weighted 3000 m, 4 weeks (kg)	14 (0 – 37)	0.12*	0.09†
Agricultural Mn Pesticide Use Wind weighted 3000 m, 8 weeks (kg)	39 (10 – 75)	0.11*	0.10*
Traffic density (VKT) 1000 m	1,370 (430 – 48,643)	-0.06	0.01
Particulate Matter < 2.5 µm 4 weeks (µg/m^3)	7 (6 – 9)	0.02	0.03
Particulate Matter < 10 µm 4 weeks (µg/m^3)	18 (15 – 22)	0.04	0.06
Percentage of Bare Soil 3000 m (%)	17 (14 – 22)	-0.01	0.00
Dust loading (g/m^2)	3.5 (1.0 – 10.7)	0.40**	0.94**

* $p<0.05$; ** $p<0.01$

Table 2.4. Determinants of manganese dust concentrations (μg/g) from mixed-effects model using samples collected at prenatal and postnatal visits (n=465).

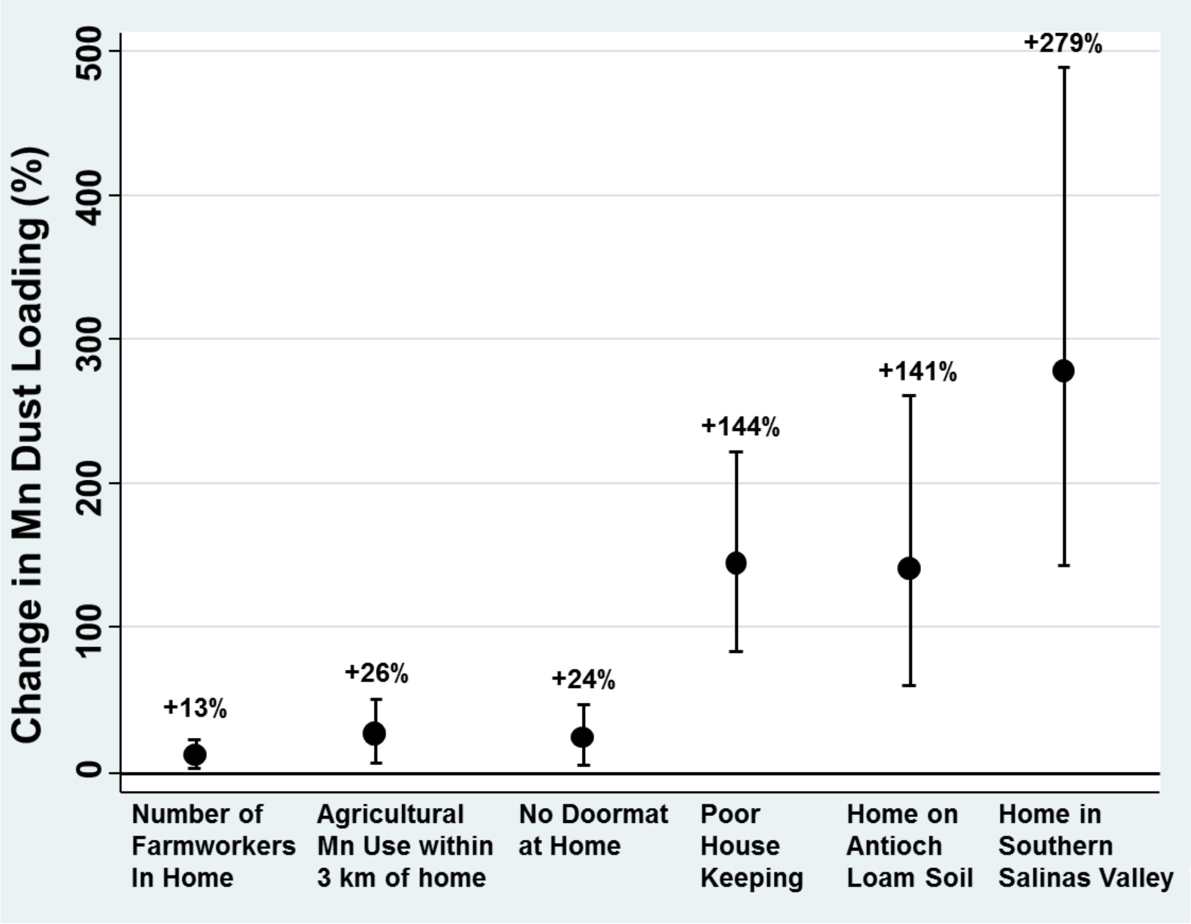
Predictor Variable	β (95% CI)	p-value	Partial R^2
Dust loading (per IQR=8.7 g/m^2)	9.8 (6.3, 13.4)	<0.0001	6.4%
Residence in South County	35.3 (7.7, 36.0)	<0.0001	4.0%
Housekeeping (Poor/average vs. excellent)	18.0 (8.9, 27.1)	<0.0001	3.6%
Antioch Loam soil type (yes vs. no)	21.9 (7.7, 36.0)	0.002	2.7%
Farm worker in the home	15.3 (2.1, 28.6)	0.02	1.9%
Wind weighted Agricultural Mn Use 3000 m, 4 weeks (per IQR =37 kg)	6.1 (0.6, 11.5)	0.03	0.9%
Sample collection time (Prenatal vs. postnatal)	20.4 (10.1, 30.7)	<0.0001	0.9%
Overall model		<0.00001	22.2%

Table 2.5. Summary of studies that have measured manganese concentrations in house dust.

Study and Year Published	Location of Residences	Samples	Median Mn (μg/g)	Sources of Mn
Gunier et al. 2013	Salinas, California	371	178	Agricultural fungicides
Gunier et al. 2013	Salinas, California	291	182	Farmworker in home
Gunier et al. 2013	Salinas, California	106	198	Located in southern County
Callan et al. 2013	Western Australia	156	53[a]	None
Kurt-Kurakos et al. 2012	Istanbul, Turkey	39	136	None
Zota et al. 2011	Ottawa, Oklahoma	55	121	None
Zota et al. 2011	Ottawa, Oklahoma	13	156	Agricultural fields < 0.5 km
Turner et al. 2006	United Kingdom	32	501	None
Chattopadhyay et al. 2003	Sydney, Australia	82	48	None
Rasmussen et al. 2001	Ottawa, Canada	48	267	MMT in gasoline
Tong et al. 2000	Hong Kong, China	151	216	None
Tong et al. 2000	Hong Kong, China	34	283	Heavy industry and traffic

[a] Geometric mean concentration, median not reported.

Figure 2.1. Percentage change in manganese dust loading (μg/m^2) for select predictor variables estimated from multivariable mixed effects models (n=464).



*Adjusted for maternal education, room and surface sampled.

Figure 2.2. Predicted manganese dust loading (μg/m^2) from co-kriging model for prenatal visit (n=371) in the Salinas Valley, California.

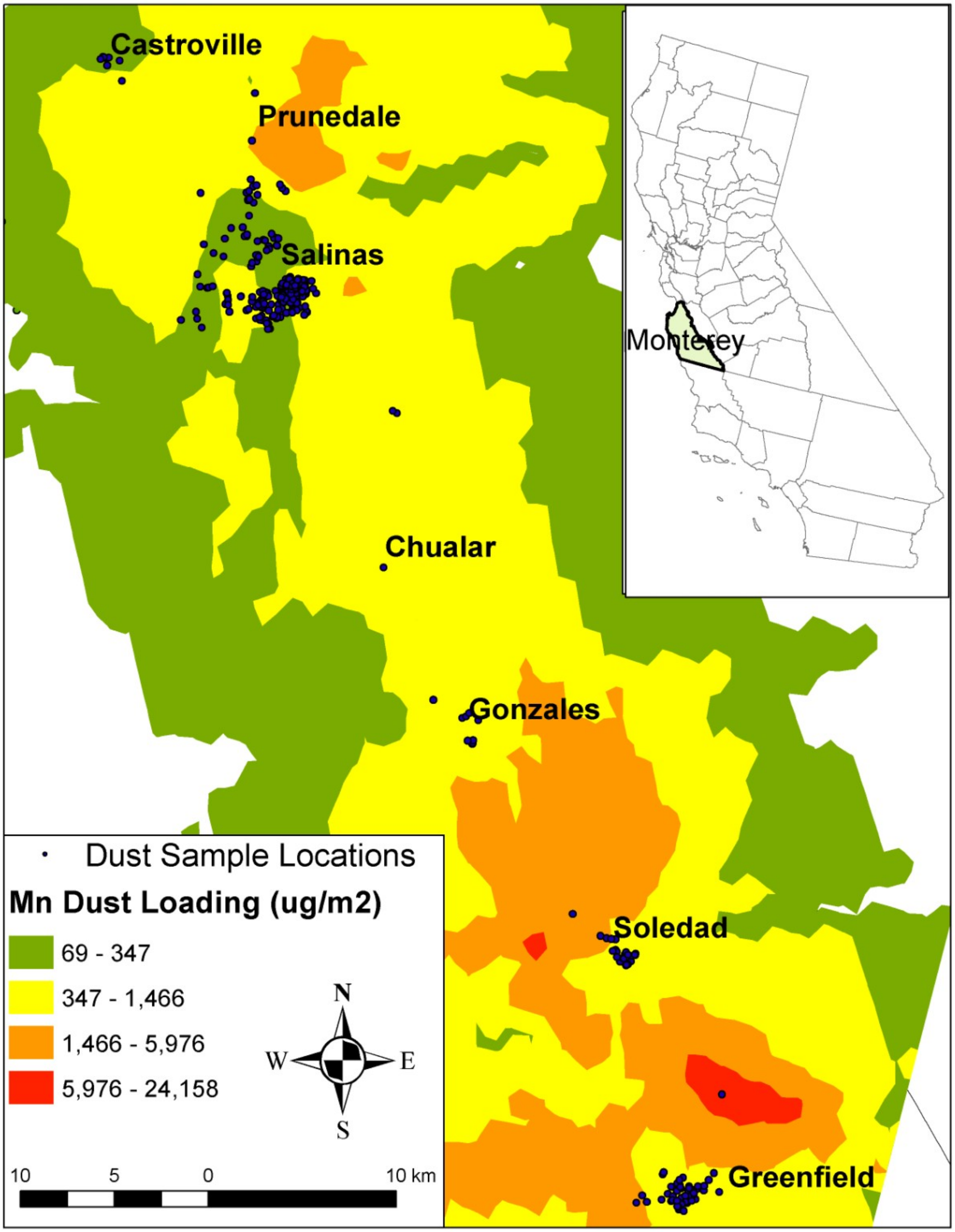


Figure 2.3. Average manganese dust concentration (µg/g) and total agricultural Mn-fungicide use (kg) in the Salinas Valley by month of dust sample collection.

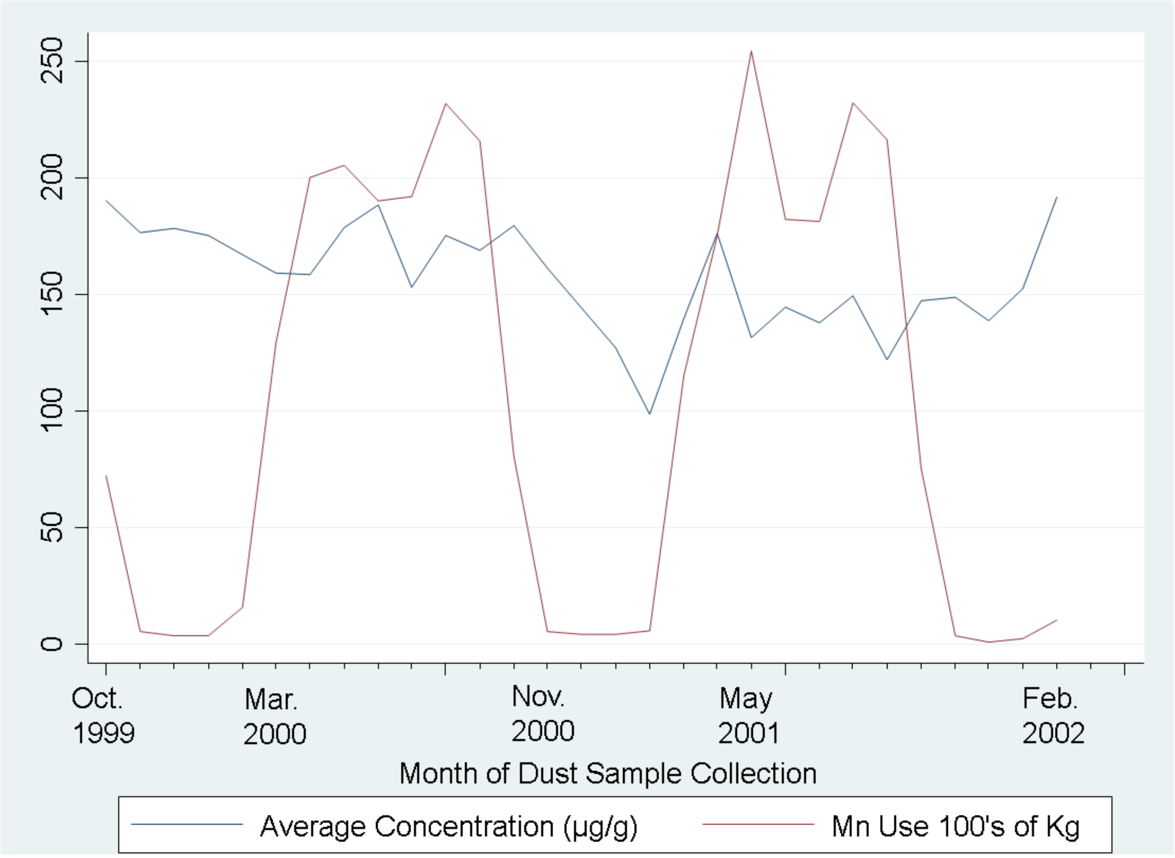


Figure 2.4. Average manganese dust loading (µg/m^2) and total agricultural Mn-fungicide use (kg) in the Salinas Valley by month of dust sample collection.

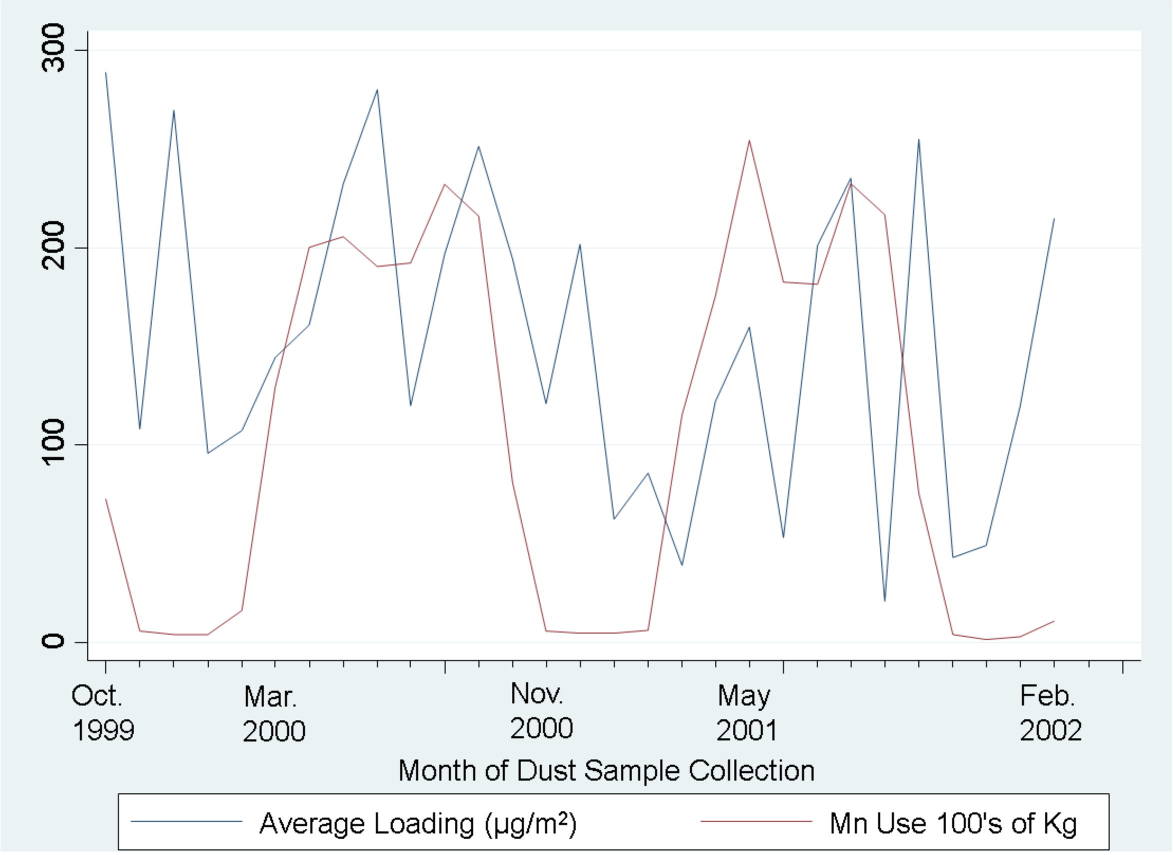


Figure 2.5. Scatter plot of dust manganese concentrations (μg/g) for repeated measurements from dust samples collected at the same residences (n=90) during the second trimester of pregnancy and at 6 months of age.

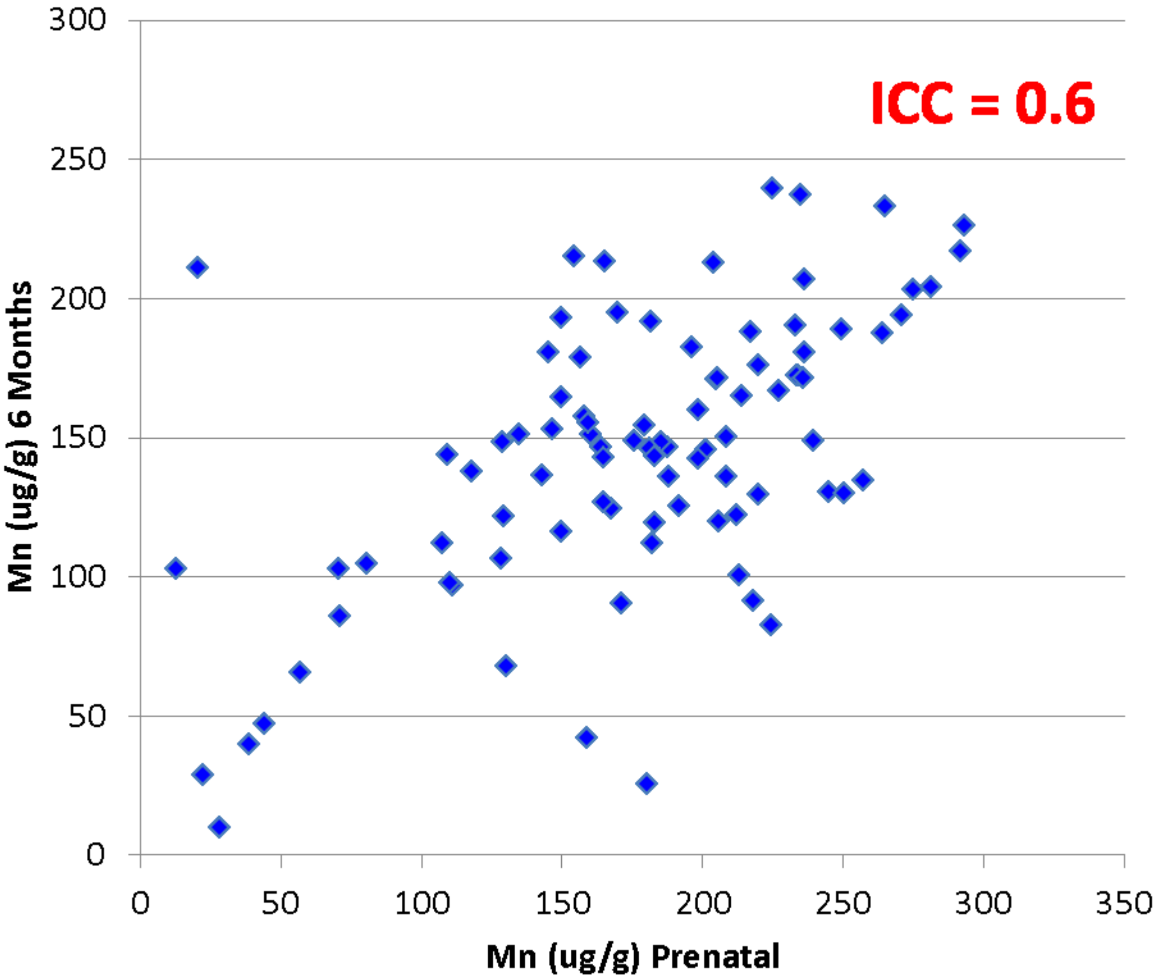
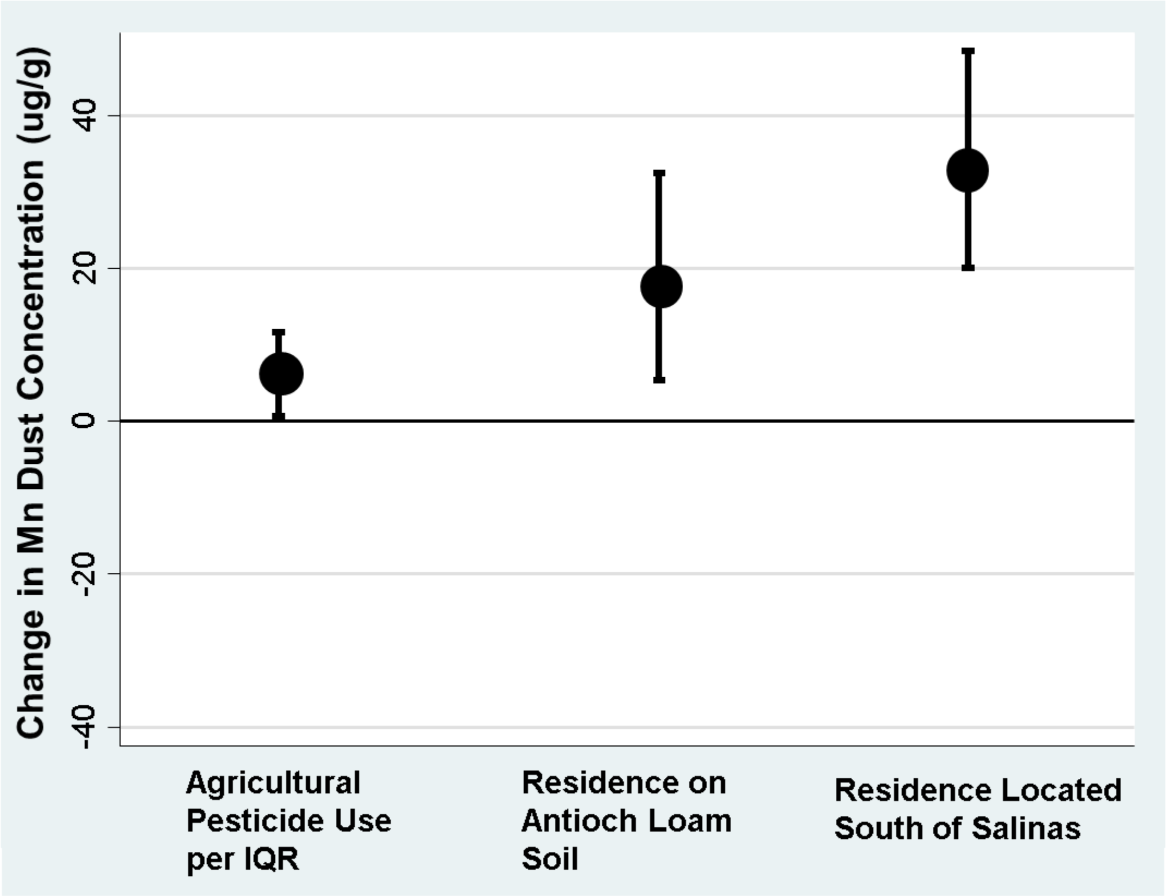


Figure 2.6. Change in manganese dust concentration (µg/g) for select predictor variables estimated from multivariable mixed effects models (n=464).



CHAPTER 3

Determinants of manganese in prenatal dentin of shed teeth from CHAMACOS children living in an agricultural community[1]

3.1. INTRODUCTION

Manganese (Mn) is a naturally-occurring element found in soil, food and water. It is mined for use in metal industries, as a gasoline additive and as an agricultural fungicide (Agency for Toxic Substances and Disease Registry 2008). Mn is an essential nutrient but at high doses it is neurotoxic and can result in a syndrome of neurologic deficits called manganism (Agency for Toxic Substances and Disease Registry 2008). There is a growing body of evidence that early life exposure to Mn, at much lower doses than those that cause manganism, may have detrimental effects on the developing organism (Martin 2006; Roels et al. 2012). In school-aged children, lower cognitive scores have been associated with higher levels of Mn in water (Wasserman et al. 2006), in blood (Kim et al. 2009; Claus-Henn et al. 2010) and in hair (Bouchard et al. 2011; Menezes-Filho et al. 2011; Riojas-Rodriguez et al. 2010; Roels et al. 2012). Pregnancy and the first year of life are potentially vulnerable periods of exposure because Mn crosses the placenta during pregnancy, and young children have increased absorption efficiency and reduced excretion via bile compared to adults (Yoon et al. 2009).

There is no consensus about the best biomarker to assess human exposure to Mn. Occupational studies have generally found no association between Mn inhalation exposure and urinary Mn concentrations (Smith et al. 2007; Laohaudomchok et al. 2011). Blood Mn has been the most commonly used biomarker of exposure, but the short half-life of Mn in blood may miss periods of peak exposure and Mn is well regulated by homeostatic mechanisms in adults (Agency for Toxic Substances and Disease Registry 2008). Higher hair Mn levels have been observed in children living near environmental sources of Mn (Menezes-Filho et al. 2009; Riojas-Rodriguez et al. 2010). However, hair is susceptible to exogenous contamination and methods used for cleaning hair samples prior to analysis may affect the accuracy of Mn measurement in hair (Eastman et al. 2013). Mn levels in nails may be a valid biomarker of cumulative occupational Mn exposure 7 – 12 months earlier (Laohaudomchok et al. 2011). In a rodent study, Mn levels in nail clippings were strongly correlated (R^2=0.93) with Mn levels in the brain (Sriram et al. 2012).

Available biomarkers have a limited ability to assess prenatal exposure to the fetus. Even maternal blood Mn levels measured during pregnancy do not accurately reflect exposure to the fetus as cord blood Mn concentrations are consistently much higher than concentrations in maternal delivery blood Mn (Takser et al. 2004; Zota et al. 2009). Measurement of Mn in deciduous teeth offers a promising biomarker to characterize prenatal and early postnatal Mn exposure. Mn is incorporated directly into developing dentin and current analytical techniques allow for detailed Mn measurements that can be related to specific time periods of neonatal development beginning in the second trimester of pregnancy for incisors (13 to 16 weeks

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[1] A similar version of this manuscript has been published: Gunier, RB, Bradman, A, Jerrett, M, Smith, DR, Harley, KG, Austin, C, Vedar, M, Arora, M, Eskenazi, B. Determinants of manganese in prenatal dentin of shed teeth from CHAMACOS children living in an agricultural community. Environmental Science and Technology. 2013. 47: 11249-11257. Available at: http://pubs.acs.org/doi/abs/10.1021/es4018688.

gestation) and ending 10 - 11 months after birth for primary coronal dentin in molars (Arora et al. 2012).

In this study, Mn in prenatal dentin of shed teeth was analyzed from children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a birth cohort of children living in the Salinas Valley. The fungicides maneb and mancozeb contain approximately 21% Mn by weight. Agricultural use of these Mn fungicides averages 160,000 kg per year in the Salinas Valley of California and more than 90% is used on lettuce (California Department of Pesticide Regulation 2011). The goal was to determine whether Mn levels in dentin during the entire prenatal period (Mn_{PN}) were related to environmental, occupational and dietary sources of Mn exposure. The contribution to Mn_{PN} was evaluated from nearby agricultural Mn fungicide use, soil type, estimated concentrations of Mn in ambient air, farm work by the mother or other members of the household, Mn levels in house dust samples, and estimated prenatal Mn intake from maternal diet and tap water consumption.

3.2. METHODS

Study population

Between September 1999 and November 2000, the CHAMACOS study enrolled 601 pregnant women from health clinics in the Salinas Valley primarily serving low-income families. Participants were eligible if they spoke English or Spanish and qualified for state funding of well-pregnancy care (within 200% of the Federal poverty level). A total of 537 liveborns were followed to delivery, of which 353 participated in a visit when the child reached 7-years. There were 324 teeth collected from 282 children and 237 (73%) of these teeth were analyzed for Mn that were free of obvious defects such as caries and extensive attrition. Analyses for this paper include children who provided a shed incisor with Mn levels measured in prenatal dentin (n=207). Written informed consent was obtained from all participants and all research was approved by the University of California, Berkeley Committee for the Protection of Human Subjects prior to commencement of the study.

Interviews

Mothers were interviewed twice during pregnancy (~13 and ~26 weeks gestation) and shortly after delivery. Trained bilingual bicultural interviewers obtained information on maternal age, country of birth, education level and household poverty level. Information was also obtained regarding potential sources of Mn exposure including maternal farm work during pregnancy, number of farmworkers in the home, number of farmworkers that stored their clothes or shoes indoors and glasses per day of tap water consumed by the mother. Information on the mother's hematocrit to hemoglobin ratio was abstracted from prenatal medical records for a subset of participants (n=161) to assess maternal iron status during pregnancy.

Home visits

A home inspection was conducted during pregnancy (mean gestational age = 14 ± 3 weeks). Latitude and longitude coordinates were collected using global positioning system units and evaluated housekeeping characteristics. House dust samples were also collected and the methods are described in more detail elsewhere (Harnly et al. 2009). Briefly, dust was collected from one square meter area of the residence using a High Volume Surface Sampler (HVS3, Envirometrics, Inc., Seattle, WA) which allows for the calculation of dust loading in grams per square meter of floor area to better characterize Mn in dust available for contact by children (Roberts et al. 2009).

Tooth Mn measurements

Deciduous teeth were collected beginning with the 7-year visit. Participants either mailed or brought in teeth as they were naturally exfoliated. The method for measuring Mn in human teeth has been described in detail elsewhere (Arora et al. 2012; Arora et al. 2011). Briefly, teeth are sectioned in a vertical plane, and microscopy is used to visualize the neonatal line and incremental markings in sectioned teeth samples. The concentrations and spatial distribution of Mn were determined using laser ablation inductively coupled plasma mass spectroscopy. Levels of tooth Mn were characterized by normalizing to measured tooth calcium levels (^{55}Mn:^{43}Ca ratio) to provide a measure independent of variations in tooth mineral density. Values are the area under the curve (AUC x 10,000) for points measured during the 2nd trimester and 3rd trimesters separately, and combined into a prenatal average value (Mn_{PN}). The coefficient 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 dentin measurements.

Mn dust measurements

Of the 207 children with a tooth analyzed for Mn, 131 had dust samples collected from the maternal residence during pregnancy. Dust samples were stored at -80 °C for approximately ten years before shipping them on dry ice for analysis. The dust samples were passed through a 150 µm sieve and digested them overnight in 7.5 N nitric acid. Mn concentrations were quantified in dust (µg/g) using inductively coupled plasma optical emission spectroscopy with a limit of detection of 0.1 µg Mn/g dust. Mn dust loading (µg/m^2) was calculated by multiplying the Mn concentration (µg/g) by the dust loading (g/m^2) obtained by weighing the sieved dust sample and dividing by the area sampled.

Mn fungicide use

The California Department of Pesticide Regulation maintains the comprehensive California Pesticide Use Report (PUR) system (California Department of Pesticide Regulation 2011). Pesticide applicators are legally required to report the active ingredient, quantity applied, acres treated, crop treated, date and location to one square mile in area (Public Land Survey Section or Section) for all agricultural pesticide applications. Geographic information system (GIS) software (ArcInfo 10, ESRI, Redlands, CA) was used to geocode residential locations using the latitude and longitude coordinates and to calculate kilograms of maneb and mancozeb reported in the PUR data for combinations of distance from the residence (buffer radii of 1, 3 and 5 kilometers) and trimester of pregnancy based on gestational age (Figure 3.1). Fungicide use near homes was weighted based on the proportion of each square-mile Section that was within the buffer around a residence (Gunier et al. 2011). To account for the potential downwind transport of fungicides from the application site, data was obtained from the five closest meteorological stations in the study area on wind direction to determine the percentage of time during each trimester that the wind blew from each of eight directions. The direction of each section centroid was determined relative to residences and weighted fungicide use in a Section by the percentage of time that the wind blew from that direction for each trimester. Since 90% of agricultural Mn fungicides are used on lettuce in the Salinas Valley, we used Monterey County crop maps for spring, summer and fall of 1997 (California Department of Water Resources 1997) to estimate the acres of lettuce within 1, 3 and 5 km of residences during each trimester.

Mn intake from tap water

The geocoded residential locations were linked to the appropriate drinking water system using customer service area boundaries provided by local drinking water companies and the state of California (California Department of Public Health 2012) Public drinking water systems provide monitoring data on Mn concentrations (µg/L) sampled at water distribution points (California Department of Public Health 2012). However, Mn was not frequently detected in the study area during the pregnancy period for our cohort. Therefore, the average Mn concentration (µg/L) of all available samples from a water system was used to estimate long-term average concentrations of Mn in tap water. Tap water consumption (L/day) was estimated using questionnaire data on the number of glasses of tap water consumed per day (8 ounces per glass). Consumption was multiplied by the average Mn concentration to estimate the average daily Mn intake (µg/day) from tap water during each trimester.

Dietary Mn and iron intake

Mothers were interviewed about their dietary intake at the time of the second prenatal interview (27 ± 3 weeks gestation) using a modified Spanish-language Block food frequency questionnaire (Block 1990) specifically adapted for this study population (Harley et al. 2005). For each food item, frequency of consumption (ranging from never up to 4+/day) and usual portion size (small, medium, or large relative to a given standard portion) were assessed for the previous year. The mean Mn concentration for each food/beverage item and the daily Mn intake (mg/day) for each women was estimated using the average frequency and portion-size of each food and beverage reportedly consumed in a day in combination with food-specific Mn estimates from the Total Diet Study data from 1991 – 2005 (United States Food and Drug Administration 2007). Mn intake from dietary supplements was also included. Because iron deficiency might increase Mn uptake, daily iron intake was estimated using similar methods for use as a covariate in the models (Claus-Henn et al. 2011). For a subset of participants (n=159), hematocrit to hemoglobin ratios were also available as a measure of anemia.

Other Mn sources

Exposure to other potential sources of Mn was estimated, including soil type at the residence, estimated Mn concentration in outdoor air, and motor vehicle traffic. To account for variations in soil Mn concentrations, each residence was linked, based on latitude and longitude coordinates, to detailed soil maps (United States Department of Agriculture 2009). To account for exposure via air inhalation, residences were assigned to a 2000 census tract and linked them to estimated 2002 Mn concentrations in ambient air from U.S. EPA (United States Environmental Protection Agency 2009). Mn emissions from vehicle traffic were estimated at each residence by calculating the traffic density using previously published methods that involve summing vehicle kilometers traveled for all major roads (California Department of Transportation 2007) by the length of the road segments within 500 m of the residence (Gunier et al. 2003).

Statistical analysis

ANOVA was used for bivariate analysis of categorical predictor variables and the Spearman correlation coefficient to evaluate continuous predictor variables Potential explanatory variables were identified for inclusion in multivariable regression models that were associated with Mn_{PN} levels with $p<0.2$. Mn tooth levels were skewed to the right and were natural log-transformed to normalize the distributions for regression models. Manual forward selection was used to derive final multivariable linear regression models to determine which Mn exposure sources (proximity to maneb and mancozeb fungicide use, soil type at the residence, estimated dietary and drinking water Mn intake, motor vehicle traffic density, estimated concentration of Mn in outdoor air, maternal smoking, maternal hematocrit to hemoglobin ratio, etc.) were significantly associated ($p<0.1$) with Mn levels in dentin during the prenatal period. Backwards elimination was also used as an alternative method to identify significant predictor variables. The percentage change associated with each exposure source was estimated by exponentiating the regression coefficients, subtracting one and multiplying by 100. Outliers were identified and models were rerun excluding one participant with a studentized t-score >3 that also had the lowest measured Mn_{PN} level (0.06).

The final models included one using data available for all children with Mn_{PN} measured in teeth (n=206) and another for the subset that had both tooth and house dust Mn measurements (n=130). Model fit was evaluated using residual plots, log likelihood tests and Aikake's Information Criterion. Non-linear relationships were identified between continuous predictor variables and tooth Mn levels using penalized splines with 3 degrees of freedom in general additive models. Moran's Global I was used to assess residual spatial autocorrelation of Mn_{PN} levels for the final models. Mn levels in prenatal dentin from the 2nd trimester were compared to levels from the 3rd trimester using a paired t-test and ran separate regression models by trimester to evaluate significant predictors by trimester. Since Mn dust loading is likely to be on the casual pathway for some of our predictors of exposure, such as farmworker shoes in the home and proximity to agricultural use of Mn fungicides, a structural equation model was used to evaluate casual pathways of exposure in the model that included participants with Mn dust measurements (Davis et al. 2012). A structural equation model was constructed to simultaneously estimate Mn tooth levels and Mn dust loading as outcome variables, with Mn dust loading also included as a predictor variable in the Mn tooth model.

2.3 RESULTS

Descriptive statistics and bivariate analyses

Most mothers included in the analyses were not born in the U.S. (88.9%), did not finish high school (77.8%) and lived at or below the poverty line (59.3%). The distributions and Spearman correlations (ρ_s) between Mn_{PN} and continuous Mn sources are provided in Table 3.1. The mean and median Mn_{PN} was 0.51 (^{55}Mn:^{45}Ca AUC x 10^4) and the interquartile range was 0.38 – 0.58. The median ^{55}Mn:^{45}Ca ratio corresponds to approximately 0.04 µg/g dentine based on calibration using dissolved dentin in solution (Arora et al. 2012). The mean concentration and loading of Mn in house dust were 165 µg/g and 1,705 µg/m^2 respectively. The mean agricultural Mn fungicide use within 3 km of the residence during the 2nd trimester and 3rd trimester was 627 kg. Mn dust loading was most highly correlated with Mn_{PN} ($\rho_s = 0.27$, $p<0.01$). Agricultural Mn fungicide use during the 2nd and 3rd trimesters within 3 km ($\rho_s =0.16$) and 5 km ($\rho_s=0.14$) were also significantly correlated ($p<0.05$) with Mn_{PN}. Accounting for the percentage of time that the residence was down wind of fungicide applications during the 2nd and 3rd trimesters (to weight agricultural Mn fungicide use within 3 km) did not improve the correlation with Mn_{PN} ($\rho_s=0.11$). Significant correlations between Mn_{PN} and Mn fungicide use within 1 km or Mn fungicide use during the first trimester were not observed (data not shown). Estimated prenatal Mn exposures from traffic density within 500 m of home, estimated outdoor Mn air concentrations, maternal dietary Mn intake, Mn tap water concentration and maternal Mn tap water intake were not significantly correlated with Mn_{PN}.

Significantly higher ($p<0.01$) Mn prenatal tooth levels were observed in children whose mothers were born outside the U.S., had less than a high school education, and did farm work during pregnancy (Table 3.2). Children living in homes with farmworker shoes stored indoors or located on Antioch Loam soil also had higher Mn tooth levels. Maternal smoking during pregnancy, although rare (<5%), was associated with significantly lower Mn levels in prenatal dentin and the largest difference in mean Mn_{PN} was among children whose mother's smoked during pregnancy (0.36 ± 0.17) compared those who did not (0.52 ± 0.19).

Determinants of Mn_{PN}

The percentage change and 95% Confidence Intervals (95% CI) of Mn_{PN} for predictor variables from multivariable regression models are presented in Table 3.3. In the model including all children with prenatal Mn tooth measurements (n=206), Mn_{PN} levels significantly increased with maternal farm work during the prenatal period (10.1%; 95% CI=0.1%, 21.5%), the number of farm workers storing their shoes in the home (8.1% per farm worker; 95% CI=4.3%, 12.0%), prenatal residence located on Antioch Loam soil (15.1%; 95% CI=4.6%, 26.6%) and agricultural use of Mn fungicides within 3 km of maternal residences during the 2nd and 3rd trimesters (4.9% per interquartile range (809 kg); 95% CI=0.9%, 9.1%). In the model that included children with both tooth and house dust Mn measurements (n=130), Mn_{PN} levels increased significantly with maternal farm work (15.8%; 95% CI=1.9%, 31.6%), prenatal residence located on Antioch Loam soil (20.7%; 95% CI=6.3%, 37.1%) and Mn dust loading. An increase in Mn house dust loading equivalent to the interquartile range (1,465 $\mu g/m^2$) was associated with a 3.3% increase (95% CI=0.3%, 6.4%) in Mn_{PN}. Maternal smoking during pregnancy was associated ($p<0.01$) with a 34% and 40% decrease in Mn_{PN} levels in models with and without Mn house dust loading, respectively. Variables that were not significant predictors of Mn_{PN} ($p>0.1$) were excluded from multivariable models, including traffic density, Mn outdoor air concentration, acres of lettuce near the home, estimated total dietary Mn and iron intake, tap water consumption, estimated prenatal Mn tap water concentration and Mn tap water intake, maternal country of birth, maternal education, household income, housekeeping practices, and maternal hematocrit to hemoglobin ratio during pregnancy. The coefficient of determination (R^2) was 22% for the model with Mn tooth measurements and 29% for the model including both Mn tooth and house dust measurements. The final models were identical using either manual forward selection or backwards elimination. There was no spatial autocorrelation between the residuals for either model (Moran's I=0.03, p=0.7).

Table 3.3 also provides the proportion of the variance explained (partial r^2) for the predictor variables from multivariable models of Mn_{PN} for all children with tooth measurements (n=206) and those with Mn measured in both teeth and dust (n=130). The number of farm workers storing shoes in the home (8.4%), maternal smoking during pregnancy (6.7%), prenatal residence on Antioch Loam soil (4.0%) and agricultural use of Mn fungicides within 3 km of residence (2.8%) explained the greatest amount of variability of Mn_{PN} in the model without Mn house dust loading. Maternal smoking (9.0%), prenatal residence on Antioch Loam soil (6.4%), the number of farm workers storing shoes in the home (5.2%), Mn house dust loading (3.6%) and maternal farm work during pregnancy (3.9%) explained the largest proportion of variability of Mn_{PN} in the model for children that also had Mn measured in prenatal house dust.

Using structural equation models, the same predictor variables were significant and no new significant predictors of Mn levels in teeth were identified. The percentage change and significance level was nearly identical for maternal smoking, maternal farm work and residence on Antioch Loam soil which were predictors of Mn levels in teeth. However, agricultural use of Mn fungicides near the home and the number of farmworker shoes stored in the home were significant predictors of Mn dust loading. As a result, the percentage change associated with an increase in Mn dust loading corresponding to the interquartile range was 17.4% in the structural equation model compared to 3.4% in the ordinary regression model because Mn dust loading now included the effects of agricultural Mn fungicide use near the home and farmworker shoes stored in the home (Figure 3.5).

Determinants of trimester specific Mn dentin levels

Median Mn levels were significantly higher ($p<0.001$) in dentin formed during the 2nd trimester (0.59 $^{55}Mn:^{45}Ca$ AUC x 10^4) than dentin formed during the 3rd trimester (0.35) (Table 3.1). The coefficient of variation (standard deviation/mean) was much higher for Mn in 3rd trimester dentin (49%) than Mn in 2nd trimester dentin or Mn_{PN} (36%) indicating relatively more variability. In models of Mn in 2nd trimester dentin with the same potential predictor variables as used in models of Mn_{PN}, maternal smoking and farm work during pregnancy were no longer significant predictors and the R^2 values were slightly lower than the Mn_{PN} models (data not shown). In models of Mn levels in 3rd trimester dentin, only farmworker shoes in the home, agricultural Mn use and Mn house dust loading remained significant ($p<0.05$) predictors and the R^2 values were about half of those from models of Mn in prenatal dentin (data not shown). There were fewer children with Mn measurements in 2nd and 3rd trimester dentin, reducing the number of participants who smoked or did farm work during pregnancy and the corresponding power to detect an association.

3.4. DISCUSSION

Mn levels measured in prenatal dentin using laser ablation inductively coupled plasma mass spectroscopy were associated with estimates of prenatal environmental Mn exposure. These findings suggest that deciduous teeth provide a biomarker of prenatal Mn exposure that is available retrospectively for the study of Mn related health effects, which would be especially useful in case-control studies. Agricultural applications of widely used Mn-containing fungicides, maneb and mancozeb, were found to contribute to higher Mn tooth levels in this population of children living in an agricultural community. This is the first study to evaluate Mn measurements in deciduous teeth as an age-specific indicator of exposure from agricultural or industrial use of Mn. The only previous study that assessed Mn exposure from fungicides found that pregnant women who reported pesticide spraying less than a kilometer from their house had significantly higher blood Mn concentrations in a community where apple orchards were sprayed with mancozeb (Takser et al. 2004). An evaluation of Mn concentrations in house dust found higher levels in residences located within 500 meters of agricultural fields than residences located farther from fields (Zota et al. 2011), Ethylenethiourea measured in urine has been used as an indicator of occupational exposure to maneb and mancozeb (Colosio et al. 2002). In the CHAMACOS cohort, ethylenethiourea was detected in 24% of maternal urine samples collected near the beginning of the second trimester suggesting maternal exposure to maneb occurred during pregnancy in this cohort (Castorina et al. 2010).

The results presented here add to the existing evidence that household proximity to farmland and parental occupational take-home increases children's exposure to other classes of pesticides (Bradman et al. 2009; Curl et al. 2002; Lu et al. 2000; Harnly et al. 2009). Importantly, agricultural-related variables such as farm work by the mother, storage of farmworker's shoes indoors and agricultural use of Mn containing fungicides within 3 km of the residence were significantly associated with increased tooth Mn levels and along with maternal smoking explained the largest proportion of the variance in this cohort. Including Mn house dust loading in the ordinary regression model reduced the amount of variability explained by the number of farmworkers storing shoes in the home and agricultural use of Mn fungicides, and based on a structural equation model this was a result of Mn dust loading being on the casual exposure pathway for Mn from these sources. Nevertheless, the predictors that were identified explained only 22 – 29% of the variability in Mn levels in prenatal dentin suggesting that other unknown factors contributed to Mn body burden.

Iron status and iron metabolizing genes such as hemochromatosis (HFE) and transferrin (TF) may play an important role in Mn biomarker levels. Mn levels in blood were 12% lower among women carrying any variant allele of HFE than women with no variant alleles and these results were replicated in a knockout mice model, suggesting that HFE contributes to variability in Mn exposure biomarkers (Claus-Henn et al. 2011). Mn levels in hair and estimated ambient Mn air concentrations near a ferromanganese refinery in Ohio were significantly correlated only when HFE or TF genotypes were included in the models (Haynes et al. 2010). Women with low serum ferritin levels had higher blood Mn levels than the normal group in Korea (Kim et al. 2011). A limitation of the present study is that information was not available for iron-metabolizing genes HFE and TF or serum ferritin levels. A relationship was not observed between maternal hematocrit to hemoglobin ratio or estimated dietary iron intake during pregnancy and Mn_{PN}.

Although there were few mothers that smoked in this population (<5%), significantly lower Mn_{PN} levels were observed in children whose mother smoked during pregnancy in multivariable models. One previous study also observed a negative relationship between smoking and Mn blood levels in the second trimester but not at delivery (Takser et al. 2004), while a national study in Korea also found lower Mn blood concentrations among current and former smokers (Lee et al. 2011). Similar findings have previously been reported in relation to placental transfer of zinc; umbilical cord blood zinc levels were lower in mothers who smoked during pregnancy compared to non-smokers (Kuhnert et al. 1987). Mn is an essential nutrient that protects against oxidative stress (Aguirre et al. 2012). As a result, Mn levels in blood may be lower in smokers and less available for fetal transfer in pregnant smokers. Further studies are needed to evaluate the relationship between smoking and biomarkers of Mn and to identify the mechanisms by which smoking reduces Mn transfer to the fetus. We also found higher Mn_{PN} levels in children whose prenatal residence was located on Antioch Loam, soil which can be high in manganese content (United States Department of Agriculture 1977) A previous study found an association between Mn levels in soil outside the residence and Mn concentrations in house dust, showing that Mn levels in the home can be influenced by Mn soil concentrations (Zota et al. 2011).

Previous exposure studies found that Mn levels in children's hair decreased with residential distance from a ferromanganese alloy plant, and residential duration and proximity to the plant explained 37% of the variance (Menezes-Filho et al. 2009). A recent study using a new method for cleaning hair prior to analysis found significantly higher Mn levels in children living in the vicinity of active, but not historic, ferroalloy plant emissions (Eastman et al. 2013). Mn measured in personal air for 38 children living near a ferromanganese refinery were associated with distance to the refinery but Mn in blood and hair were not (Haynes et al. 2012). Higher nitrogen dioxide concentrations, a proxy for motor vehicle emissions, have been associated with higher Mn levels in cord blood (Lin et al. 2012). There was no association between Mn_{PN} and traffic density in this analysis, which is relatively low in our study area, but there was a borderline significant (p=0.16) increase in Mn_{PN} with estimated outdoor Mn air concentrations in the model that included Mn house dust loading.

Higher Mn levels were observed in teeth during the 2nd trimester than the 3rd trimester while previous studies have found higher maternal blood Mn concentrations later in pregnancy (Takser et al. 2004; Zota et al. 2009). While maternal blood Mn levels fluctuate during pregnancy, they do not necessarily reflect variations in fetal exposure. The use of dentin Mn allows the measure fetal Mn exposure directly and higher Mn levels were observed in dentin formed during the 2nd trimester in comparison to dentin formed later in gestation. There are no known variations in tooth mineralization over this period that would affect Mn uptake in dentin, and it is possible that the higher Mn levels in dentin formed during the 2nd trimester reflect increased fetal uptake.

Future studies should assess the within person variability in Mn_{PN} using multiple teeth per child and evaluate Mn levels in different types of teeth that develop at slightly different times. This study had a number of other limitations. Information on time-activity patterns for the mothers was not available for this study and using only residential locations to assess proximity to Mn fungicide use and other Mn sources could result in misclassification of exposure. Personal environmental or duplicate diet samples were not collected to measure Mn exposure. Drinking water quality data was collected for regulatory purposes not to determine exposure levels and sampling occurred irregularly over time. Most of the study population (>82%) drank less than one glass per day of tap water and Mn was not detected frequently in public water supplies in the study area. Future studies should collect tap water samples for Mn analysis to better characterize potential exposure from drinking water. Data from the Total Diet Study was used to estimate Mn intake via food items but this study may not be representative of Mn levels in food consumed by our population, however, the primary source of dietary intake in our population was from prenatal vitamin supplements.

Strengths of this study include extensive prenatal questionnaire data and prenatal house dust samples with measured Mn concentrations and loadings for a subset of participants. Mn levels in dentin were measured for specific prenatal time points using knowledge of tooth mineralization instead of digesting the entire tooth and combining prenatal and postnatal exposures. A previous study used measurements in tooth enamel to estimate Mn exposure (Ericson et al. 2007); however measurements in enamel cannot be readily linked to developmental timing of exposure because, unlike dentin, initial deposits of enamel matrix are not completely mineralized immediately but rather more slowly and diffusely during maturation. An additional strength of this study is the availability of prenatal latitude and longitude coordinates which allowed the use of GIS methods and publically-available data on agricultural pesticide use, drinking water, hazardous air pollutants and traffic density resulting in limited exposure information bias. The current study was also able to evaluate a comprehensive set of exposure predictors including occupational information, household and demographic characteristics, dietary intake, drinking water consumption, outdoor air concentrations and house dust levels.

Table 3.1. Distributions and Spearman correlations for Mn in prenatal dentin (^{55}Mn:^{43}Ca AUC) and continuous prenatal Mn sources.

Variable	N	Mean ± SD	Percentiles 25th	50th	75th	$\rho_{Spearman}$ With Mn_{PN}
Mn in Dentin						
Prenatal Mn Dentin	207	0.51 ± 0.19	0.38	0.51	0.58	1.00
2nd Trimester Mn Dentin	178	0.62 ± 0.22	0.47	0.59	0.70	0.86**
3rd Trimester Mn Dentin	188	0.39 ± 0.19	0.26	0.35	0.44	0.76**
Potential sources of Mn exposure						
Lettuce fields within 3 km (acres)[a]	207	68 ± 23	54	71	80	0.02
Agricultural Mn within 1km (kg)[b]	207	34 ± 55	0.8	14	42	0.03
Agricultural Mn within 3km (kg)[b]	207	627 ± 533	148	539	957	0.16**
Agricultural Mn within 5km (kg)[b]	207	2,120 ± 1,660	461	2,070	3,430	0.14*
Mn Outdoor Air (ng/m^3)[c]	207	1.3 ± 0.4	0.9	1.3	1.5	0.08
Dietary Manganese (mg/day)[d]	195	4.1 ± 1.4	3.3	4.0	5.0	-0.01
Mn Tap Water (µg/L)[e]	194	29 ± 81	15	15	24	-0.03
Mn House Dust Concentraion (µg/g)	131	165 ± 75	126	176	202	0.25**
Mn House Dust Load (µg/m^2)	131	1,705 ± 3,343	99	415	1564	0.27**

[a] Determined from detailed crop maps (California Department of Water Resources, 1997). [b] Calculated using agricultural fungicide use reporting data for maneb and mancozeb during the second and third trimester of pregnancy (California Department of Pesticide Regulation, 2011). [c] Estimated outdoor air concentration for 1999 by U.S. census tract (United States Environmental Protection Agency, 2009). [d] Estimated from prenatal food frequency questionnaire data combined with Mn levels in food items from Total Diet Study (United States Food and Drug Administration, 2007). [e] Estimated from water service area boundaries (California Department of Public Health, 2012a) combined with drinking water data for Mn (California Department of Public Health, 2012b).
* $p<0.05$; ** $p<0.01$.

Table 3.2. Mean and standard deviation of manganese in prenatal dentin (^{55}Mn:^{43}Ca AUC) by categorical demographic and household characteristics (n=207).

Characteristic	N (%)	Mn Prenatal Dentine Mean ± SD
Child gender		
Boy	88 (42.5)	0.50 ± 0.17
Girl	119 (57.5)	0.52 ± 0.20
Maternal Age (years)		
18 – 24	79 (38.2)	0.48 ± 0.17
25 – 29	76 (36.7)	0.55 ± 0.20
30 – 34	34 (16.4)	0.53 ± 0.20
35 – 45	18 (8.7)	0.47 ± 0.13†
Mother smoked during pregnancy		
Yes	10 (4.8)	0.36 ± 0.17
No	197 (95.2)	0.52 ± 0.19**
Mother born in United States		
Yes	23 (11.1)	0.39 ± 0.13
No	184 (88.9)	0.53 ± 0.19**
Maternal Education		
≤ 6th– 12th Grade	161 (77.8)	0.53 ± 0.19
≥ High School Graduate	46 (22.2)	0.44 ± 0.16**
Family Income		
≤ Poverty line	115 (59.3)	0.54 ± 0.20
> Poverty line	79 (40.7)	0.48 ± 0.18†
Housekeeping practices		
Average/Poor	119 (60.1)	0.54 ± 0.23
Excellent	79 (39.9)	0.47 ± 0.16*
Farmworker shoes stored inside home		
Yes	68 (32.9)	0.57 ± 0.22
No	139 (67.1)	0.48 ± 0.16**
Drank ≥1 glass tap water/day		
Yes	38 (18.4)	0.55 ± 0.23
No	169 (81.6)	0.50 ± 0.18
Mother farmwork during pregnancy		
Yes	78 (37.7)	0.56 ± 0.21
No	129 (62.3)	0.48 ± 0.17**
Residence on Antioch Loam soil[a]		
Yes	77 (37.2)	0.56 ± 0.20
No	130 (62.8)	0.48 ± 0.18**

[a] Determined from soil survey data (USDA, 2009).

†p<0.1; *p<0.05; **p<0.01

Table 3.3. Percent change of Mn in prenatal[a] dentine and partial coefficient of determination (r^2) for prenatal predictor variables in multivariable models.

	Children with Tooth Mn Levels (n=206)			Children with Tooth and Dust Mn Levels (n=130)		
Predictor Variable	% Change[b] (95% CI)	p-value	Partial r^2 (%)	% Change[b] (95% CI)	p-value	Partial r^2 (%)
Maternal farmwork (Prenatal yes vs. no)	10 (0.1, 21)	0.05	1.8	16 (1.9, 32)	0.03	3.9
Farm worker shoes in home (Prenatal per worker)	8.1 (4.3, 12)	<0.001	8.4	6.4 (1.5, 11)	0.01	5.2
Agricultural Fungicide Use Prenatal within 3 km (per IQR[c] = 809 kg)	4.9 (0.9, 9.1)	0.02	2.8	3.4 (-1.6, 8.6)	0.19	1.4
Soil Type (Antioch Loam vs. other)	15 (4.6, 27)	0.004	4.0	21 (6.3, 37)	0.004	6.4
Mother smoked (Prenatal yes vs. no)	-34 (-47, -18)	<0.001	6.7	-40 (-55, -20)	0.001	9.0
Mn dust loading (per IQR[c] = 1465 μg/m^2)	-		-	3.3 (0.3, 6.4)	0.03	3.6
R^2 for Model			22%			29%

[a] Prenatal = 2nd and 3rd trimesters. [b] Percent change = (exp(β)-1)*100; [c] IQR = interquartile range.

Figure 3.1. Map of agricultural manganese fungicide use in the Salinas Valley, California for 1999 – 2001 by Public Land Survey System section grid, prenatal residential locations (◦) and illustration of 1, 3 and 5 kilometer radius buffers around a residence (•).

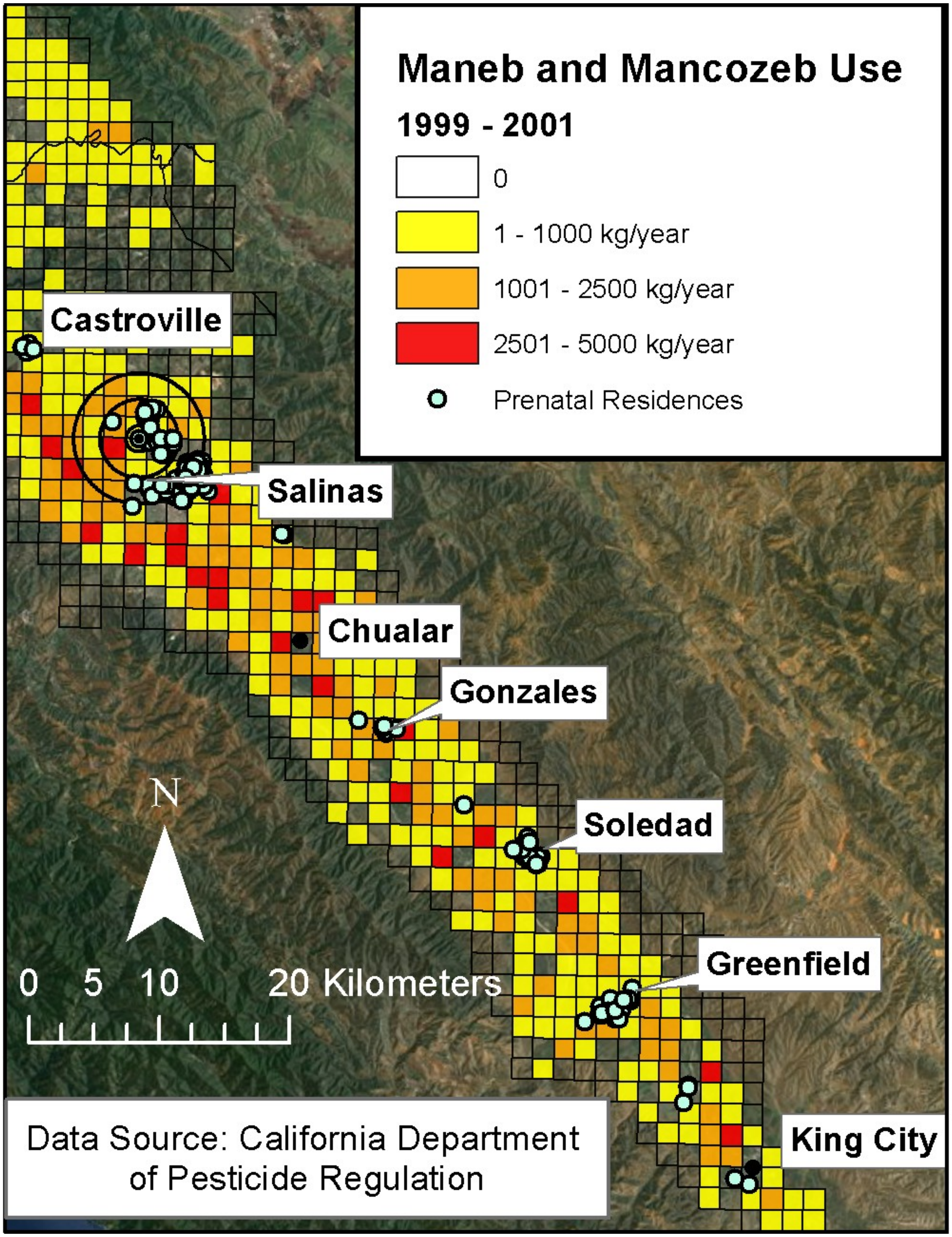


Figure 3.2. Manganese levels in teeth (Mn:Ca AUC x 10,000) by time period.

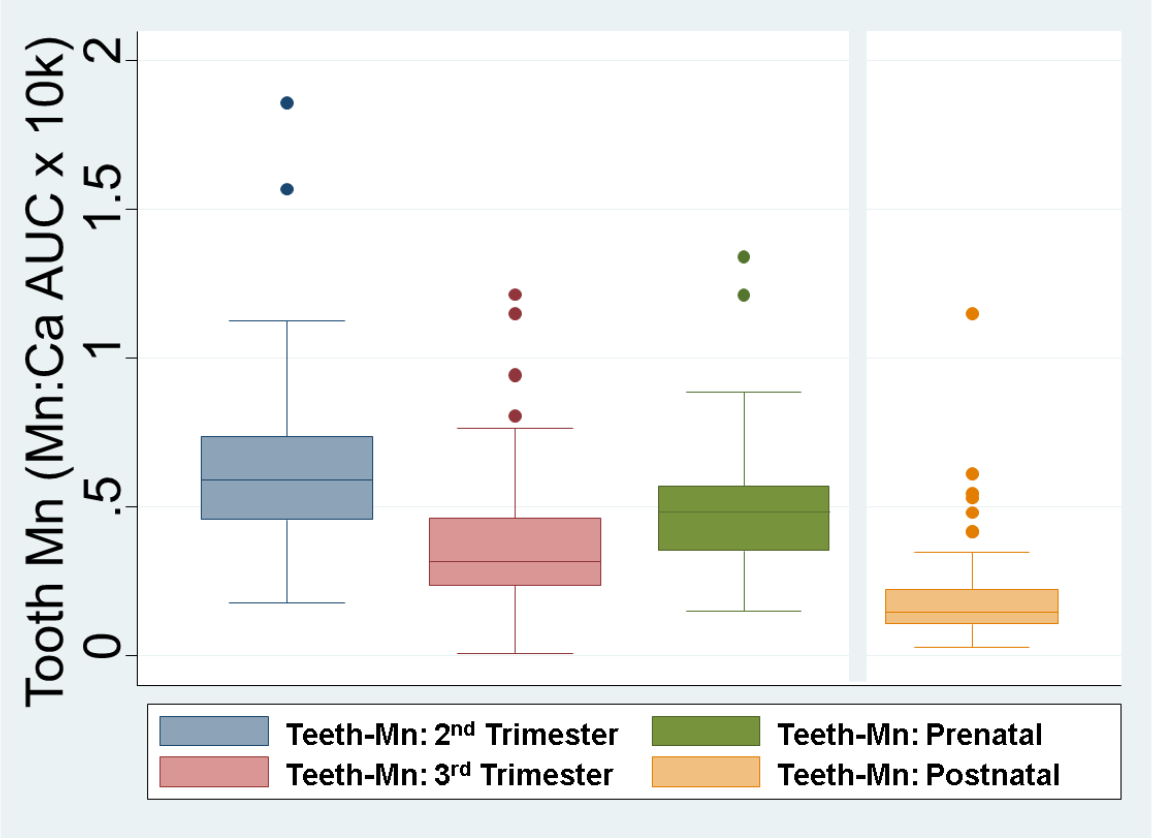


Figure 3.3. Scatter plot of prenatal and postnatal manganese concentrations (Mn:Ca AUC x 10,000) for repeated measurements from shed incisors (n=199).

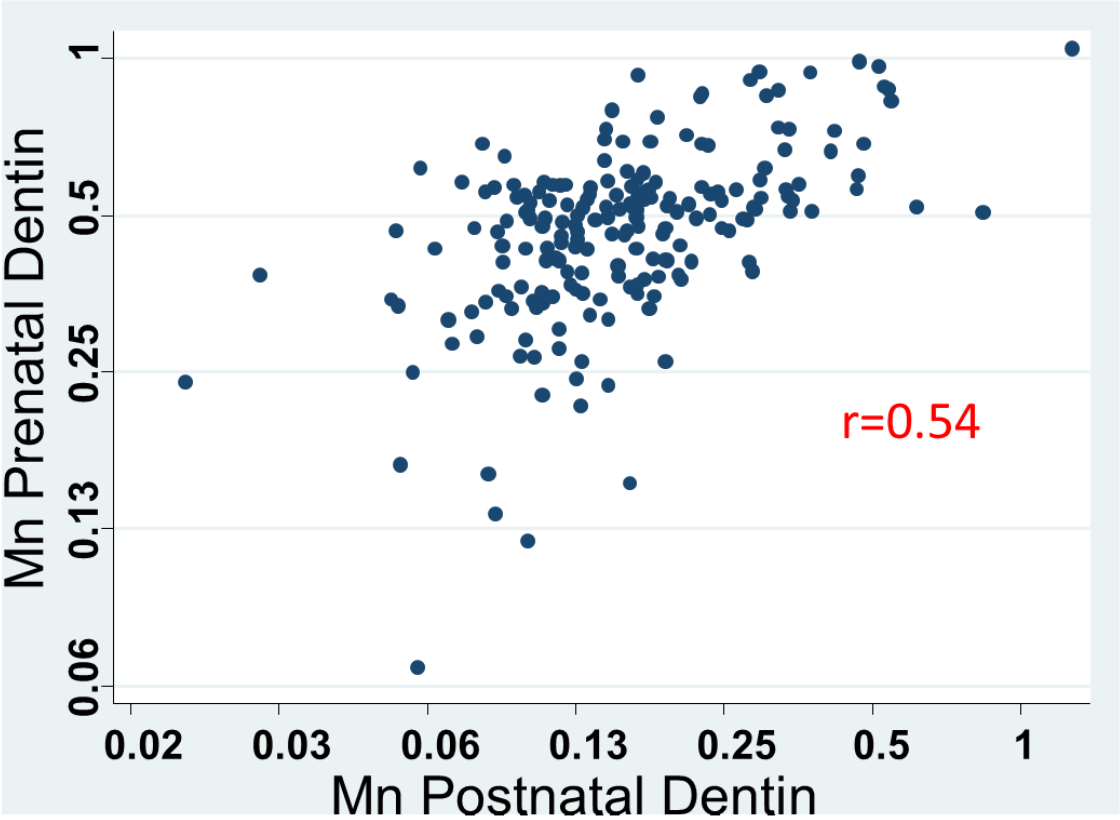


Figure 3.4. Percentage change in manganese in prenatal dentin for select predictor variables in model with all teeth (n=206) and in model with both teeth and dust samples (n=130).

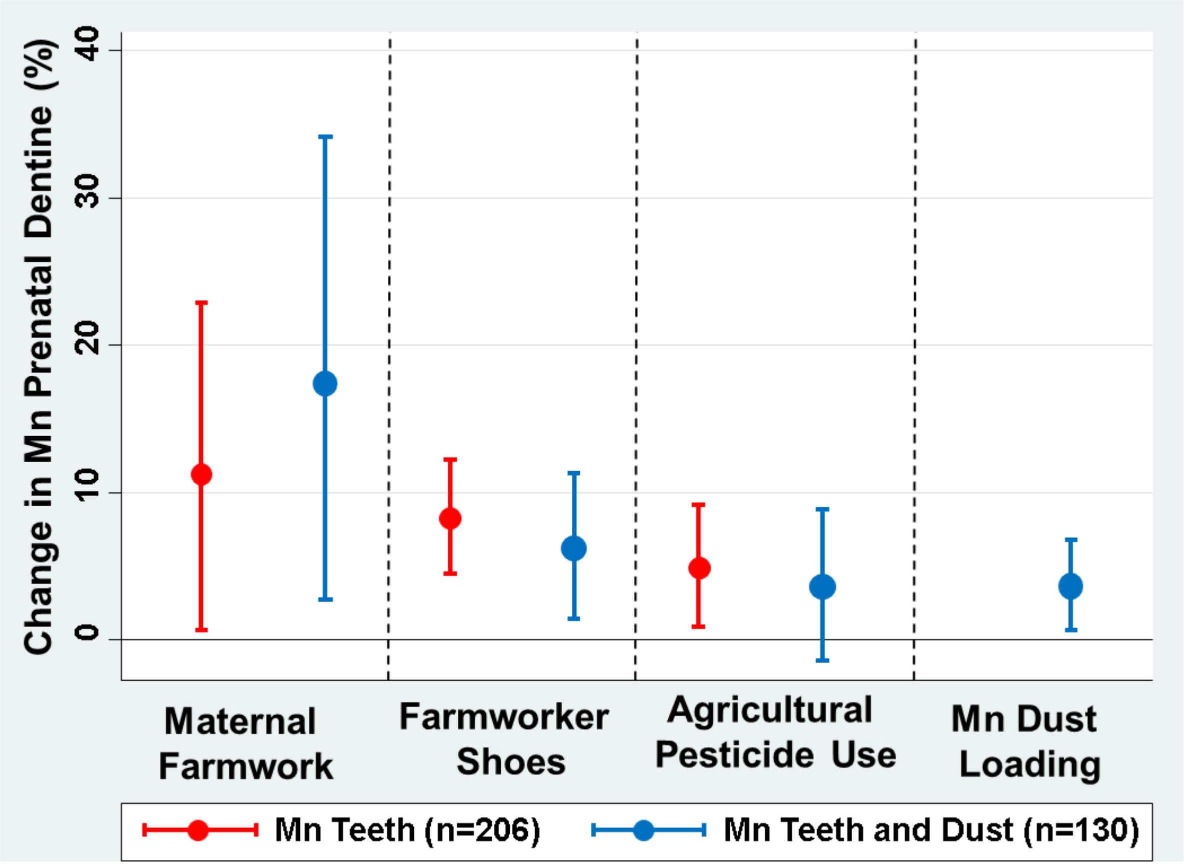
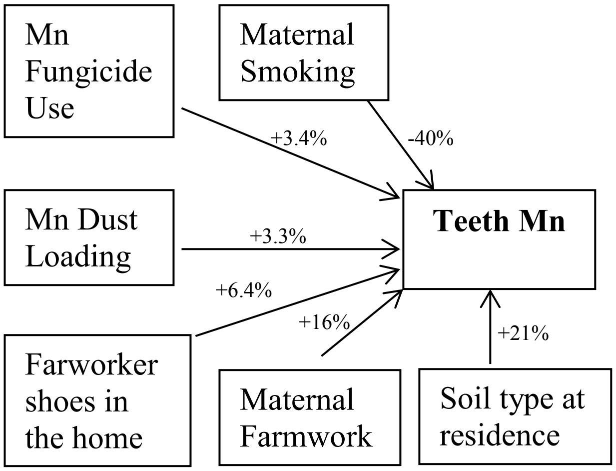
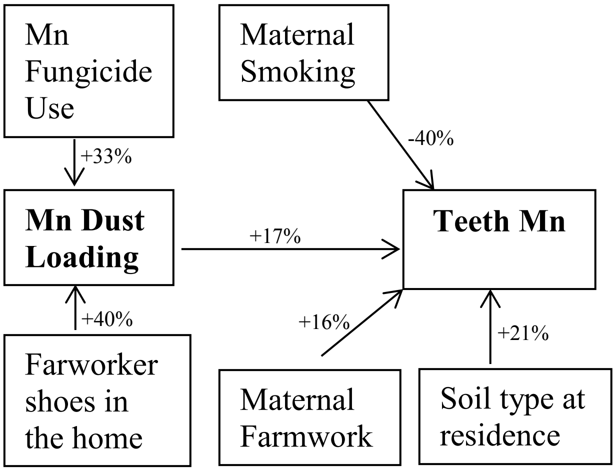


Figure 3.5. Percentage change in manganese in prenatal dentin per interquartile range increase in manganese dust loading from multivariate regression model and simultaneous equation model.

(i) Results from standard regression model:



(ii) Results from structural equation model:



CHAPTER 4

Manganese levels in teeth and Neurodevelopment in Young Mexican-American Children

4.1. INTRODUCTION

Manganese (Mn) is an essential nutrient but can be neurotoxic in children at high exposure levels (Roels et al. 2012). In particular, exposure from inhalation is more likely to result in Mn being oxidized to the more toxic manganic ion (Mn^{3+}) due to differences in uptake characteristics between dietary and inhalation exposures because inhalation exposure bypasses intestinal and hepatic Mn control processes (Teeguarden et al. 2007). Numerous studies have reported associations between Mn biomarkers and cognitive deficits or behavioral problems in children (Rodriguez-Barranco et al. 2013). In cross-sectional studies of school-aged children, higher Mn in hair, blood and water has been associated with lower full-scale and verbal intelligence quotients (Bouchared et al. 2011; Kim et al. 2009; Menezes-Filho et al. 2011; Riojas-Rodriguez et al. 2010; Wasserman et al. 2006; Wasserman et al. 2011; Wright et al. 2006), lower verbal learning and memory scores (Torres-Agustin et al. 2013), increased internalizing and externalizing behavior problems (Khan et al. 2011), and increased oppositional and hyperactive behaviors (Bouchard et al. 2007). Case-control studies of school-aged children have reported increased odds of attention deficit hyperactivity disorder among children with higher Mn concentrations in blood (Farias et al. 2010; Yousef et al. 2011).

Prospective cohort studies of infants and young children have observed inverse associations between Mn concentrations in cord blood and cognitive and language development at 2-years of age (Lin et al. 2013), and attention, non-verbal memory and hand skills in 3-year olds (Takser et al. 2003). A prospective cohort study of children 1 – 3 years of age reported an inverted U-shaped relationship between the child's Mn blood concentration at 12-months and concurrent mental development with deficits at both the lowest and highest quintiles of blood Mn compared to the middle three quintiles (Claus Henn et al. 2010). A study of 27 children observed a positive correlation between Mn levels in tooth enamel corresponding to prenatal exposure at 20 weeks gestation and behavioral disinhibition at 3 years of age, as well as parental and teacher report of internalizing and externalizing behavior problems in 1st and 3rd grades (Ericson et al. 2007).

Several studies have found evidence that exposure to other heavy metals may modify the relationship between Mn and neurodevelopment. For example, Mn levels in blood modified the relationship between lead and neurodevelopment in children at one to three years of age resulting in steeper slopes for children with high manganese coexposure (Claus Henn et al. 2012; Lin et al. 2013) and school-aged children (Kim et al. 2009). Iron status may also modify the relationship since perinatal iron deficiency has also been associated with poorer cognitive development in young children (Chang et al. 2013; Radlowski et al. 2013). Higher blood Mn concentrations have been reported among iron deficient infants and young children likely because Mn and iron appear to share the same absorption pathways (Park et al. 2013; Smith et al. 2013), and blood Mn levels in pregnant women at delivery have been related to iron metabolizing genes (Claus Henn et al. 2011).

Blood Mn concentrations have been used most often as a biomarker of exposure in studies of neurodevelopment (Rodriguez-Barranco et al. 2013). Blood Mn levels, however, represent recent exposure since Mn has a half-life of less than 30 days in blood (Smith et al. 2007). Maternal Mn blood concentrations do not accurately reflect *in utero* exposure to the fetus as evidenced by the differences between Mn levels in maternal delivery blood and cord blood (Smargiassi et al. 2002; Takser et al. 2004; Zota et al. 2009). Hair Mn levels have also been used to assess exposure in many studies of neurodevelopment, but is only representative of exposure during the period of hair growth. Hair is also susceptible to exogenous contamination and measured Mn levels in hair depend on the methodology used to clean the samples prior to analysis (Eastman et al. 2013).

In this study, we use a novel matrix to estimate exposure, Mn concentration in prenatal and postnatal dentin from children's shed teeth (Arora et al. 2012), and examine concentrations in relationship to infant neurodevelopment in the Center for the Health Assessment of Mothers' and Children of Salinas (CHAMACOS) study, a prospective birth cohort in the agricultural Salinas Valley of California. In the Salinas Valley, an average of 160,000 kg of Mn-containing fungicides is applied annually (CDPR 2013). We previously reported a relationship between Mn levels in prenatal dentin and residential proximity to use of these fungicides and the number of farmworkers storing clothes and shoes inside the home in this cohort (Gunier et al. 2013).

4.2. METHODS

Study population

For this study, 601 pregnant women were enrolled between October 1999 and October 2000 as part of the CHAMACOS study. Women were eligible if they were ≥ 18 years of age, < 20 weeks gestational age, eligible for California's low-income health care program, spoke English or Spanish and were planning to deliver at the county hospital. We followed the women through the delivery of 537 liveborn children. Children were excluded from the analyses who did not have a neurodevelopmental assessment (n=76) and who did not have prenatal or postnatal Mn measurements from a shed incisor (n=257). Children were excluded with medical conditions that could affect assessment (n=3 with seizures and 1 later diagnosed with autism). Children were also excluded with Mn measurements that were greater than four standard deviations from the mean value in prenatal (n=1 low) or postnatal (n=1 high) dentin. The final study population for this analysis was 198 children and their mothers. Neurodevelopmental assessments included 183 children at 6-months, 189 children at 12-months and 187 at 24-months. The mothers of children included in these analyses were older at delivery (mean=26.7 years) than the mothers of children that were not included in these analyses (mean=24.7 years), otherwise the two populations were similar demographically. Written informed consent was obtained from all women and all research was approved by the University of California, Berkeley, Committee for the Protection of Human Subjects prior to commencement of the study.

Neurodevelopmental outcomes

The Bayley Scales of Infant Development-Second Edition were used to assess the developmental functioning of the children at 6 months and 1 and 2 years (Bayley 1993). The Mental Developmental Index (MDI) characterizes cognitive abilities and the Psychomotor Developmental Index (PDI) assesses large muscle and fine motor coordination. Trained psychometricians that were blind to Mn exposure administered both scales in Spanish or English at the CHAMACOS research office in Salinas or in a recreational vehicle utilized as a mobile testing facility. Both the MDI and PDI scales are age-standardized to a mean of 100 with a SD of 15. We assessed the children on average (mean ± SD) at 6.6 ± 0.9 months, 12.8 ± 1.6 months and 24.6 ± 1.0 months of age. At each time point, there was one child that only had a valid score for either the MDI or PDI.

Manganese measurements in teeth

Deciduous teeth were collected beginning with the 7-year visit. Participants either mailed or brought in teeth as they were naturally exfoliated. The method for measuring Mn in human teeth has been described in detail elsewhere (Arora et al. 2011; Arora et al. 2012). The teeth were sectioned in a vertical plane, and microscopy was used to visualize the neonatal line and incremental markings in sectioned teeth samples. The neonatal line distinguishes between dentin formation during the prenatal and postnatal periods. Formation of prenatal dentin begins at approximately 3-months gestation continuing until birth and postnatal dentin formation occurs from birth until around 2.5-months of age for incisors (Ash and Nelson 2003). The concentrations and spatial distribution of Mn were determined using laser ablation inductively coupled plasma mass spectroscopy. Levels of tooth Mn were characterized by normalizing to measured tooth calcium levels (^{55}Mn:^{43}Ca ratio) to provide a measure independent of variations in tooth mineral density. Values are the area under the curve (AUC x 10,000) for points measured during the prenatal (Mn_{pre}) and postnatal (Mn_{post}) periods separately. The coefficient 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 dentin measurements. There were eight children with Mn measurements in prenatal dentin that were missing postnatal Mn measurements due to attrition of the tooth.

Maternal interviews, assessments and chemical measurements

Mothers were interviewed twice during pregnancy (mean=14 and 27 weeks gestation), at delivery and when their children were 6, 12 and 24 months of age. Information was collected on maternal characteristics and lifestyle factors including age at delivery, country of birth, education, smoking, alcohol and drug use during pregnancy. Information was also gathered to determine housing density, household poverty status and father's presence in the home at each time point. The Peabody Picture Vocabulary Test (PPVT) was administered to the mother at the 6-month visit to assess verbal ability and scholastic aptitude (Dunn and Dunn 1981), and the Center for Epidemiologic Studies Depression Scale (CES-D) (Radloff 1977) at the 12-month visit. The Infant-Toddler Home Observation for Measurement of the Environment (HOME) instrument (Caldwell and Bradley 1984) was administered at the 6 and 12-month visit and 32 of the 45 items at the 24-month visit.

Sensitivity analyses were conducted including prenatal exposure to organochlorine and organophosphate insecticides in the models because both have been previously associated with infant neurodevelopment in this cohort (Eskenazi et al. 2006; Eskenazi et al. 2007). Prenatal exposure to *p,p'*-dichlorodiphenyltrichloroethylene (DDT) and *p,p'*-dichlorodiphenyl-dichloroethylene (DDE) was measured in blood samples collected from the mother at the time of the second pregnancy interview (n=104) or just prior to delivery (n=37) using gas chromatography-high resolution mass spectrometry methods that have been described previously (Barr et al. 2003). Lipid adjusted, $\log_{10}$ transformed DDT and DDE concentrations were used in the analyses. To assess exposure to organophosphate insecticides, dialkyl phosphate metabolites (DAPs) were measured in maternal urine samples collected at approximately 13 and 26 weeks gestation using high-resolution gas chromatography-tandem mass spectrometry with isotope dilution quantification (Olsson et al. 2003). The two prenatal DAP measurements were averaged and used $\log_{10}$ transformed creatinine adjusted concentrations in our analyses. Measurements of blood hemoglobin concentration (g/dL) were abstracted from medical records of prenatal visits during the second trimester as an indicator of maternal iron status during pregnancy. Blood lead concentrations (μg/dL) were measured using graphite furnace atomic absorption spectrophotometry in cord blood samples (n=139), maternal delivery blood samples (n=15) or maternal blood samples collected at the second prenatal visit around 26 weeks gestation (n=15). Blood lead concentrations for children at 1-year (n=158) and 2-years (n=172) were collected from healthy child visits to health clinics.

Data analysis

Mn teeth levels were log-transformed in the models to reduce heteroskedasticity and the influence of outliers. A $\log_2$ based transformation was used so that a one unit change represented a two-fold increase of Mn levels measured in dentin. A paired t-test was used to compare $\mathrm{Mn}_{\mathrm{pre}}$ and $\mathrm{Mn}_{\mathrm{post}}$ levels and calculated the intraclass correlation coefficient to assess the within-person variability of Mn exposures over time. The MDI and PDI values were approximately normally distributed and were modeled as continuous outcomes, therefore the results reflect the change in Bayley scores for a doubling in Mn teeth levels.

Covariates were evaluated using directed acyclic graphs. Several covariates were selected *a priori* that have been associated with infant neurodevelopment in previous studies for inclusion in multivariable regression models including the child's exact age at assessment, sex, maternal PPVT score and maternal education (< high school vs. ≥ high school). Other potential covariates were considered for inclusion in the models separately in bivariate analyses using Analysis of Variance for categorical variables and Spearman correlation coefficients for continuous variables. Maternal age at delivery, parity, breastfeeding duration, maternal years in the U.S., maternal smoking, alcohol consumption and drug use during pregnancy, maternal depression and Cesarean delivery were considered for inclusion in the models. Covariates collected at each visit were also considered including housing density, HOME score, household poverty level (comparing the federal poverty threshold to household income divided by the number of people supported), father presence in the home, maternal work status, psychometrician, location of assessment (field office or recreational vehicle), and season of assessment (winter=December-February; spring=March-May; summer=June-August; fall=September-November). Covariates were added to the multivariate models that were associated in bivariate analyses ($p<0.2$) with an outcome or exposure at any time point. Backwards elimination methods were used and the same covariates (psychometrician, location of assessment, household poverty status and HOME score) were retained in all models if they were at $p<0.2$ in multivariable models at any time point.

Generalized additive models (GAMs) with a three-degrees-of-freedom cubic spline function were used to evaluate the shape of the relationships between Mn teeth levels and Bayley scores and to test for linearity because altered neurodevelopment was hypothesized to occur at both high and low levels of Mn in teeth following an inverted U-shaped dose response. Models were run using tertiles of Mn teeth levels for outcomes with $p<0.1$ for digression from linearity using the middle tertile as the reference category since neurodevelopment was hypothesized to follow an inverse U-shaped association with potential decrements at the lowest and highest Mn levels in teeth. Sensitivity analyses were conducted including $\log_{10}$ transformed prenatal DAPs, DDT or DDE in the models for participants with these measurements. Models were rerun excluding preterm (n=10) and other low birthweight (n=8) infants since these factors may be on the causal pathway. Models were fit for Mn_{post} that included Mn_{pre} values as a confounder for participants with both measurements. Models were run excluding outliers identified using the Generalized Extreme Studentized Deviates Many-Outlier procedure (Rosner 1983). To control for potential selection bias of those with tooth measures, models were run with weights determined as the inverse probability of inclusion in our analyses at each time point (Hogan et al. 2004). Probability of inclusion was determined based on multiple logistic regression models using baseline covariates as potential predictors. Model selection for probability of inclusion was conducted using the SuperLearner statistical package since easily interpretable coefficients were not necessary only finding the best predictive model. SuperLearner combines user determined algorithms to find the best combination of variables that minimizes the cross-validated risk (van der Laan and 2007). The following learners from R packages were used for the prediction model: generalized linear models (glm), generalized additive models (gam), Bayesian generalized linear models (bayesglm), random forest models (randomForest) and Lasso and elastic-net regularized generalized linear models (glmnet).

Interactions with prenatal blood lead concentrations and Mn_{pre}, and child blood lead concentrations at 12 and 24 months and Mn_{post} were evaluated using models stratified by blood low and high lead concentrations (< 2 µg/dL vs. ≥ 2 µg/dL). Potential interactions with prenatal iron status were assessed by running models for Mn_{pre} stratified by low and high hemoglobin levels (< 12 g/dL vs. ≥ 12 g/dL).

4.3. RESULTS

Distributions of neurodevelopment scores

The Bayley Scales of Infant Development at 6, 12 and 24 months were normally distributed with similar mean and median scores (Table 4.2). The mean scores were mostly between 96 and 101, although the mean was lower for MDI at 24-months (85.8) and higher for PDI at 12-months (105.9). The standard deviations ranged from 7.3 for MDI at 6-months to 13.6 for PDI at 12-months.

Distributions of Mn levels in teeth

Mothers who participated in this study were mostly under 30 years of age (74.8%), Latina (97.5%), born in Mexico (88.4%) and few (22.2%) had completed high school (Table 4.1). Most women lived below the poverty level (59.6%), were multiparous (66.7%), did not smoke (94.9%) and drank less than one serving of alcohol per week (94.9%) during pregnancy. More of the children were girls (57.6%) than boys, and few were born preterm (9.1%) or of low birthweight (5.0%). The range of Mn_{pre} was 0.12 – 1.34 and the range of Mn_{post} was 0.02 – 1.27. The mean ± SD of Mn in prenatal and postnatal dentin (^{55}Mn:^{45}Ca AUC x 10^4) were 0.51 ± 0.18 and 0.19 ± 0.16 respectively (Table 2). Based on a paired t-test of the 190 children with both measurements, Mn_{pre} levels were significantly higher than Mn_{post} values ($p<0.001$). There was moderate within-person correlation between Mn_{pre} and Mn_{post} (ICC=0.5). The geometric mean levels of Mn_{pre} were higher in children with Latina mothers, mothers that did not complete high school and who did not smoke during pregnancy (Table 4.1). Both Mn_{pre} and Mn_{post} were higher in children of women born in Mexico. There was an inverse U-shaped relationship with Mn_{pre} and maternal age at delivery and somewhat lower Mn_{pre} levels if the child's mother had more than one drink per week of alcohol.

Relationship between Mn levels in teeth and neurodevelopment

A doubling of Mn_{post} was associated with a -2.6 decrease (95% CI: -4.5, -0.6) in PDI at 6-months of age with Mn_{pre} was included as a confounder in model (Table 4.3). However, the linear association between Mn_{post} and PDI at 6-months was also no longer significant after excluding two outliers from the model with a -1.5 point decrease (95% CI: -3.4, 0.3) for a two-fold increase in Mn_{post}. There was no association between Mn_{post} and MDI at 6-months, and either MDI or PDI at 12 or 24-months (Table 4.3). There was no relationship between Mn_{pre} and MDI or PDI at 6, 12 or 24 months. Results using GAMs showed significant departures from linearity ($p<0.1$) for Mn_{pre} and MDI at 6-months and PDI at 12-months, and also for Mn_{post} and both MDI and PDI at 6-months. Suggestive inverse U-shaped relationships were observed between tertiles of Mn_{post} with the 2nd tertile as the reference category and both MDI and PDI at 6-months (Figure 4.1). However, the only significant association using tertiles of Mn exposure was a -4.6 point decrease for PDI (95% CI: -8.0, -1.3) for children in the 3rd tertile (highest) of Mn_{post}. The relationships between Mn_{pre} and MDI at 6-months and PDI at 12-months were not inverse U-shaped, and there were no associations with tertiles of exposure (data not shown).

Among children whose mothers were iron deficient (hemoglobin ≤ 12 g/dL), there was a significant decrease of -4.8 points for MDI at 6-months of age (95% CI: -8.9, -0.7) compared to a change of 0.0 points (95% CI: -3.6, 3.6) among children whose mothers were iron sufficient (hemoglobin > 12 g/dL) during pregnancy (Figure 4.2). A non-significant decrease of 5.3 points for PDI at 6-months (95% CI: -12.1, 0.5) was observed among children whose mothers were iron deficient compared to a decrease of -1.4 points (95% CI: -6.5, 3.6) among iron replete mothers. There was no relationship between prenatal iron status and MDI or PDI at 12 and 24 months. There were no significant associations between Mn levels in teeth and MDI or PDI at any time point when stratifying by blood lead level using ≤ 2 μg/dL as low and > 2 μg/dL as high. There was a suggestion of inverse relationships with Mn levels in teeth among those with high blood lead compared to those with low blood lead. For example, there was an insignificant decrease of -4.3 points (95% CI: -9.4, 0.9) for MDI at 6-months among those with high prenatal blood lead compared to a decrease of -1.7 points (95%CI: -4.1, 0.7) among the relatively few participants (n=27) with low prenatal blood lead in this cohort.

Sensitivity analyses

The estimates for MDI and PDI at each time point were very similar after adjusting for prenatal organophosphate metabolites in urine and DDT or DDE in blood (Table 4.4). The results were similar for Mn_{post} after excluding children who were born preterm (n=10) or full-term but of low birthweight (n=8) but the associations became slightly stronger and in some instances significant for Mn_{pre} with a 1.9 point decrease in MDI at 6-months (95% CI: -3.8, -0.1).

4.4. DISCUSSION

An association between higher Mn levels measured in postnatal dentin of children's shed teeth and a deficit in psychomotor development at 6-months of age was observed when comparing the highest tertile to the middle tertile. The observed relationship suggests a modest decrease from a mean PDI score of 96 for the middle tertile of Mn teeth levels to a score of 94 at the highest tertile of Mn. These results lend some support to the hypothesis that as an essential nutrient Mn exposure would follow an inverted U-shaped association. The association remained significant for postnatal Mn levels in teeth with prenatal levels included in the models suggesting that early postnatal levels were more relevant to psychomotor development at 6-months of age in our cohort. Postnatal dentin formation occurs from birth through 2.5 months of age for incisors (Ash and Nelson 2003) and therefore the association observed with psychomotor development represents Mn exposure that occurred 3-6 months prior to the assessment. An inverse relationship was also observed between Mn_{pre} and both mental and psychomotor development at 6-months among children whose mothers were iron deficient during pregnancy. There was no significant interaction between Mn and blood lead in our cohort. There was no relationship between prenatal or postnatal Mn dentin levels and neurodevelopment at 12 or 24-months of age.

The only previous study of Mn exposure that also used the Bayley Scales of Infant Neurodevelopment found an inverted U-shaped association between Mn in children's blood at 12-months and concurrent mental development, and a diminished effect on neurodevelopment after 12-months and no relationship with psychomotor development or 24-month blood Mn concentrations (Claus Henn et al. 2010). Slightly lower, but non-significant, decreases in mental development scores at 6-months (β=-1.2: 95% CI=-2.7, 0.3) and 12-months (β=-1.2: 95% CI=-2.6, 0.2) were observed with Mn_{post}. Taken together, these findings suggest an association between infant neurodevelopment and concurrent Mn exposure from 6 – 12 months, but not as the child grows older and homeostatic mechanisms that control Mn levels become more developed (Dorner et al. 1989).

Previous studies have observed an interaction effect between Mn and lead exposure and neurodevelopment in young children. A study of children from Mexico City observed an interaction between the highest quintile of Mn blood levels and continuous blood lead concentrations for both mental and psychomotor development using longitudinal models for assessments conducted every 6-months between the ages of 12 and 36 months of age (Claus Henn et al. 2012). A study of 2-year old children in Taiwan also found a significant interaction between the highest quartiles of Mn and lead concentrations in cord blood with whole and cognitive developmental quotients (Lin et al. 2013). Although point estimates were lower among children with higher blood lead levels in our cohort, the interaction terms between Mn in teeth and blood lead were not significant either categorically or continuously at any time point for either mental or psychomotor development. Children in this cohort, however, had much lower mean blood lead concentrations at 12-months (1.7 µg/dL) and 24-months (1.9 µg/dL) than the children from Mexico City who had mean concentrations of 5.1 and 5.0 µg/dL respectively (Claus Henn et al. 2012). Children in this cohort also had lower median cord blood lead levels (0.7 µg/dL) than the median cord blood concentration (1.3 µg/dL) in children from Taiwan (Lin et al. 2013). Lead exposures may therefore have been too low to observe an interaction effect in our cohort.

The only previous study that evaluated Mn levels in a small number of children's teeth (n=27) measured Mn in tooth enamel instead of dentin and reported a correlation between prenatal tooth Mn levels and behavioral problems at 3-years of age and in 1st and 3rd grades (Ericson et al. 2007). Mn levels in dentin were evaluated in this study instead of enamel because initial deposits of enamel matrix, unlike dentin, are not completely mineralized initially but rather more diffusely during maturation; thus, enamel measurements cannot be linked as reliably to developmental timing of exposure. Relationships between Mn levels in teeth and neurodevelopment may be related to iron deficiency because Mn uptake increases with low iron stores (Ericson et al. 2007; Park et al. 2013; Smith et al. 2013). Stronger effects of Mn_{pre} on neurodevelopment at 6-months of age were observed among children whose mothers' were iron deficient than iron sufficient during pregnancy. A direct relationship was not observed between prenatal maternal iron levels expressed categorically or continuously and infant neurodevelopment (data not shown). Hemoglobin concentrations were used to assess iron deficiency not serum ferritin measurements, which is considered a better indicator of iron status (Radlowski et al. 2013).

An important limitation of this study is that we had a relatively small number of participants in our analyses, especially for evaluating interactions between Mn exposure and blood lead levels or iron status. Measurements of iron status in children after birth were not available to evaluate potential interactions with postnatal Mn exposure. Mn measurements in blood or hair were not available for this study, which would enable better comparisons to previous studies of Mn exposure and neurodevelopment. Nevertheless both prenatal and early postnatal exposures were evaluated using Mn measurements in teeth. Neurodevelopment was assessed at several time points using Bayley Scales in a prospective cohort of children and had extensive data on potential confounders including prenatal exposure to other pesticides.

Table 4.1. Geometric mean and standard deviation of prenatal and postnatal manganese levels (AUC Mn:Ca) in dentin by demographic characteristics of the CHAMACOS study population.

	Prenatal Dentin (n=198)			Postnatal Dentin (n=190)		
Characteristic	N (%)	GM ± GSD		N (%)	GM ± GSD	
All participants	198 (100.0)	0.48 ± 1.5		190 (100.0)	0.16 ± 1.8	
Mothers						
Age (years)						
18 - 24	75 (37.9)	0.44 ± 1.5		72 (37.9)	0.15 ± 1.8	
25 - 29	73 (36.9)	0.52 ± 1.4		70 (36.8)	0.17 ± 1.8	
30 - 34	32 (16.2)	0.49 ± 1.4		31 (16.3)	0.18 ± 1.8	
35 - 45	18 (9.1)	0.45 ± 1.4	*	17 (9.0)	0.13 ± 1.7	
Race/Ethnicity						
White	2 (1.0)	0.24 ± 1.8		2 (1.0)	0.09 ± 1.0	
Latina	193 (97.5)	0.48 ± 1.5		185 (97.4)	0.16 ± 1.8	
Other	3 (1.5)	0.40 ± 1.1	*	3 (1.6)	0.13 ± 1.4	
Education						
≤ 6th grade	94 (47.5)	0.51 ± 1.4		89 (46.8)	0.17 ± 1.9	
7 - 12 grade	60 (30.3)	0.48 ± 1.4		59 (31.1)	0.14 ± 1.6	
≥ High School	44 (22.2)	0.40 ± 1.6	**	42 (22.1)	0.15 ± 1.9	
Income (% poverty)						
< 100	118 (59.6)	0.49 ± 1.5		111 (58.4)	0.17 ± 1.9	
100 - 200	72 (36.4)	0.45 ± 1.5		71 (37.4)	0.15 ± 1.6	
> 200	8 (4.0)	0.47 ± 1.3		8 (4.2)	0.13 ± 2.5	
Country of birth						
United States	21 (10.6)	0.36 ± 1.5		21 (11.1)	0.12 ± 1.8	
Mexico	175 (88.4)	0.49 ± 1.4		167 (87.9)	0.16 ± 1.8	
Other	2 (1.0)	0.40 ± 1.1	**	2 (1.0)	0.12 ± 1.5	*
Time in USA (years)						
≤ 5	41 (20.7)	0.49 ± 1.3		39 (20.5)	0.16 ± 1.9	
6 - 10	53 (26.8)	0.50 ± 1.6		51 (26.9)	0.18 ± 1.9	
≥ 11	104 (52.5)	0.46 ± 1.4		100 (52.6)	0.15 ± 1.8	
Parity						
0	66 (33.3)	0.48 ± 1.4		64 (33.7)	0.15 ± 1.7	
≥ 1	132 (66.7)	0.48 ± 1.5		126 (66.3)	0.16 ± 1.9	
Smoking during pregnancy						
No	188 (94.9)	0.49 ± 1.4		180 (94.7)	0.16 ± 1.8	
Yes	10 (5.1)	0.32 ± 2.4	**	10 (5.3)	0.14 ± 1.8	
Alcohol during pregnancy						

No	188 (98.4)	0.48 ± 1.4		181 (98.4)	0.16 ± 1.8
Yes	3 (1.6)	0.32 ± 2.4	†	3 (1.6)	0.11 ± 1.2
Children					
Sex					
Boy	84 (42.4)	0.48 ± 1.4		79 (41.6)	0.15 ± 1.9
Girl	114 (57.6)	0.48 ± 1.5		111 (58.4)	0.17 ± 1.8
Birthweight (g)					
< 2500	10 (5.0)	0.40 ± 1.6		10 (5.3)	0.15 ± 1.9
2500 - 3500	93 (47.0)	0.48 ± 1.4		90 (47.4)	0.16 ± 1.8
> 3500	95 (48.0)	0.49 ± 1.5		90 (47.4)	0.16 ± 1.9
Gestational duration (weeks)					
< 37	18 (9.1)	0.43 ± 1.5		18 (9.5)	0.17 ± 2.0
37 - 42	180 (90.9)	0.48 ± 1.5		172 (90.5)	0.16 ± 1.8

*p<0.05; **p<0.01

Table 4.2. Distributions of manganese in prenatal and postnatal dentin and Bayley Scales of Infant Development at 6, 12 and 24 months in CHAMACOS cohort participants.

Variable	N	Min.	p25	p50	p75	Max.	Mean± SD
Mn Prenatal Dentin	198	0.12	0.39	0.5	0.57	1.34	0.51 ± 0.18
Mn Postnatal Dentin	190	0.02	0.11	0.15	0.22	1.27	0.19 ± 0.16
MDI 6-months	182	67	92	96	100	121	95.9 ± 7.3
PDI 6-months	183	64	90	97	104	120	96.4 ± 10.4
MDI 12-months	189	72	96	102	107	126	101.3 ± 9.1
PDI 12-months	188	69	97	105	116	134	105.9 ± 13.6
MDI 24-months	186	58	76	84	94	117	85.8 ± 11.8
PDI 24-months	187	72	92	100	106	125	97.9 ± 10.2

Table 4.3. Association between a two-fold increase in manganese levels (AUC Mn:Ca) and Bayley Scales of Infant Development at 6, 12 and 24 months in CHAMACOS study participants.

Outcome	Manganese in Prenatal Dentine				Manganese in Postnatal Dentine			
	N	β	(95% CI)	p-value	N	β	(95% CI)	p-value
MDI 6-months	183	-1.8	(-3.6, 1)	0.25	176	-1.2	(-2.7, 0.3)	0.11
PDI 6-months	183	-1.8	(-4.9, 1.3)	0.25	176	-2.3	(-4.3, -0.3)	0.02
MDI 12-months	189	-0.8	(-2.9, 1.3)	0.45	183	-1.2	(-2.6, 0.2)	0.09
PDI 12-months	188	1.4	(-2.3, 5.1)	0.45	182	0.9	(-1.2, 2.9)	0.40
MDI 24-months	186	0.3	(-2.7, 3.2)	0.86	178	1.1	(-0.8, 2.9)	0.26
PDI 24-months	187	0.4	(-2.2, 3.1)	0.74	179	0.4	(-1.1, 1.9)	0.58

*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status and HOME score.

MDI=Mental Development Index; PDI=Psychomotor Development Index

Table 4.4. Association between a two-fold increase in manganese levels (AUC Mn:Ca) and Bayley Scales of Infant Development at 6, 12 and 24 months in CHAMACOS study participants including prenatal DDT blood concentrations in models.

Outcome	Manganese in Prenatal Dentine				Manganese in Postnatal Dentine			
	N	β	(95% CI)	p-val.	N	β	(95% CI)	p-val.
MDI 6-months	105	-1.8	(-2.8, 3.0)	0.35	103	-1.4	(-3.3, 0.5)	0.16
PDI 6-months	105	-1.8	(-5.5, 2.0)	0.35	103	-3.1	(-5.6, -0.5)	0.02
MDI 12-months	107	-1.4	(-4.9, 2.1)	0.44	105	-1.0	(-3.3, 1.4)	0.42
PDI 12-months	107	-1.2	(-6.2, 3.8)	0.64	105	-0.4	(-3.8, 3.1)	0.84
MDI 24-months	106	-0.2	(-4.7, 4.3)	0.93	104	1.9	(-1.3, 5.1)	0.25
PDI 24-months	106	-2.3	(-6.4, 1.8)	0.28	104	-0.1	(-3.0, 2.9)	0.96

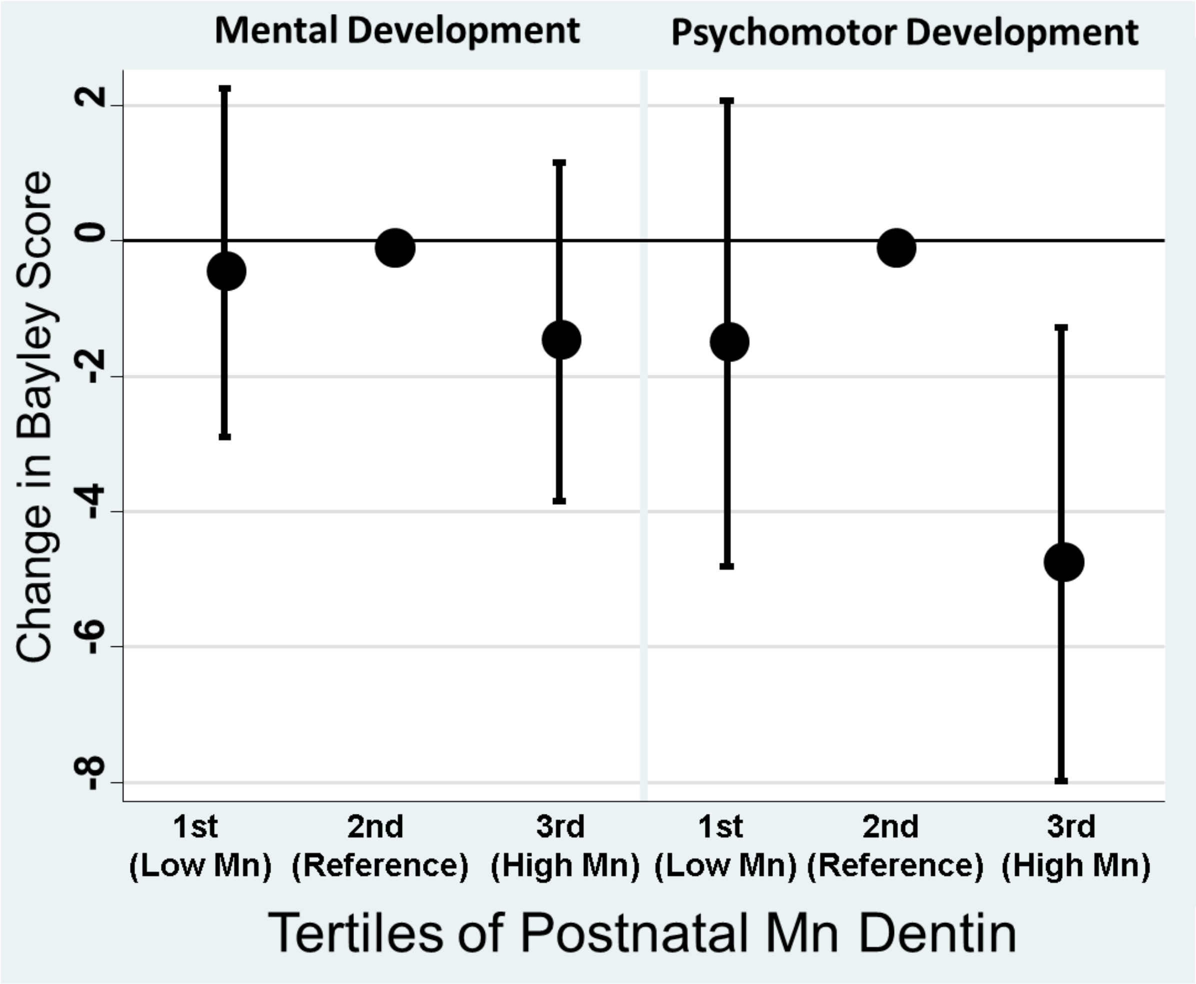
*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status, HOME score and prenatal DDT.
MDI=Mental Development Index; PDI=Psychomotor Development Index

Table 4.5. Association between a two-fold increase in manganese levels (AUC Mn:Ca) and Bayley Scales of Infant Development at 6, 12 and 24 months in CHAMACOS study participants with inverse probability of inclusion weights.

Outcome	Manganese in Prenatal Dentine				Manganese in Postnatal Dentine			
	N	β	(95% CI)	p-value	N	β	(95% CI)	p-value
MDI 6-months	183	-1.8	(-3.0, 1.5)	0.29	176	-1.2	(-2.7, 0.2)	0.09
PDI 6-months	183	-1.8	(-5.2, 1.5)	0.29	176	-2.4	(-4.4, -0.3)	0.02
MDI 12-months	189	-0.7	(-3.1, 1.7)	0.56	183	-1.5	(-2.9, 0.0)	0.05
PDI 12-months	188	1.6	(-2.1, 5.2)	0.40	182	0.9	(-1.2, 2.9)	0.41
MDI 24-months	186	0.1	(-2.9, 3.1)	0.94	178	1.0	(-0.8, 2.8)	0.29
PDI 24-months	187	0.9	(-2.0, 3.8)	0.54	179	0.1	(-1.6, 1.8)	0.88

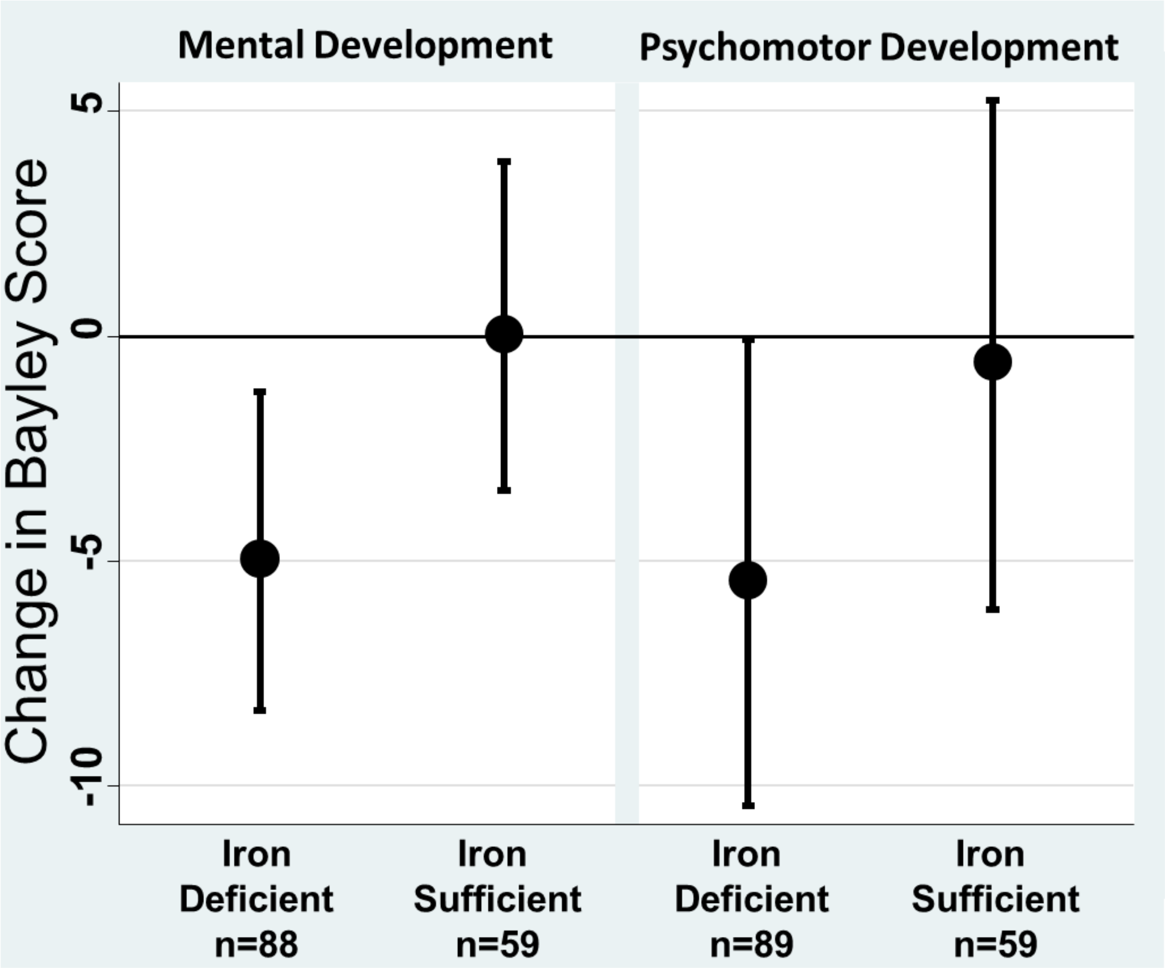
*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status, HOME score and prenatal DDT.
MDI=Mental Development Index; PDI=Psychomotor Development Index

Figure 4.1. Association between tertiles of postnatal manganese level (Mn:CA AUC) and Bayley Scales of Infant Development at 6-months.



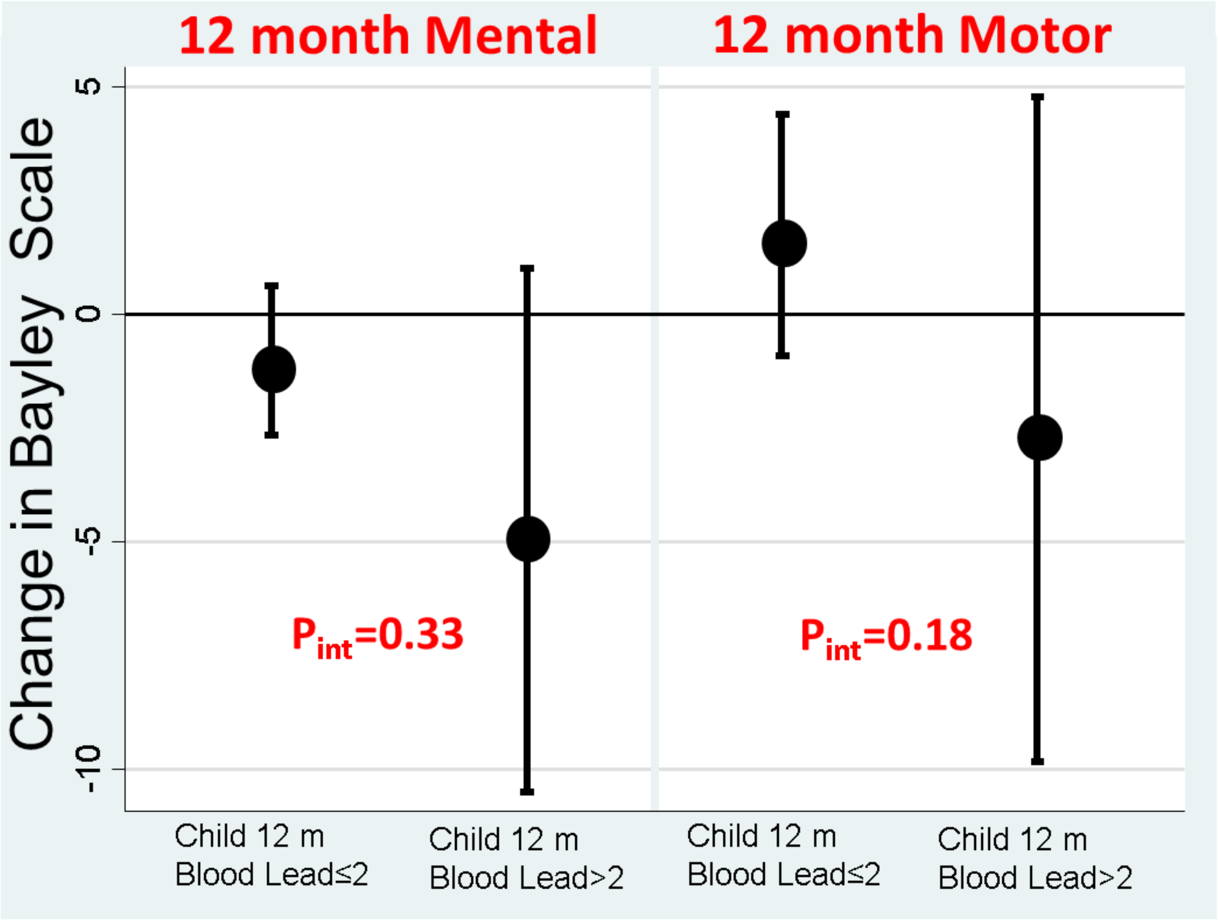
*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status and HOME score.

Figure 4.2. Association between a two-fold increase in prenatal manganese level (Mn:CA AUC) and Bayley Scales of Infant Development at 6-months stratified by prenatal iron status.



*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status and HOME score.

Figure 4.3. Association between a two-fold increase in postnatal manganese level in dentin (Mn:CA AUC) and Bayley Scales of Infant Development at 12-months stratified by child's blood lead concentration at 12-months.



*Adjusted for child's exact age, sex, maternal education, maternal IQ, psychometrician, location of assessment, household poverty status and HOME score.

Figure 4.4. Generalized Additive Model with 3-degrees of freedom for log2(Mn Postnatal Dentin) and Bayley Psychomotor Development Index at 6-months (n=176).

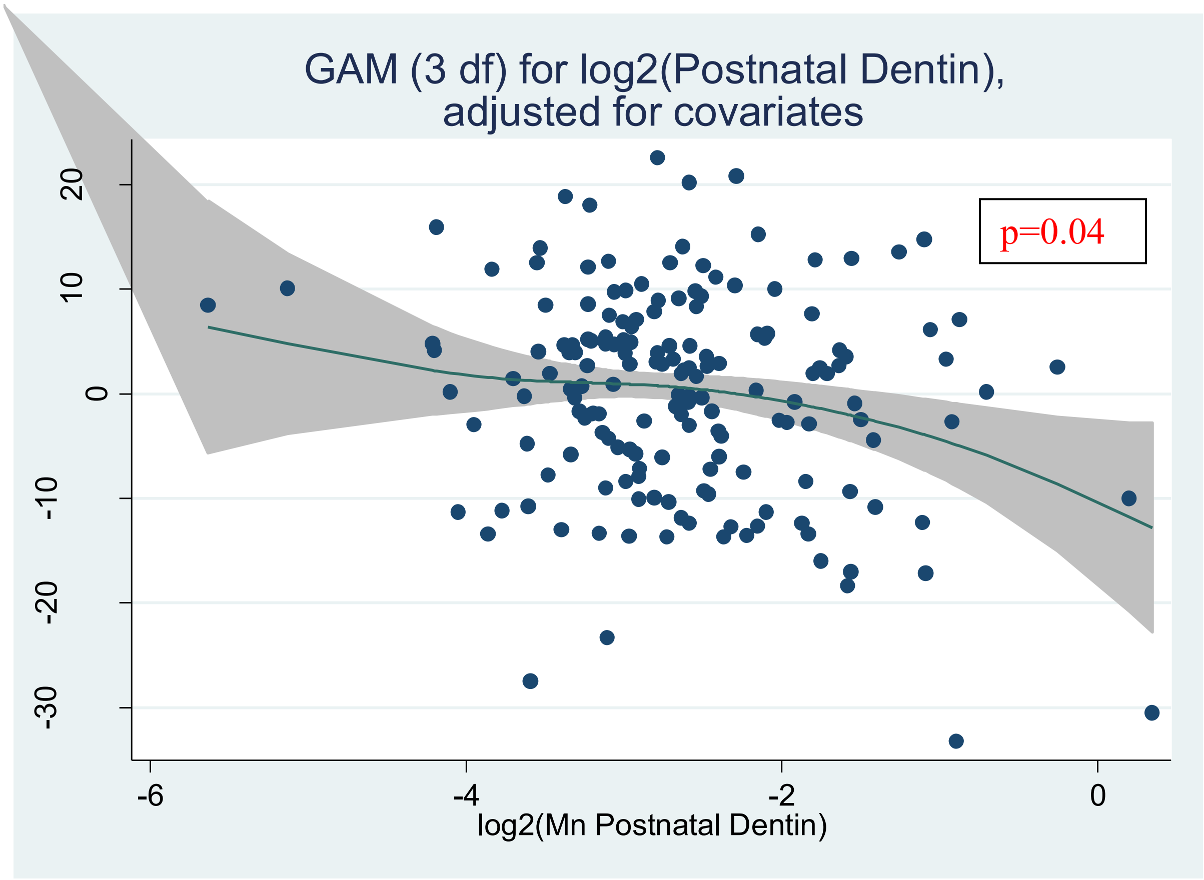
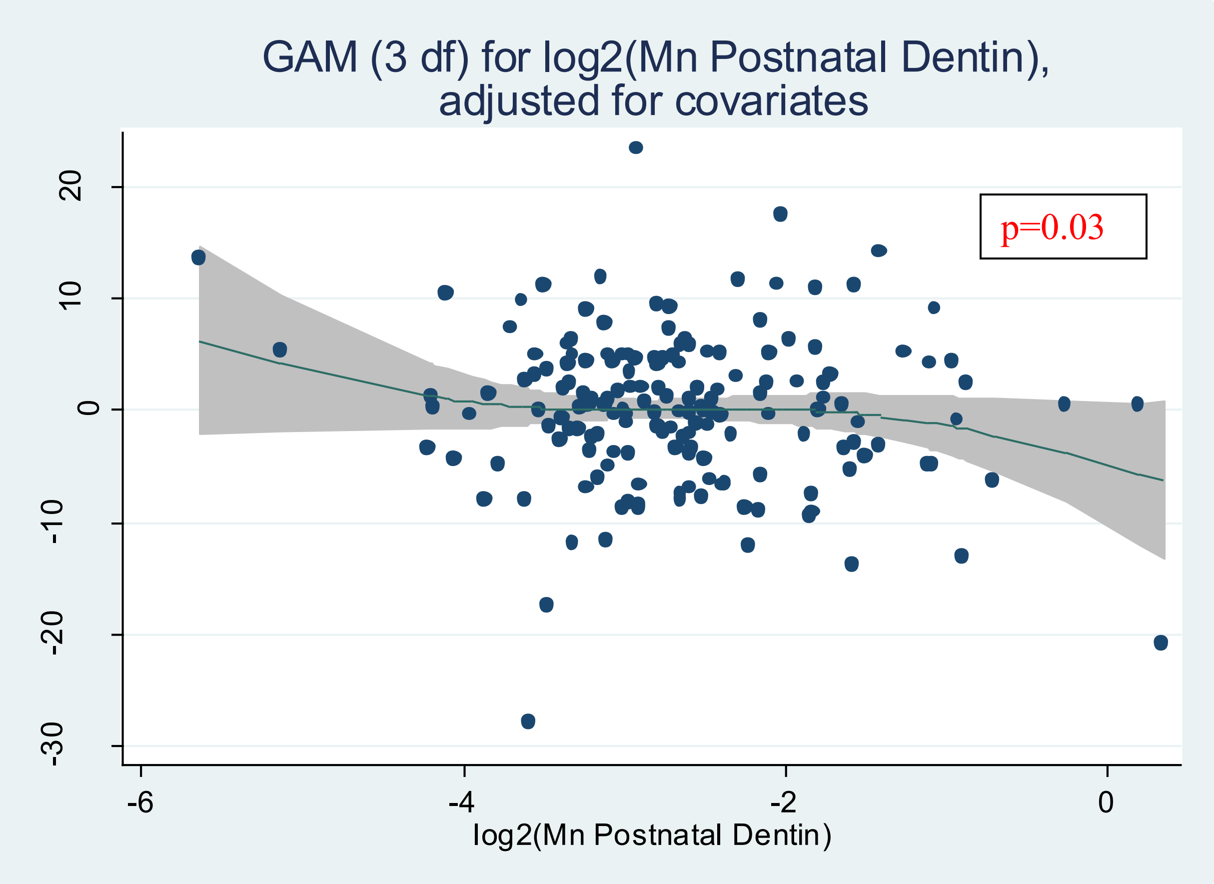


Figure 4.5. Generalized Additive Model with 3-degrees of freedom for log2(Mn Postnatal Dentin) and Bayley Mental Development Index at 6-months (n=176).



CHAPTER 5

Summary, conclusions and future research needs

Summary

The purpose of this study was to determine whether (1) the amount of Mn in children's homes was increased from nearby agricultural use of Mn-containing fungicides, (2) the body burden of Mn in children as measured in shed teeth was also increased by agricultural use of Mn fungicides, and (3) higher Mn levels in teeth were associated with deficits in neurodevelopment in infants and toddlers. Based on previously published studies of Mn exposure and health effects, the hypothesis was that the use of Mn-containing fungicides would increase levels of Mn in children's homes and shed teeth and adversely affect early neurodevelopment. A total of 468 house dust samples were analyzed to measure Mn dust concentrations (µg/g). Mn dust loading ($\mu g/m^2$) was calculated using the concentration of Mn in dust, total mass of sieved dust collected and the floor area that was sampled. Mn levels were quantified for prenatal and postnatal periods of tooth development and quantified as the AUC of Mn:Ca for 207 children enrolled in the CHAMACOS study. Among children with Mn levels measured in teeth, between 170 and 180 children competed neurodevelopmental assessments at 6, 12 or 24 months using the Bayley Scales of Infant Development (second edition).

In this study, the concentrations and loadings of Mn in house dust were related to agricultural applications of Mn fungicides within 3 km of the residence and the number of farmworkers living in the home. Mn levels in dust were also higher in residences located on Antioch Loam soil type and residences located in the southern portion of the Salinas Valley. This analysis provides further evidence of the parental occupational take-home exposure pathway for pesticides and suggests that pesticide levels in homes could be reduced by storing shoes and clothes of agricultural workers outside the home, cleaning the home more frequently or effectively, and by using doormats. Concentrations of Mn in house dust provide an indicator of potential exposure, especially for young children, but house dust does not allow for the separation of non-dietary exposure from inhalation exposure, which is important for Mn. Much of the variability in Mn dust concentrations and loadings were not explained by the predictors included in our models.

This study provided evidence that Mn measurements in dentin from shed incisors provides time specific indicators of the body burden during the prenatal and early postnatal periods. Mn levels in prenatal dentin increased with maternal farm work, the number of farmworkers in the home, and agricultural use of fungicides containing Mn within 3 km of the home. Levels of Mn in prenatal dentin were also higher among children living in residences located on Antioch Loam soil and in homes with higher Mn dust loadings. Children whose mother's smoked during pregnancy had lower Mn levels in prenatal dentin. Much of the variability in Mn levels in prenatal dentin was not explained by the predictors included in our models, and we did not have Mn measurements for multiple teeth from the same child to assess the reproducibility of the measurements.

Prenatal Mn exposure levels were associated with modest deficits in mental and psychomotor development at 6-months of age only among children whose mothers' were iron deficient during pregnancy. An inverse association was observed between postnatal Mn exposure measured in shed teeth and psychomotor development at 6-months. There was no association between Mn concentrations in teeth prenatally or postnatally and neurodevelopment at 12 or 24-months, suggesting that the observed effects of Mn on neurodevelopment either did not persist or were spurious findings. There was no significant effect modification of blood lead concentrations on the relationship between Mn levels in teeth and early neurodevelopment in this analysis, but the blood lead concentrations in this study population may have been too low and the sample size too small to observe such an effect. Although agricultural use of Mn fungicides resulted in higher Mn levels in home and children's teeth, Mn exposure in this population may have been below the levels that cause significant, long-term deficits in neurodevelopment.

Conclusions

This study used unique data on agricultural pesticide use reporting and a GIS to show that Mn levels in homes and children's bodies were higher if the residence was located within 3 km of treated fields, providing further evidence that pesticide exposure levels are higher in agricultural communities. The number of farmworkers' storing their clothes and shoes in the home was also related to higher levels of Mn in the home and children's teeth which lends additional support to the importance of parental occupational take-home as an exposure pathway for persistent pesticides. This study was exceptional in that it combined environmental samples, biomarkers of exposure and neurodevelopmental outcomes for a prospective cohort of children. This was the first study to identify predictors of Mn levels in teeth from sources of Mn and to use Mn levels in teeth in an evaluation of early child neurodevelopment. By providing measures of both prenatal and early postnatal Mn exposure, Mn levels in dentin enable the evaluation of the most important time period of exposure. In models including both prenatal and postnatal Mn dentin levels, postnatal Mn levels were more strongly associated with neurodevelopment at 6-months. Evidence that iron status may be an important effect modifier of neurodevelopmental deficits associated with Mn exposure was provided by the findings of an inverse relationship between prenatal Mn levels in dentin and neurodevelopment at 6-months of age only among children whose mothers' were iron deficient during pregnancy.

This study adds important information regarding a new biomarker of Mn exposure and the relationship of this biomarker with environmental sources of Mn. This is the first study to use Mn levels in teeth to assess infant neurodevelopment. Although a modest decrease in neurodevelopment was observed in children at 6-months of age, the lack of an association at later time points is consistent with the only other prospective study that measured Mn in blood and infant neurodevelopment. Levels of Mn in prenatal dentin are likely to reflect exposure for a narrow time period during the second and third trimester of pregnancy, while Mn in postnatal dentin reflect exposure from birth through 9-months of age when dentin formation is complete. Previous studies that have observed an association between Mn exposure and neurodevelopment have used Mn concentrations in blood and hair as exposure measures and have mainly conducted cross-sectional evaluations of school aged children. It is also possible that Mn only effects neurodevelopment at very high exposure levels, in combination with high exposure to lead or only in iron deficient populations.

Future directions

Future studies should include house dust samples from non-agricultural areas to provide an unexposed group for comparison to those from agricultural areas. It would also be informative to collect and analyze of air samples for PM_{10} and Mn concentrations during the month prior to sample collection to evaluate the contribution of windblown dust from nearby agricultural fields in comparison to take-home dust from agricultural workers. Air samples collected at participating homes would be helpful for assessing the spatial variability of windblown dust and the contribution from longer range transport of particles into homes downwind of agricultural fields. Air samples could also potentially provide information for evaluating the differences in toxicity associated with exposure from inhalation compared to non-dietary ingestion.

More information is needed on the relationship between Mn measurements in teeth and those in blood and hair. Future analyses of Mn levels in dentin from shed teeth should include multiple teeth per child to evaluate the within-person variability of this exposure biomarker. Bayesian measurement error models could be used to improve prenatal and postnatal Mn exposure estimates from tooth dentin by incorporating information from GIS exposure estimates, house dust samples and shed teeth in a unified model that is fitted jointly and accounts for missing covariates and latent variables that are unaccounted for in traditional statistical models [Molitor, 2006]. Future studies could potentially use teeth as biomarkers of exposure to other metals and evaluate models for predicting exposure to metals by combining data from interview and GIS based exposure estimates.

In the future, analyses should be conducted evaluating the relationship between prenatal and postnatal Mn measurements in teeth and neurodevelopment and behavior in older children. Large studies with information on Mn exposure levels, prenatal and postnatal iron status, as well as iron metabolizing genes, would also be helpful for exploring effect modification by iron status and potential gene-environment interactions. Children living in agricultural communities are simultaneously exposed to many pesticides. Future studies need to incorporate exposure to multiple pesticides into the risk analysis. Bayesian hierarchical regression models provide an effective method for simultaneously evaluating a large number of pesticide exposures while reducing the possibility of false positive results [Rull, 2006].

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