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Determinants of Manganese in Prenatal Dentin of Shed Teeth from CHAMACOS Children Living in an Agricultural Community

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Abstract

Manganese (Mn) is an essential nutrient, but overexposure can be neurotoxic. Over 800 000 kg of Mn-containing fungicides are applied each year in California. Manganese levels in teeth are a promising biomarker of perinatal exposure. Participants in our analysis included 207 children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS), a longitudinal birth cohort study in an agricultural area of California. Mn was measured in teeth using laser-ablation-inductively coupled plasma-mass spectrometry. Our purpose was to determine environmental and lifestyle factors related to prenatal Mn levels in shed teeth. We found that 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 ($\mu g/m^2$ 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. Our results suggest that Mn measured in prenatal dentin provides retrospective and time specific levels of fetal exposure resulting from environmental and occupational sources.

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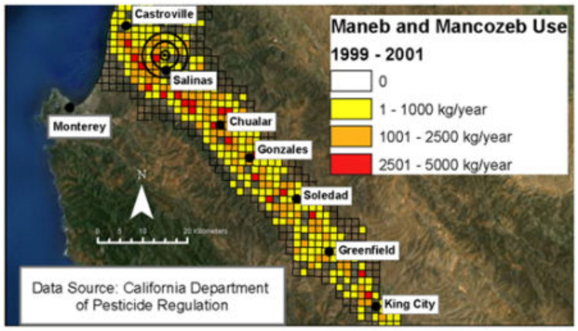
Notes

The authors declare no competing financial interest.



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.[1] Mn is an essential nutrient but at high doses it is neurotoxic and can result in a syndrome of neurologic deficits called manganism.[1] 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.[2,3] In school-aged children, lower cognitive scores have been associated with higher levels of Mn in water,[4] in blood[5,6] and in hair.[3,7–9] 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.[10]

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.[11,12] 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.[1] Higher hair Mn levels have been observed in children living near environmental sources of Mn.[9,13] 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.[14] Mn levels in nails may be a valid biomarker of cumulative occupational Mn exposure 7–12 months earlier.[12] In a rodent study, Mn levels in nail clippings were strongly correlated (R^2 = 0.93) with Mn levels in the brain.[15]

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.[16,17] 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 (13–16 weeks gestation) and ending 10–11 months after birth for primary coronal dentin in molars.[18]

In this study, we analyzed Mn in prenatal dentin of shed teeth 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.[19] Our 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. We evaluated the contribution to Mn_{PN} 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.

MATERIALS AND 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. We collected 324 teeth from 282 children. We analyzed 237 (73%) of these teeth 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. We abstracted information on the mother's hematocrit to hemoglobin ratio from prenatal medical records for a subset of participants (n = 161) to assess maternal iron status during pregnancy.

Home Visits

We conducted a home inspection during pregnancy (mean gestational age =14 ± 3 weeks). We collected latitude and longitude coordinates using global positioning system units and evaluated housekeeping characteristics. We also collected house dust samples described in more detail elsewhere.[20] Briefly, we collected dust 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.[21]

Tooth Mn Measurements

We collected deciduous teeth 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.[18,22] Briefly, teeth are sectioned in a vertical plane, and microscopy is used to visualize the neonatal line and incremental markings in sectioned teeth samples. We determined the concentrations and spatial distribution of Mn 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 × 10 000) for points measured during the second trimester and third 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. We stored dust samples at −80 °C for approximately ten years before shipping them on dry ice for analysis. We passed the dust samples through a 150 μm sieve and digested them overnight in 7.5 N nitric acid. We quantified Mn concentrations in dust (μg/g) using inductively coupled plasma optical emission spectroscopy with a limit of detection of 0.1 μg Mn/g dust. We calculated Mn dust loading (μg/m^2) 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.[19] 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. We used geographic information system (GIS) software (ArcInfo 10, ESRI, Redlands, CA) 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 km) and trimester of pregnancy based on gestational age (Figure 1). We weighted fungicide use near homes based on the proportion of each square-mile Section that was within the buffer around a residence.[23] To account for the potential downwind transport of fungicides from the application site, we obtained data 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. We determined the direction of each section centroid 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[24] to estimate the acres of lettuce within 1, 3, and 5 km of residences during each trimester.

Mn Intake from Tap Water

We linked the geocoded residential locations to the appropriate drinking water system using customer service area boundaries provided by local drinking water companies and the state of California.[25] Public drinking water systems provide monitoring data on Mn concentrations (μg/L) sampled at water distribution points.[26] However, Mn was not frequently detected in the study area during the pregnancy period for our cohort. Therefore, we used the average Mn concentration (μg/L) of all available samples from a water system to estimate long-term average concentrations of Mn in tap water. We estimated tap water consumption (L/day) using questionnaire data on the number of glasses of tap water consumed per day (8 ounces per glass). We multiplied consumption 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[27] specifically adapted for this study population.[28] 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. We estimated the mean Mn concentration for each food/beverage item and the daily Mn intake (mg/day) for each women 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 to 2005.[29] We also included Mn intake from dietary supplements. Because iron deficiency might increase Mn uptake, we also estimated daily iron intake using similar methods for use as a covariate in the models.[30] For a subset of participants (n = 159), we also had hematocrit to hemoglobin ratios as a measure of anemia.

Other Mn Sources

We estimated exposure to other potential sources of Mn, including soil type at the residence, estimated Mn concentration in outdoor air, and motor vehicle traffic. To account for variations in soil Mn concentrations, we linked each residence, based on latitude and longitude coordinates, to detailed soil maps.[31] To account for exposure via air inhalation, we assigned residences to a 2000 census tract and linked them to estimated 2002 Mn concentrations in ambient air from U.S. EPA.[32] We also estimated Mn emissions from vehicle traffic at each residence by calculating the traffic density using previously published methods that involve summing vehicle kilometers traveled for all major roads[33] by the length of the road segments within 500 m of the residence.[34]

Statistical Analysis

We used ANOVA for bivariate analysis of categorical predictor variables and the Spearman correlation coefficient to evaluate continuous predictor variables We identified potential explanatory variables 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 we natural log-transformed the values to normalize the distributions for regression models. We used manual forward selection 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. We also used backward elimination as an alternative method to identify significant predictor variables. We estimated the percentage change associated with each exposure source by exponentiating the regression coefficients, subtracting one and multiplying by 100. We evaluated outliers and reran models excluding one participant with a studentized t-score >3 that also had the lowest measured Mn_{PN} level (0.06). Our 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). We evaluated model fit using residual plots, log likelihood tests and Aikake's Information Criterion. We investigated nonlinear relationships between continuous predictor variables and tooth Mn levels using penalized splines with 3 degrees of freedom in general additive models. We used Moran's Global I to assess residual spatial autocorrelation of Mn_{PN} levels for the final models. We compared Mn levels in prenatal dentin from the second trimester to levels from the third 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, we used a structural equation model to evaluate casual pathways of exposure in the model that included participants with Mn dust measurements. [35] We constructed a structural equation model 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.

RESULTS

Descriptive Statistics and Bivariate Analyses

Most mothers included in our 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 1. The mean and median Mn_{PN} was 0.51 ($^{55}Mn{:}^{45}Ca$ AUC × 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 1705 μg/m^2 respectively. The mean agricultural Mn fungicide use within 3 km of the residence during the second trimester and third 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 second and third 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 second and third trimesters (to weight agricultural Mn fungicide use within 3 km) did not improve the correlation with Mn_{PN} (ρ_s = 0.11). We did not observe significant correlations between Mn_{PN} and Mn fungicide use within 1 km or Mn fungicide use during the first trimester (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}.

We observed significantly higher ($p < 0.01$) Mn prenatal tooth levels in children whose mothers were born outside the U.S., had less than a high school education, and did farm work during pregnancy (Table 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. 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% to 21.3%), 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 second and third 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. We excluded from the multivariable models those variables that were not significant predictors of Mn_{PN} ($p > 0.1$), 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 backward elimination. There was no spatial autocorrelation between the residuals for either model (Moran's I = 0.03, p = 0.7).

Table 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.

Determinants of Trimester Specific Mn Dentin Levels

Median Mn levels were significantly higher ($p < 0.001$) in dentin formed during the second trimester (0.59 ^{55}Mn:^{45}Ca AUC × 10^4) than dentin formed during the third trimester (0.35) (Table 1). The coefficient of variation (standard deviation/mean) was much higher for Mn in third trimester dentin (49%) than Mn in second trimester dentin or Mn_{PN} (36%) indicating relatively more variability. In models of Mn in second 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 third 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 second and third trimester dentin, reducing the number of participants who smoked or did farm work during pregnancy and the corresponding power to detect an association.

DISCUSSION

We report that Mn levels measured in prenatal dentin using laser ablation inductively coupled plasma mass spectroscopy were associated with estimates of prenatal environmental Mn exposure. Our 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. We observed that agricultural applications of widely used Mn-containing fungicides, maneb, and mancozeb, 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.[16] An evaluation of Mn concentrations in house dust found higher levels in residences located within 500 m of agricultural fields than residences located farther from fields.[36] Ethylenethiourea measured in urine has been used as an indicator of occupational exposure to maneb and mancozeb.[37] 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.[38]

Our results add to the existing evidence that household proximity to farmland and parental occupational take-home increases children's exposure to other classes of pesticides. [20,39–41] 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 we 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.[30] 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.[42] Women with low serum ferritin levels had higher blood Mn levels than the normal group in Korea.[43] A limitation of the present study is that we did not have information on iron-metabolizing genes HFE and TF or serum ferritin levels. We did not observe a relationship between maternal hematocrit to hemoglobin ratio or estimated dietary iron intake during pregnancy and Mn_{PN}.

Although we had few mothers that smoked in our population (<5%), we observed significantly lower Mn_{PN} levels 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,[16] while a national study in Korea also found lower Mn blood concentrations among current and former smokers.[44] 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 nonsmokers.[45] Mn is an essential nutrient that protects against oxidative stress.[46] 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.[47] 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.[36]

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.[13] 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.[14] 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.[48] Higher nitrogen dioxide concentrations, a proxy for motor vehicle emissions, have been associated with higher Mn levels in cord blood.[49] We did not observe an association between Mn_{PN} and traffic density, which is relatively low in our study area, but we did see 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.

We observed higher Mn levels in teeth during the second trimester than the third trimester while previous studies have found higher maternal blood Mn concentrations later in pregnancy.[16,17] While maternal blood Mn levels fluctuate during pregnancy, they do not necessarily reflect variations in fetal exposure. The use of dentin Mn allows us to measure fetal Mn exposure directly and we observed higher Mn levels in dentin formed during the second 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 second 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. We did not have information on time-activity patterns for the mothers and using only residential locations to assess proximity to Mn fungicide use and other Mn sources could result in misclassification of exposure. We did not collect personal environmental or duplicate diet samples 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 our study population (>82%) drank less than one glass per day of tap water and Mn was not detected frequently in public water supplies in our study area. Future studies should collect tap water samples for Mn analysis to better characterize potential exposure from drinking water. We used data from the Total Diet Study 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. We measured Mn levels in dentin for specific prenatal time points using knowledge of tooth mineralization instead of digesting the entire tooth and combining prenatal and postnatal exposures. Previous studies[50] have used measurements in tooth enamel to estimate Mn exposure; 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 our 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. We were 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.

In future analyses, we will evaluate measurements of Mn_{PN} for children newly enrolled in the CHAMACOS study at 9-years of age, utilizing multilevel Bayesian measurement error models to improve exposure estimates.[51] We will also evaluate the relationship between Mn levels in teeth and neurodevelopment in the CHAMACOS cohort. In conclusion, we found that exposure variables related to Mn containing fungicides are related to higher levels of Mn body burden in children. Further, deciduous teeth are relatively easy to obtain and store and measurements in dentin provide a unique opportunity to retrospectively assess prenatal exposure.

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ABBREVIATIONS

AUC	area under the curve
CHAMACOS	Center for the Health Assessment of Mothers and Children of Salinas
GIS	Geographic information system
HFE	hemochromatosis
Mn	manganese
Mn_{PN}	manganese in prenatal dentin
PUR	pesticide use report
TF	transferrin

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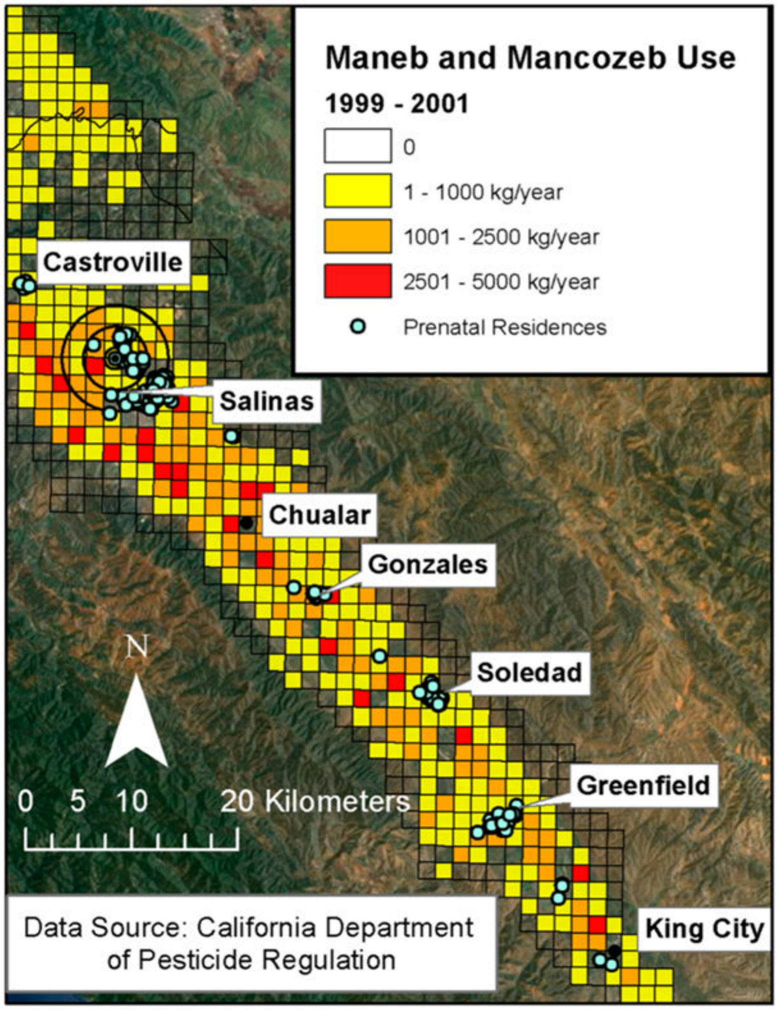

Figure 1.

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 km radius buffers around a residence (●).

Table 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 ($\mu g/m^2$)	131	1,705 ± 3,343	99	415	1564	0.27**

[a] Determined from detailed crop maps.[24]

[b] Calculated using agricultural fungicide use reporting data for maneb and mancozeb during the second and third trimester of pregnancy.[19]

[c] Estimated outdoor air concentration for 2002 by U.S. census tract.[32]

[d] Estimated from prenatal food frequency questionnaire data combined with Mn levels in food items from Total Diet Study.[29]

[e] Estimated from water service area boundaries[25] combined with drinking water data for Mn.[26]

* $p < 0.05$.

** $p < 0.01$.

Table 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[b]
Mother Smoked during Pregnancy		
yes	10 (4.8)	0.36 ± 0.17
no	197 (95.2)	0.52 ± 0.19[d]
Mother Born in United States		
yes	23 (11.1)	0.39 ± 0.13
no	184 (88.9)	0.53 ± 0.19[d]
Maternal Education		
≤6th–12th grade	161 (77.8)	0.53 ± 0.19
≥high school graduate	46 (22.2)	0.44 ± 0.16[d]
Family Income		
≤poverty line	115 (59.3)	0.54 ± 0.20
>poverty line	79 (40.7)	0.48 ± 0.18[b]
Housekeeping Practices		
average/poor	119 (60.1)	0.54 ± 0.23
excellent	79 (39.9)	0.47 ± 0.16[c]
Farmworker Shoes Stored Inside Home		
yes	68 (32.9)	0.57 ± 0.22
no	139 (67.1)	0.48 ± 0.16[d]
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[d]
Residence on Antioch Loam Soil[a]		
yes	77 (37.2)	0.56 ± 0.20
no	130 (62.8)	0.48 ± 0.18[d]

[a]Determined from soil survey data.[31]

[b] $p < 0.1$.

[c] $p < 0.05$.

[d] $p < 0.01$.

Table 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)	10.1 (0.1, 21.3)	0.05	1.8	15.8 (1.9, 31.6)	0.03	3.9
farm worker shoes in home (prenatal per worker)	8.1 (4.3, 12.0)	0.00003	8.4	6.4 (1.5, 11.4)	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)	15.1 (4.6, 26.6)	0.004	4.0	20.7 (6.3, 37.1)	0.004	6.4
mother smoked (prenatal yes vs no)	−33.8 (−46.6, −18.0)	0.0002	6.7	−40.3 (−55.3, −20.2)	0.001	9.0
Mn dust loading (per IQR[c] = 1465 $\mu g/m^2$)				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.