

## Biomarkers of Manganese Exposure in Pregnant Women and Children Living in an Agricultural Community in California

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**ABSTRACT:** Manganese (Mn) is an essential nutrient but at high exposure levels is a neurotoxicant. There is no well-validated biomarker to assess perinatal Mn exposure. A total of 75 mother-child pairs provided blood, urine, and/or deciduous tooth samples. We analyzed Mn in dentin and enamel of shed teeth; maternal, cord, and child blood; and maternal and child urine and examined the interrelationships of Mn levels in all matrices. We observed higher Mn levels in prenatal than postnatal dentin (geometric mean (GM) = 0.51 vs 0.16 Mn:Ca,  $p < 0.001$ ), maternal blood at delivery than 26 weeks gestation (GM = 20.7 vs. 14.6  $\mu\text{g/L}$ ,  $p = 0.001$ ), and cord blood than child blood at 24 months of age (39.9 vs 25.0  $\mu\text{g/L}$ ,  $p = 0.005$ ). There were no significant correlations between Mn in dentin and Mn concentrations in maternal blood or maternal or child urine. Levels of Mn in prenatal dentin, prenatal maternal blood, and 24 month urine were higher ( $p < 0.05$ ) among mothers and children living with a farm worker. Prenatal Mn levels in dentin were correlated with Mn loadings and concentrations in prenatal house dust. Levels of Mn measured in tooth dentin constitute a promising biomarker of perinatal exposure.



### INTRODUCTION

Manganese (Mn) is a naturally occurring element found in air, soil, water, and food.<sup>1</sup> It is commonly used in metal industries, as a gasoline additive, and in the formulation of agricultural fungicides.<sup>1</sup> Mn is an essential nutrient and a cofactor in enzymatic reactions involved in protein and energy metabolism and metabolic regulation.<sup>1</sup> However, human studies suggest that elevated early life exposures to Mn may have detrimental effects on the developing organism.<sup>2–7</sup> In school-aged children, high concentrations of Mn in drinking water, blood, and hair have been associated with low cognitive scores,<sup>8–12</sup> behavioral problems,<sup>13</sup> impaired motor function,<sup>14,15</sup> and poor memory.<sup>16</sup> Fetus and infants may be particularly vulnerable to the negative effects of high Mn concentrations due to the ability of Mn to cross the placenta and differences in Mn homeostatic mechanisms in young children, who absorb and retain a larger fraction of ingested Mn than adults.<sup>17–19</sup>

There is currently no consensus on which is the best biomarker of exposure to Mn. Urinary Mn has been used in multiple occupational studies, but it may have limited use as a direct measure of exposure due to high within person variability over time<sup>20</sup> and because the primary route of Mn excretion is via the biliary system.<sup>21,22</sup> Blood Mn has been frequently used as a

biomarker of exposure in occupational and population-based studies.<sup>3,23</sup> Nevertheless, concentrations of Mn in blood are homeostatically regulated by the hepatic portal system, have a relatively short half-life, and therefore do not serve as a reliable indicator of total body burden of Mn.<sup>1,21</sup> Hair Mn has also been used often in epidemiologic studies and is believed to reflect environmental exposures.<sup>10,13</sup> Two main limitations of using hair Mn as a predictor of Mn body burden include exogenous contamination<sup>24</sup> and the variability in hair metal concentrations between individuals due to differences in hair characteristics and personal habits.<sup>25,26</sup> More recently used and less invasive biomarkers to assess Mn exposure include saliva<sup>27</sup> and toenails,<sup>22,28</sup> however, there is limited information currently available on the relationship between Mn levels in these tissues and exposure.

Evidence suggests that available biomarkers may have a limited ability to assess prenatal Mn status and, more specifically, that biomarkers measured in maternal specimens may not accurately

**Received:** August 8, 2014

**Revised:** November 6, 2014

**Accepted:** November 12, 2014

**Published:** November 12, 2014

reflect fetal exposure. For example, maternal and cord blood Mn concentrations are only moderately correlated<sup>29</sup> with concentrations twice as high in cord than maternal blood.<sup>30–32</sup> Because Mn is taken up in the developing dentin of deciduous teeth,<sup>33</sup> Mn concentrations in teeth may serve as a useful biomarker of exposure to Mn during fetal development and early childhood. Current analytical techniques allow for Mn measurements for specific time periods of neonatal development starting in weeks 13–16 of gestation for incisors and ending 10–11 months after birth for molars.<sup>34</sup>

We previously examined the correlation of dentin Mn levels in deciduous teeth with Mn concentrations in maternal blood collected during the second trimester, cord blood, and Mn loading in house dust in a subgroup of ~80 CHAMACOS mother-child pairs ( $n = 204$  biological samples).<sup>34</sup> We found that Mn measurements in prenatally formed dentin immediately adjacent to the neonatal line, a histological feature formed in deciduous teeth at birth, were significantly associated with Mn concentrations in cord blood, and that Mn levels in dentin during the second trimester were significantly associated with house dust Mn loadings during pregnancy.

In this analysis, we measured Mn concentrations in deciduous teeth from children, and blood and urine samples from mothers and children enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study, a birth cohort study in the Salinas Valley, California. In the Salinas Valley, agricultural use of Mn-containing fungicides has averaged more than 160 000 kg per year during the previous two decades and more than 90% has been applied on lettuce crops.<sup>35</sup> Our goal was to examine the relationship of Mn levels in dentin and enamel from deciduous teeth with maternal and child blood and urine Mn concentrations measured at different time points during pregnancy and early life to evaluate the utility of perinatal biomarkers of Mn exposure. We also compared levels of Mn biomarkers among participants with and without potential sources of Mn exposure in their homes. The current analysis expands upon our previous work by examining additional Mn measurements in maternal and child urine, maternal blood at delivery, and child blood at 24 months; and more than doubling the total number of samples used for comparisons between Mn biomarkers ( $n = 423$  biological samples).

## MATERIALS AND METHODS

**Study Population.** Between October 1999 and October 2000, the CHAMACOS study enrolled 601 low-income pregnant women from six community clinics in the Salinas Valley. Women were eligible if they were  $\geq 18$  years old,  $< 20$  weeks of gestation, Spanish- or English-speaking, qualified for State funding of well-pregnancy care (within 200% of the Federal poverty level), and planning to deliver at the county hospital. A total of 526 liveborns were followed to delivery and 339 of them were followed-up until age 7 years. We collected teeth from 282 of these children. Participants were selected for this analysis based on the availability of two of the following biological samples: shed teeth; maternal blood collected at approximately 26 weeks' gestation or delivery; cord blood or child blood collected at 24 months; maternal urine at 26 weeks' gestation or child urine samples collected at 24 months. We included 75 mother-child pairs that provided blood, urine, and/or teeth samples ( $n = 423$  samples total). 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.

**Maternal Interviews and House Dust Collection.** We administered maternal interviews during pregnancy at approximately 13- and 26 weeks' gestation, shortly after delivery, and when the child was 24 months of age. Trained bilingual 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 farm workers in the home, and number of farm workers that stored their clothes or shoes indoors. House dust samples were collected during home visits shortly after enrollment (median = 17 weeks gestation).

**Mn Tooth Measurements.** We collected deciduous teeth beginning with the 7-year visit. Participants either mailed or brought in teeth when they naturally shed. The method for measuring Mn in teeth has been described elsewhere.<sup>34,36</sup> Briefly, teeth were washed with deionized water, and then sectioned in a vertical plane, and microscopy was used to visualize the neonatal line and incremental markings in sectioned teeth samples. The neonatal line is a histological feature formed at birth that distinguishes between the prenatal and postnatal periods of development in all primary human teeth.<sup>37</sup> 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 ( $^{55}\text{Mn}:^{43}\text{Ca}$  ratio) to provide a measure independent of variations in tooth mineral density. Values are the area under the curve ( $\text{AUC} \times 10\,000$ ) for points measured that correspond to the second trimester and third trimesters separately, a combined prenatal average value, and a postnatal average value. For some teeth, due to excessive wear from grinding, the trimester could not be identified and it was only possible to determine that the measurement was from the prenatal region of the tooth. Regions of dentin immediately adjacent to the attrition were not analyzed to avoid contamination. The analytical limits of detection (LOD) for Mn in tooth dentin and enamel were both  $0.04\ ^{55}\text{Mn}:^{43}\text{Ca}$ . The one sample with Mn in postnatal enamel below the LOD was set to the LOD/2. The coefficient of variation for five teeth measured on three different days ranged from 4.5% to 9.5% indicating good reproducibility of  $^{55}\text{Mn}:^{43}\text{Ca}$  measurements.

**Mn Blood Measurements.** Blood samples were collected from the mother by venipuncture at the time of the pregnancy interview (~26 weeks) and shortly before delivery. We also collected umbilical cord blood samples at birth and child blood samples by venipuncture at the 24-month visit. Blood samples were immediately processed and stored at  $-80\ ^\circ\text{C}$  prior to analysis. For analysis, blood samples were digested in Teflon vials using concentrated nitric acid, evaporated to dryness, and redissolved in 1% nitric acid for analyses.<sup>21</sup> Whole blood samples were analyzed for Mn concentrations using trace metal clean techniques and high resolution inductively coupled plasma mass spectrometry (ICP-MS). The LOD for Mn in blood was  $0.003\ \mu\text{g}/\text{L}$ , but no samples were below the detection limit.

**Mn Urine Measurements.** Spot urine samples were collected from mothers at ~26 weeks of gestation in sterile polyethylene containers and from children at 24 months of age using a standard infant urine collection bag (Hollister, Libertyville, IL). Urine samples were aliquoted into precleaned glass containers with Teflon-lined caps and stored at  $-80\ ^\circ\text{C}$  prior to analysis. Urine samples were acidified to  $\text{pH} < 2$  for direct

Table 1. Distribution of Mn Levels in Biological and Environmental Media<sup>a</sup>

biomarker (units)	time period	N	min	25th	50th	75th	max	GM (GSD)
dentin (Mn:Ca AUC × 10 000)	second trimester	57	0.18	0.46	0.59	0.74	1.86	0.60 (1.5)
	third trimester	60	0.01	0.24	0.32	0.46	1.21	0.33 (2.0)
	prenatal	62	0.15	0.35	0.48	0.57	1.34	0.51 (1.5)
	postnatal	61	0.03	0.10	0.14	0.22	1.15	0.16 (2.0)
enamel (Mn:Ca AUC × 10 000)	second trimester	58	0.10	0.18	0.27	0.42	1.35	0.30 (1.9)
	third trimester	60	0.09	0.19	0.30	0.47	1.37	0.31 (2.0)
	prenatal	61	0.10	0.18	0.28	0.51	1.31	0.30 (1.8)
	postnatal	37	<LOD	0.11	0.16	0.19	1.09	0.16 (1.8)
blood (μg/L)	26 weeks gestation	53	4.34	12.5	14.8	18.5	32.8	14.6 (1.5)
	delivery	53	6.66	16.5	20.3	27.9	35.7	20.7 (1.4)
	cord	59	20.74	31.4	40.5	50.6	72.4	39.9 (1.4)
	child 24 months	37	11.04	17.5	25.2	32.8	50.1	25.0 (1.5)
urine (μg/L)	26 weeks gestation	59	0.07	0.2	0.4	0.6	7.3	0.5 (2.4)
	child 24 months	39	<LOD	0.3	0.6	1.1	16.1	0.8 (3.6)
house dust concentration (μg/g)	18 weeks gestation	46	37	135	170	200	414	153 (1.7)
house dust loading (μg/m <sup>2</sup> )	18 weeks gestation	46	6.2	193	619	1,720	18,400	511 (5.9)

<sup>a</sup>GM = geometric mean; GSD = geometric standard deviation; LOD = limit of detection.

analyses. All digested samples were centrifuged (10,000g for 10 min) and then analyzed using ICP-MS. External standardization was via certified standards (Spex Industries, Inc., Edison, NJ). The analytical LOD for Mn in urine was 0.01 μg/L and the one sample below the LOD was set to the LOD/2. Standard reference materials (NIST SRM 1577b, bovine liver) and sample spike-recoveries were used to confirm analytical recovery which was > 95%. Urinary creatinine concentrations were measured using a commercially available diagnostic enzyme method (Vitros CREA slides; OrthoClinical Diagnostics, Raritan, NJ). The results that we observed when we used creatinine-adjusted Mn urine concentrations were very similar to those of the unadjusted Mn urine concentrations (Pearson's correlation coefficient = 0.94); therefore we only present results for unadjusted Mn urine concentrations.

**Mn House Dust Measurements.** We collected house dust samples during the second trimester (mean = 18 weeks, SD = 6 weeks gestation). Details of our dust sample collection methods are provided elsewhere.<sup>38,39</sup> Briefly, dust samples were collected from a one square meter area of floor using a high volume small surface sampler (HVS3, Envirometrics, Inc., Seattle, WA) and then stored at -80 °C before shipping on dry ice for analysis. One dust sample per residence was collected for pesticide analyses. The pesticide dust sample was taken from (in order of priority): carpet in the central living area; if none then carpet in the bedroom; if none then bare floor in the central living area; if sample volume was judged inadequate then stuffed furniture in the central living area was sampled. Samples were sieved to 150 μm and then weighed. We digested ~500 mg the dust samples overnight in 7.5 N nitric acid and quantified Mn concentrations using inductively coupled plasma optical emission spectroscopy. We used Mn standards to develop a calibration curve. The procedural LOD was 0.1 μg Mn/g dust and was derived via repeated analyses of analytical blanks within each analytical run using the formula three times the average standard deviation of blanks, then converting this analytical LOD to a procedural LOD using the typical dust sample weight that was processed,

accounting for dilutions during processing. We analyzed 26 dust samples in triplicate to determine the reproducibility of Mn measurements and the coefficient of variation for Mn among these samples was 2.7%. Since dust loading is believed to better characterize the amount of metals in dust that may be available for contact by children,<sup>40</sup> we computed Mn dust loading (μg/m<sup>2</sup>) by multiplying the Mn concentration (μg/g) by the dust loading (g/m<sup>2</sup>), obtained by weighing the sieved dust sample and dividing by the area sampled.

**Statistical Analysis.** Because the distributions of Mn biomarkers were not normally distributed and skewed to the right, we calculated the geometric means and geometric standard deviations as better measures of the central tendency and variability. We used nonparametric methods to evaluate bivariate relationships including Spearman correlation coefficients for continuous variables, the Wilcoxon rank sum test to assess the association between Mn biomarker concentrations and presence of farm workers in the home, and the Wilcoxon matched-pair signed-ranks test to compare Mn biomarker levels in mothers and children at different time points. We evaluated the correlations between Mn levels in teeth during the second and third trimesters and other prenatal biomarkers to assess whether Mn measurements in teeth provide time specific information on potential Mn exposures to the fetus. All data analyses were performed using Stata version 13.0 (StataCorp, College Station, TX).

## RESULTS

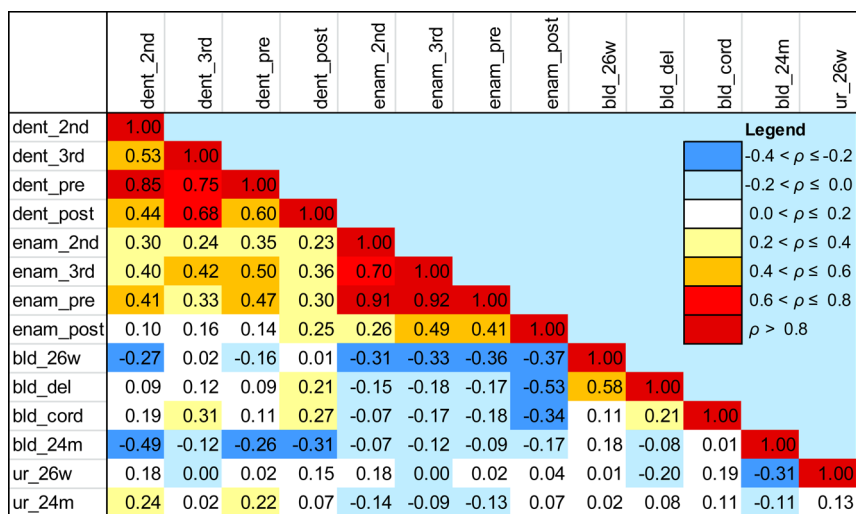
At the time of enrollment, most mothers (76%) were under 30 years of age, multiparous (69%), had a high school education or less (80%), and lived in a household at or below the poverty level (65%). Almost all mothers were born in Mexico (96%) and most had lived in the United States for five years or less (67%). Fifty-five percent of the participating children were girls (55%).

The distributions of Mn biomarker concentrations and Mn house dust levels are shown in Table 1. The geometric mean (± geometric standard deviation) Mn level in tooth dentin was

**Table 2. Spearman Correlation Coefficients and 95% Confidence Intervals (CI) for Prenatal Mn Levels in Tooth Dentin With Mn Levels in Tooth Enamel, Maternal and Cord Blood, and Maternal Urine**

biomarker	time period	spearman correlation coefficients (95% CI) with Mn levels in tooth dentin		
		second trimester	third trimester	prenatal
enamel (Mn:Ca)	second trimester	0.30 (0.05, 0.52) <sup>b</sup>	0.24 (−0.02, 0.47) <sup>a</sup>	0.35 (0.10, 0.55) <sup>c</sup>
	third trimester	0.40 (0.16, 0.60) <sup>c</sup>	0.42 (0.18, 0.60) <sup>c</sup>	0.50 (0.28, 0.67) <sup>c</sup>
	prenatal	0.41 (0.17, 0.61) <sup>c</sup>	0.33 (0.09, 0.54) <sup>c</sup>	0.47 (0.24, 0.64) <sup>c</sup>
blood (μg/L)	26 weeks gestation	−0.27 (−0.53, 0.25)	0.02 (−0.29, 0.32)	−0.16 (−0.44, 0.15)
	delivery	0.09 (−0.24, 0.40)	0.12 (−0.20, 0.42)	0.09 (−0.22, 0.39)
	cord	0.19 (−0.12, 0.47)	0.31 (0.02, 0.55) <sup>b</sup>	0.11 (−0.19, 0.38)
urine (μg/L)	26 weeks gestation	0.18 (−0.12, 0.44)	0.00 (−0.28, 0.28)	0.02 (−0.26, 0.29)

<sup>a</sup>*p* < 0.1. <sup>b</sup>*p* < 0.05. <sup>c</sup>*p* < 0.01.



**Figure 1.** Spearman correlation coefficients between levels of Mn biomarkers. Abbreviations: bld = blood; dent = dentin; enam = enamel; ur = urine; 2nd = 2nd trimester; 3rd = 3rd trimester; pre = prenatal; post = postnatal; del = delivery; cord = cord blood; 26w = 26 weeks gestation; 24m = child 24 months of age.

**Table 3. Levels of Mn Biomarkers by Farm Worker Living in Home and Maternal Farm Work at the Time Corresponding to Biomarker Measurement**

biomarker	time period	farm worker living in home				maternal farm work			
		yes		no		yes		no	
		N	GM (GSD)	N	GM (GSD)	N	GM (GSD)	N	GM (GSD)
dentin (Mn:Ca)	prenatal	45	0.51 (1.5)	17	0.39 (1.5) <sup>b</sup>	7	0.50 (1.5)	55	0.47 (1.5)
	postnatal	48	0.16 (2.0)	13	0.16 (1.9)	9	0.18 (2.1)	52	0.15 (1.8)
enamel (Mn:Ca)	prenatal	45	0.31 (1.8)	16	0.29 (2.0)				
	postnatal	30	0.15 (1.9)	7	0.17 (1.4)	2	0.16 (1.0)	35	0.15 (1.9)
blood (μg/L)	26 w gestation	40	15.7 (1.5)	13	11.9 (1.6) <sup>a</sup>	7	20.9 (1.4)	46	13.9 (1.5) <sup>c</sup>
	delivery	34	20.8 (1.5)	19	20.4 (1.4)	0	-	53	20.7 (1.4)
	cord	38	39.5 (1.4)	20	41.0 (1.4)	0	-	59	39.9 (1.4)
urine (μg/L)	child 24 m	30	24.3 (1.5)	7	23.9 (1.5)	11	25.4 (1.4)	26	24.8 (1.5)
	26 w gestation	44	0.44 (2.6)	15	0.37 (2.0)	7	0.57 (2.4)	52	0.40 (2.4)
	child 24 m	34	0.59 (3.5)	5	0.22 (3.7) <sup>a</sup>	11	0.70 (7.0)	28	0.47 (2.5)

<sup>a</sup>*p* < 0.1. <sup>b</sup>*p* < 0.05. <sup>c</sup>*p* < 0.01. GM = geometric mean; GSD = geometric standard deviation. Corresponding time for prenatal teeth = 26 weeks gestation and postnatal teeth = child 6 months of age.

higher in the second trimester ( $0.6 \pm 1.5$  Mn:Ca AUC) than the third trimester of pregnancy ( $0.33 \pm 2.0$  Mn:Ca AUC, *p* < 0.001), but Mn levels in tooth enamel were similar during these trimesters ( $0.30 \pm 1.9$  vs  $0.31 \pm 2.0$  Mn:Ca AUC). Prenatal Mn dentin levels were significantly higher than postnatal Mn levels (*p*

< 0.001) and this was also true in tooth enamel (*p* < 0.001). In tooth dentin, there was a decreasing trend in Mn levels over time (*p* < 0.001) with the second trimester > third trimester > postnatal (*p* < 0.001). Geometric mean blood Mn concentrations increased significantly in the mothers during pregnancy from 26



**Table 4. Spearman Correlation Coefficients and 95% Confidence Intervals (95% CI) between Prenatal Mn Biomarkers and Mn House Dust Concentrations and Loadings**

biomarker	time period	N	Spearman correlation coefficients (95% CI) of biomarker with dust	
			Mn concentration	Mn loading
			( $\mu\text{g/g}$ )	( $\mu\text{g/m}^3$ )
dentin (Mn:Ca AUC)	prenatal	37	0.44 (0.13, 0.67) <sup>b</sup>	0.27 (−0.06, 0.55) <sup>a</sup>
enamel (Mn:Ca AUC)	prenatal	37	0.10 (−0.23, 0.41)	−0.03 (−0.35, 0.30)
blood ( $\mu\text{g/L}$ )	26 w gestation	33	−0.14 (−0.46, 0.21)	−0.20 (−0.51, 0.15)
	delivery	25	−0.09 (−0.47, 0.32)	0.10 (−0.31, 0.48)
	cord	35	0.01 (−0.33, 0.34)	0.29 (−0.05, 0.57) <sup>a</sup>
urine ( $\mu\text{g/L}$ )	26 w gestation	32	0.39 (0.05, 0.65) <sup>b</sup>	0.26 (−0.10, 0.56)

<sup>a</sup> $p < 0.1$ . <sup>b</sup> $p < 0.05$ .

weeks' gestation ( $15 \pm 1.5 \mu\text{g/L}$ ) to delivery ( $21 \pm 1.4 \mu\text{g/L}$ ,  $p < 0.001$ ) and decreased significantly in children from birth (cord blood  $40 \pm 1.4 \mu\text{g/L}$ ) to 24 months of age ( $25 \pm 1.5 \mu\text{g/L}$ ,  $p < 0.001$ ). The geometric mean Mn concentration in cord blood ( $40 \pm 1.4 \mu\text{g/L}$ ) was nearly twice as high as the geometric mean concentration in maternal delivery blood ( $21 \pm 1.4 \mu\text{g/L}$ ,  $p < 0.001$ ). The geometric mean Mn house dust concentration was  $153 \pm 1.7 \mu\text{g/g}$  and there was a wide range of Mn house dust loading values ( $6.2$ – $18\,400 \mu\text{g/m}^2$ ) with a geometric mean of  $511 \pm 5.9 \mu\text{g/m}^2$ .

Table 2 shows the correlation between prenatal Mn levels in tooth dentin, tooth enamel, blood, and urine measured at different time points. There were no significant correlations between Mn in dentin and Mn concentrations in maternal blood or maternal or child urine. Levels of Mn in enamel were positively and significantly ( $p < 0.01$ ) correlated with Mn levels in dentin during the second trimester ( $r_s = 0.30$ , 95% CI: 0.05, 0.52), third trimester ( $r_s = 0.42$ , 95% CI = 0.18, 60), and prenatal ( $r_s = 0.47$ , 95% CI: 0.24, 0.64) time periods. Figure 1 provides the Spearman rank correlation coefficients between levels of all measured Mn biomarkers. Cord blood Mn concentrations were weakly correlated with maternal delivery blood ( $r_s = 0.21$ ,  $p = 0.19$ ) and Mn levels in tooth dentin ( $r_s = 0.31$ ,  $p < 0.05$ ). Conversely, there were significant negative correlations between prenatal Mn blood concentrations in mothers and Mn levels in enamel during the third trimester ( $r_s = -0.33$ ) and in enamel over the entire prenatal time period ( $r_s = -0.36$ ). There was no correlation between Mn levels in prenatal enamel and Mn concentrations in prenatal maternal urine. There was also no correlation between Mn concentrations in blood and urine collected concurrently at 26-weeks gestation and when the child was 24-months of age.

Table 3 compares the levels of Mn in tooth dentin, tooth enamel, blood, and urine among homes with and without a farm worker and whether the mother was a farm worker at the corresponding time (26 weeks gestation for prenatal and 6 months for postnatal). Levels of Mn were significantly higher in prenatal dentin ( $0.51 \pm 1.5$  vs  $0.39 \pm 1.5$  Mn:Ca,  $p = 0.05$ ), prenatal blood ( $15.7 \pm 1.5$  vs  $11.9 \pm 1.6 \mu\text{g/L}$ ,  $p = 0.07$ ), and child urine at 24 months ( $0.59 \pm 3.5$  vs  $0.22 \pm 3.7 \mu\text{g/L}$ ,  $p = 0.06$ ) if a farm worker was living in the home than if a farm worker was not living in the home at that time. The results were similar for homes where a farm worker stored their shoes or clothes indoors (data not shown). Prenatal blood concentrations were higher if the mother was a farm worker ( $20.9 \pm 1.4 \mu\text{g/L}$ ) than if the

mother was not a farm worker ( $13.9 \pm 1.5 \mu\text{g/L}$ ). The levels of other Mn biomarkers were similar whether the mother was a farm worker or not during the time period. Table 4 shows the Spearman correlation coefficients and 95% CI's between prenatal Mn biomarkers and Mn house dust concentrations and loadings. Spearman correlation coefficients between prenatal Mn biomarkers and prenatal Mn dust concentration ( $\mu\text{g/g}$ ) were significant for prenatal dentin ( $r_s = 0.44$ , 95% CI: 0.13, 0.67) and maternal urine at 26 weeks gestation ( $r_s = 0.39$ , 95% CI: 0.05, 0.65). Spearman correlation coefficients were borderline significant ( $p < 0.1$ ) between Mn dust loading ( $\mu\text{g/m}^2$ ) and prenatal Mn dentin ( $r_s = 0.27$ , 95% CI: −0.06, 0.55) and cord blood Mn concentrations ( $r_s = 0.29$ , 95% CI: −0.05, 0.57).

## DISCUSSION

In this study of mother-child pairs living in an agricultural area with use of Mn-containing fungicides, we found higher Mn levels in prenatal maternal blood and tooth dentin if a farm worker lived in the home and in homes with higher Mn dust loading levels. Maternal blood Mn concentrations increased during pregnancy, whereas tooth Mn levels decreased during pregnancy and from the prenatal to postnatal period, and cord blood Mn concentrations were twice as high as delivery blood Mn concentrations.

We observed increasing Mn concentrations in maternal blood over pregnancy, consistent with previous studies,<sup>31,41–43</sup> which is considered to be normal during pregnancy.<sup>44</sup> Increases in blood Mn concentrations during pregnancy could be related to an increase in Mn intestinal absorption or changes in Mn metabolism,<sup>42</sup> or differences in tissue Mn mobilization related to increased estrogen and progesterone concentrations during pregnancy.<sup>31,45</sup> Maternal Mn concentrations in blood during the second trimester of pregnancy in our cohort (median =  $14.8 \mu\text{g/L}$ ) were higher than those reported in previous studies,<sup>31,41,42,46</sup> but were lower than levels in a cohort of pregnant women (median =  $23.7 \mu\text{g/L}$ ) that lived near banana plantations treated with Mn-fungicides in Costa Rica.<sup>43</sup> One possible explanation for the higher blood levels in the cohort from Costa Rica is that Mn-containing fungicides are applied by tractor in the Salinas Valley but aerielly in Costa Rica. Higher Mn blood concentrations were also observed among exposed school-aged children living in a mining area of Mexico<sup>9</sup> and in cord blood samples from children in Taiwan living in areas with higher nitrogen dioxide concentrations, which was used as a marker of motor vehicle emissions of Mn from fuel.<sup>47</sup>

In contrast to maternal blood Mn concentrations, we found a significant decrease in Mn levels in tooth dentin between the second and third trimesters of pregnancy. It is possible that uptake of Mn in dentin varies with fetal development. Based on our data, a single measure of maternal blood Mn concentrations during pregnancy may not be an accurate biomarker of fetal exposure since maternal blood Mn concentrations were not correlated with Mn in cord blood or prenatal Mn levels in teeth, the trends in levels of these biomarkers during pregnancy were in opposite directions, and information on sources of Mn exposure were more highly correlated with Mn in dentin. Our results suggest that more frequent measurements of maternal blood Mn during pregnancy might be required to provide a more accurate measure of fetal Mn exposure.

Cord blood Mn concentrations were nearly two times higher than Mn concentrations in maternal delivery blood (40 vs 21  $\mu\text{g}/\text{L}$ ), which is consistent with reports from previous studies.<sup>30–32,44,48</sup> Concentrations of Mn could be higher in cord blood than adult blood partially due to a higher fraction of erythrocytes that store much of the Mn in the body.<sup>31</sup> However, there are also active transport mechanisms that may be regulated to ensure adequate supply of the essential element Mn to the developing fetus while continuing to maintain maternal homeostasis.<sup>18</sup> Notably, the correlation between cord blood and delivery blood Mn concentrations, although positive ( $r = 0.3$ ), was not significant in our study population. Previous studies have reported similar correlations between cord blood and delivery blood, ranging from 0.28 to 0.38.<sup>31,32</sup> These findings suggest that maternal Mn biomarkers may not accurately reflect Mn levels in fetal tissues over time. However, we observed a significant correlation between cord blood Mn concentrations and third trimester dentin levels ( $r_s = 0.31$ ). In a subset of this cohort where the teeth were analyzed at a higher temporal resolution that allowed us to estimate exposure over the week prior to birth (as opposed to the entire trimester), the correlation was even stronger between cord blood Mn concentrations and dentin Mn levels ( $r_s = 0.70$ ) indicating the time-specific resolution of Mn in dentin.<sup>34</sup>

In our cohort, blood Mn concentrations in cord blood were higher than those measured at 24 months. A similar decline was also observed between 12 and 24 months in a cohort of children from Mexico.<sup>5</sup> We also observed a significant decrease in both dentin and enamel Mn levels from the prenatal and postnatal periods. A study of 27 children that measured Mn deposits in tooth enamel also reported decreasing Mn levels between the prenatal and postnatal periods.<sup>49</sup> The decrease in Mn levels in blood and teeth between birth and age two years likely reflect changes in homeostatic control and developmental requirements for Mn during early childhood.<sup>19</sup> Levels of Mn in prenatal enamel were moderately correlated with Mn levels in prenatal dentine in our cohort, which reflects the differences in mineralization patterns of these compartments. Unlike dentin which deposits almost entirely during tooth development from 13–16 weeks gestation through 10–11 months of age, enamel has a protracted maturation process with initial deposits representing only ~30% of the total enamel<sup>50,51</sup> and therefore measurement of Mn at any single location in enamel cannot be directly linked to timing of exposure.<sup>34</sup>

We found higher Mn levels in prenatal dentin, prenatal maternal blood, and children's urine collected at 24 months of age in homes with a farm worker than in homes without a farm worker. We also observed significant correlations between prenatal Mn levels in dentin and maternal urine with Mn dust

concentration and loadings, and with Mn dust loadings and cord blood Mn concentrations. In a previous analysis of the teeth included in this study plus additional shed teeth from participants who did not provide blood or urine samples, we observed higher Mn levels in prenatal dentin among children whose mothers worked in agriculture during pregnancy, lived with a farm worker, had higher agricultural use of Mn-containing fungicides within three kilometers of their home (as determined using pesticide use registry data), or had higher Mn dust loadings in their home.<sup>52</sup> Overall, our findings in this report and previous analyses suggest that agricultural use of Mn-containing fungicides results in higher Mn levels in biomarkers and house dust. Our findings are consistent with research in an agricultural community near Quebec where higher Mn blood concentrations were observed during pregnancy when pesticides (unspecified, but possibly Mn-containing fungicides) were applied less than one kilometer from participant homes,<sup>31</sup> and in Costa Rica, where higher Mn concentrations were observed in hair of pregnant women who worked in agriculture and among those who lived within 50 m of a banana plantation.<sup>43</sup>

Our study had several limitations. In spite of the laboratory analysis of a large number of specimens for each participant, we lacked statistical power due to the relatively small sample size, but were still able to detect moderate correlations ( $r_s > 0.27$ ). Because primary teeth are not shed until approximately 6–7 years of age, Mn levels in dentin are most valuable for research studies that require retrospective exposure assessment of perinatal Mn exposure. Since our study population was low-income and primarily Hispanic, further research is needed in populations that include a broader range of household income and ethnicities to determine whether our results are generalizable. We did not have information on iron metabolizing genes or serum ferritin levels. In Korea, women with low serum ferritin levels had higher blood Mn concentrations than those with normal ferritin levels.<sup>53</sup> A recent study found that children with iron deficiency anemia had higher blood Mn concentrations than children without anemia.<sup>54</sup> Women carrying any variant allele of hemochromatosis had 12% lower Mn blood concentrations than women with no variant alleles and these results were replicated in a knockout mice model.<sup>55</sup> We did not measure Mn levels in hair. Hair has been used in other studies as a biomarker of Mn exposure for children and adults and may integrate changes in circulating Mn levels and body burden over time better than blood.<sup>24</sup> We collected hair samples at 10.5 years of age in this cohort and are currently measuring Mn concentrations, however, these samples do not represent the same time period as the teeth, blood, and urine samples presented in this analysis.

Despite these limitations, this study includes measurements of Mn in hundreds of biological and environmental samples and is the largest sample to date of Mn measurements in both teeth and blood and is the first study to compare Mn levels in teeth and urine. Deciduous teeth have the advantage that they provide retrospective information on perinatal Mn levels. However, measurement of Mn in dentin is an intensive process that requires expertise in tooth histology. Our study was conducted in an agricultural community with high use of Mn-containing fungicides and had Mn measurements in both mothers and children at multiple time points. We also had Mn dust concentration and loading measurements available for a subset of the participants to compare biomarkers to environmental samples.

In summary, both maternal and fetal Mn biomarker levels change during pregnancy and maternal biomarker levels do not

appear to be strongly related to fetal Mn levels. Levels of Mn measured in tooth dentin offers a promising biomarker of early life exposure that may better reflect fetal Mn levels than a single measure of Mn in maternal blood and provides an integrated measure of exposure over time. Future studies are needed that compare Mn levels measured in noninvasive biomarkers such as hair and toenails collected early in life to Mn levels in dentin from shed teeth. Measurements of Mn in multiple teeth of the same type (e.g., incisors) from the same individuals are also needed to assess within and between person variability of these biomarkers.

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### Notes

The authors declare the following competing financial interest(s): One of the authors (AB) has served as a consultant on cases unrelated to the issues covered in this paper and has participated as a member of the Science Advisory Board for The Organic Center, a non-profit organization that provides information for scientific research about organic food and farming. B.E. has provided expert opinion on a pesticide poisoning case (more than three years ago). The other authors declare they have no actual or potential competing financial interests.

## ACKNOWLEDGMENTS

This work was funded by research grant numbers RD 82670901, RD 83451301, RD 83171001 from EPA and PO1 ES009605 from NIEHS. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or EPA. We gratefully acknowledge the CHAMACOS staff, students, community partners, and, especially, the CHAMACOS participants and their families.

## LIST OF ABBREVIATIONS

AUC = area under the curve

Ca = calcium

CHAMACOS = Center for the Health Assessment of Mothers and Children of Salinas

CI = confidence interval

GM = geometric mean

GSD = geometric standard deviation

HVS3 = high volume small surface sampler

ICP-MS = high resolution inductively coupled plasma mass spectrometry

LOD = limit of detection

Mn = manganese

$r_s$  = Spearman correlation coefficient

## REFERENCES

- (1) *Toxicological Profile for Manganese*; Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2012.
- (2) Roels, H. A.; Bowler, R. M.; Kim, Y.; Claus Henn, B.; Mergler, D.; Hoet, P.; Gocheva, V. V.; Bellinger, D. C.; Wright, R. O.; Harris, M. G.; Chang, Y.; Bouchard, M. F.; Riojas-Rodriguez, H.; Menezes-Filho, J. A.; Tellez-Rojo, M. M. Manganese exposure and cognitive deficits: A growing concern for manganese neurotoxicity. *Neurotoxicology* **2012**, *33* (4), 872–80.
- (3) Takser, L.; Mergler, D.; Hellier, G.; Sahuquillo, J.; Huel, G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. *Neurotoxicology* **2003**, *24* (4–5), 667–674.

- (4) Ericson, J. E.; Crinella, F. M.; Clarke-Stewart, K. A.; Allhusen, V. D.; Chan, T.; Robertson, R. T. Prenatal manganese levels linked to childhood behavioral disinhibition. *Neurotoxicol. Teratol.* **2007**, *29* (2), 181–7.

- (5) Claus-Henn, B. C.; Ettinger, A. S.; Schwartz, J.; Tellez-Rojo, M. M.; Lamadrid-Figueroa, H.; Hernandez-Avila, M.; Schnaas, L.; Amarasiwardena, C.; Bellinger, D. C.; Hu, H.; Wright, R. O. Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology* **2010**, *21* (4), 433–439.

- (6) Rink, S. M.; Ardoino, G.; Queirolo, E. I.; Cicariello, D.; Manay, N.; Kordas, K. Associations between hair manganese levels and cognitive, language, and motor development in preschool children from Montevideo, Uruguay. *Arch. Environ. Occup. Health* **2014**, *69* (1), 46–54.

- (7) Lin, C. C.; Chen, Y. C.; Su, F. C.; Lin, C. M.; Liao, H. F.; Hwang, Y. H.; Hsieh, W. S.; Jeng, S. F.; Su, Y. N.; Chen, P. C. In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. *Environ. Res.* **2013**, *123*, 52–7.

- (8) Wasserman, G. A.; Liu, X.; Parvez, F.; Ahsan, H.; Levy, D.; Factor-Litvak, P.; Kline, J.; van Geen, A.; Slavkovich, V.; LoIacono, N. J.; Cheng, Z.; Zheng, Y.; Graziano, J. H. Water manganese exposure and children's intellectual function in Arahazar, Bangladesh. *Environ. Health Perspect* **2006**, *114* (1), 124–9.

- (9) Riojas-Rodriguez, H.; Solis-Vivanco, R.; Schilman, A.; Montes, S.; Rodriguez, S.; Rios, C.; Rodriguez-Agudelo, Y. Intellectual function in Mexican children living in a mining area and environmentally exposed to manganese. *Environ. Health Perspect* **2010**, *118* (10), 1465–70.

- (10) Menezes-Filho, J. A.; Novaes, C. D.; Moreira, J. C.; Sarcinelli, P. N.; Mergler, D. Elevated manganese and cognitive performance in school-aged children and their mothers. *Environ. Res.* **2011**, *111* (1), 156–163.

- (11) Kim, Y.; Kim, B. N.; Hong, Y. C.; Shin, M. S.; Yoo, H. J.; Kim, J. W.; Bhang, S. Y.; Cho, S. C. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. *Neurotoxicology* **2009**, *30* (4), 564–71.

- (12) Bouchard, M. F.; Sauve, S.; Barbeau, B.; Legrand, M.; Brodeur, M. E.; Bouffard, T.; Limoges, E.; Bellinger, D. C.; Mergler, D. Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ. Health Perspect.* **2011**, *119* (1), 138–143.

- (13) Bouchard, M.; Laforest, F.; Vandelay, L.; Bellinger, D.; Mergler, D. Hair manganese and hyperactive behaviors: Pilot study of school-age children exposed through tap water. *Environ. Health Perspect* **2007**, *115* (1), 122–7.

- (14) Hernandez-Bonilla, D.; Schilman, A.; Montes, S.; Rodriguez-Agudelo, Y.; Rodriguez-Dozal, S.; Solis-Vivanco, R.; Rios, C.; Riojas-Rodriguez, H. Environmental exposure to manganese and motor function of children in Mexico. *Neurotoxicology* **2011**, *32* (5), 615–21.

- (15) Lucchini, R. G.; Guazzetti, S.; Zoni, S.; Donna, F.; Peter, S.; Zacco, A.; Salmistraro, M.; Bontempi, E.; Zimmermann, N. J.; Smith, D. R. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. *Neurotoxicology* **2012**, *33* (4), 687–96.

- (16) Torres-Agustin, R.; Rodriguez-Agudelo, Y.; Schilman, A.; Solis-Vivanco, R.; Montes, S.; Riojas-Rodriguez, H.; Cortez-Lugo, M.; Rios, C. Effect of environmental manganese exposure on verbal learning and memory in Mexican children. *Environ. Res.* **2013**, *121*, 39–44.

- (17) Aschner, J. L.; Aschner, M. Nutritional aspects of manganese homeostasis. *Molecular Aspects of Medicine* **2005**, *26* (4–5), 353–62.

- (18) Yoon, M.; Nong, A.; Clewell, H. J., 3rd; Taylor, M. D.; Dorman, D. C.; Andersen, M. E. Evaluating placental transfer and tissue concentrations of manganese in the pregnant rat and fetuses after inhalation exposures with a PBPK model. *Toxicol. Sci.* **2009**, *112* (1), 44–58.

- (19) Yoon, M.; Schroeter, J. D.; Nong, A.; Taylor, M. D.; Dorman, D. C.; Andersen, M. E.; Clewell, H. J., 3rd Physiologically based pharmacokinetic modeling of fetal and neonatal manganese exposure in humans: Describing manganese homeostasis during development. *Toxicol. Sci.* **2011**, *122* (2), 297–316.



- (20) Baker, M. G.; Simpson, C. D.; Sheppard, L.; Stover, B.; Morton, J.; Cocker, J.; Seixas, N. Variance components of short-term biomarkers of manganese exposure in an inception cohort of welding trainees. *J. Trace Elem. Med. Biol.* **2014**, DOI: 10.1016/j.jtemb.2014.05.004.
- (21) Smith, D.; Gwiazda, R.; Bowler, R.; Roels, H.; Park, R.; Taicher, C.; Lucchini, R. Biomarkers of Mn exposure in humans. *Am. J. Ind. Med.* **2007**, *50* (11), 801–811.
- (22) Laohaudomchok, W.; Lin, X.; Herrick, R. F.; Fang, S. C.; Cavallari, J. M.; Christiani, D. C.; Weisskopf, M. G. Toenail, blood, and urine as biomarkers of manganese exposure. *J. Occup Environ. Med.* **2011**, *53* (5), 506–10.
- (23) Mergler, D.; Baldwin, M.; Belanger, S.; Larribe, F.; Beuter, A.; Bowler, R.; Panisset, M.; Edwards, R.; de Geoffroy, A.; Sassine, M. P.; Hudnell, K. Manganese neurotoxicity, a continuum of dysfunction: Results from a community based study. *Neurotoxicology* **1999**, *20* (2–3), 327–42.
- (24) Eastman, R. R.; Jursa, T. P.; Benedetti, C.; Lucchini, R. G.; Smith, D. R. Hair as a biomarker of environmental manganese exposure. *Environ. Sci. Technol.* **2013**, *47* (3), 1629–37.
- (25) Chojnacka, K.; Gorecka, H.; Gorecki, H. The effect of age, sex, smoking habit and hair color on the composition of hair. *Environ. Toxicol. Pharmacol.* **2006**, *22* (1), 52–7.
- (26) Kempson, I. M.; Lombi, E. Hair analysis as a biomonitor for toxicology, disease and health status. *Chem. Soc. Rev.* **2011**, *40* (7), 3915–40.
- (27) Olmedo, P.; Pla, A.; Hernandez, A. F.; Lopez-Guarnido, O.; Rodrigo, L.; Gil, F. Validation of a method to quantify chromium, cadmium, manganese, nickel and lead in human whole blood, urine, saliva and hair samples by electrothermal atomic absorption spectrometry. *Anal. Chim. Acta* **2010**, *659* (1–2), 60–7.
- (28) Grashow, R.; Zhang, J.; Fang, S. C.; Weisskopf, M. G.; Christiani, D. C.; Cavallari, J. M. Toenail metal concentration as a biomarker of occupational welding fume exposure. *J. Occup Environ. Hyg* **2013**, *11*, 397–405.
- (29) Yazbeck, C.; Moreau, T.; Sahuquillo, J.; Takser, L.; Huel, G. Effect of maternal manganese blood levels on erythrocyte calcium-pump activity in newborns. *Sci. Total Environ.* **2006**, *354* (1), 28–34.
- (30) Smargiassi, A.; Takser, L.; Masse, A.; Sergerie, M.; Mergler, D.; St-Amour, G.; Blot, P.; Heliier, G.; Huel, G. A comparative study of manganese and lead levels in human umbilical cords and maternal blood from two urban centers exposed to different gasoline additives. *Sci. Total Environ.* **2002**, *290* (1–3), 157–64.
- (31) Takser, L.; Lafond, J.; Bouchard, M.; St-Amour, G.; Mergler, D. Manganese levels during pregnancy and at birth: Relation to environmental factors and smoking in a Southwest Quebec population. *Environ. Res.* **2004**, *95* (2), 119–25.
- (32) Zota, A. R.; Ettinger, A. S.; Bouchard, M.; Amarasiwardena, C. J.; Schwartz, J.; Hu, H.; Wright, R. O. Maternal blood manganese levels and infant birth weight. *Epidemiology* **2009**, *20* (3), 367–73.
- (33) Hillson, S. *Dental Anthropology*; Cambridge University Press, 1996.
- (34) Arora, M.; Bradman, A.; Austin, C.; Vedar, M.; Holland, N.; Eskenazi, B.; Smith, D. R. Determining fetal manganese exposure from mantle dentine of deciduous teeth. *Environ. Sci. Technol.* **2012**, *46* (9), 5118–25.
- (35) Regulation, C. D. o. P. California Pesticide Use Reporting Data. <http://www.cdpr.ca.gov/docs/pur/purmain.htm> (accessed May 7, 2014).
- (36) Arora, M.; Hare, D.; Austin, C.; Smith, D. R.; Doble, P. Spatial distribution of manganese in enamel and coronal dentine of human primary teeth. *Sci. Total Environ.* **2011**, *409* (7), 1315–9.
- (37) Schour, I. The neonatal line in enamel a dentine of human deciduous and first permanent molars. *J. Am. Dent. Assoc.* **1936**, *23*, 1946–1955.
- (38) Harnly, M. E.; Bradman, A.; Nishioka, M.; McKone, T. E.; Smith, D.; McLaughlin, R.; Kavanagh-Baird, G.; Castorina, R.; Eskenazi, B. Pesticides in dust from homes in an agricultural area. *Environ. Sci. Technol.* **2009**, *43* (23), 8767–74.
- (39) Gunier, R. B.; J, M.; Smith, D. R.; Jursa, T.; Yousefi, P.; Camacho, J.; Hubbard, A.; Eskenazi, B.; Bradman, A. Determinants of manganese levels in house dust samples from the CHAMACOS cohort. *Sci. Total Environ.* **2014**, DOI: 10.1016/j.scitotenv.2014.08.005.
- (40) Roberts, J. W.; Wallace, L. A.; Camann, D. E.; Dickey, P.; Gilbert, S. G.; Lewis, R. G.; Takaro, T. K. Monitoring and reducing exposure of infants to pollutants in house dust. *Rev. Environ. Contam. Toxicol.* **2009**, *201*, 1–39.
- (41) Spencer, A. Whole blood manganese levels in pregnancy and the neonate. *Nutrition* **1999**, *15* (10), 731–4.
- (42) Tholin, K.; Sandstrom, B.; Palm, R.; Hallmans, G. Changes in blood manganese levels during pregnancy in iron supplemented and non supplemented women. *J. Trace Elem. Med. Biol.* **1995**, *9* (1), 13–7.
- (43) Mora, A. M.; van Wendel de Joode, B.; Mergler, D.; Cordoba, L.; Cano, C.; Quesada, R.; Smith, D. R.; Menezes-Filho, J. A.; Lundh, T.; Lindh, C. H.; Bradman, A.; Eskenazi, B. Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. *Environ. Sci. Technol.* **2014**, *48* (6), 3467–76.
- (44) Kopp, R. S.; Kumbartski, M.; Harth, V.; Bruning, T.; Kafferlein, H. U. Partition of metals in the maternal/fetal unit and lead-associated decreases of fetal iron and manganese: An observational biomonitoring approach. *Arch. Toxicol.* **2012**, *86* (10), 1571–81.
- (45) Das, K.; Chowdhury, A. R. Metallic ion concentration during menstrual cycle in normally menstruating women. *Ind. J. Med. Sci.* **1997**, *51* (2), 52–4.
- (46) Hansen, S.; Nieboer, E.; Sandanger, T. M.; Wilsgaard, T.; Thomassen, Y.; Veyhe, A. S.; Odland, J. O. Changes in maternal blood concentrations of selected essential and toxic elements during and after pregnancy. *J. Environ. Monit* **2011**, *13* (8), 2143–52.
- (47) Lin, Y. Y.; Hwang, Y. H.; Chen, P. C.; Chen, B. Y.; Wen, H. J.; Liu, J. H.; Guo, Y. L. Contribution of gestational exposure to ambient traffic air pollutants to fetal cord blood manganese. *Environ. Res.* **2012**, *112*, 1–7.
- (48) Rudge, C. V.; Calderon, I. M.; Rudge, M. V.; Volpato, G.; Silva, J. L.; Duarte, G.; Neto, C. M.; Sass, N.; Mattar, R.; Rollin, H. B.; Thomassen, Y.; Odland, J. O. Toxic and essential elements in blood from delivering women in selected areas of Sao Paulo State, Brazil. *J. Environ. Monit* **2011**, *13* (3), 563–71.
- (49) Ericson, J. E.; Rinderknecht, A.; Gonzalez, E. J.; Crinella, F. M.; Kleinman, M. T. Measurements of manganese with respect to calcium in histological enamel cross sections: Toward a new manganese biomarker. *Environ. Res.* **2001**, *86* (1), 46–50.
- (50) Berkovitz, B.; Holland, G. R.; Moxhain, B. J. *A Colour Atlas and Text of Oral Anatomy, Histology and Embryology*; Wolfe Publishing: London, 1992.
- (51) Smith, C. Cellular and chemical events during enamel maturation. *Crit Rev. Oral Biol. Med.* **1998**, *9*, 128–161.
- (52) Gunier, R. B.; Bradman, A.; Jerrett, M.; Smith, D. R.; Harley, K. G.; 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. *Environ. Sci. Technol.* **2013**, *47* (19), 11249–57.
- (53) Kim, Y.; Lee, B. K. Iron deficiency increases blood manganese level in the Korean general population according to KNHANES 2008. *Neurotoxicology* **2011**, *32* (2), 247–54.
- (54) Smith, E. A.; Newland, P.; Bestwick, K. G.; Ahmed, N. Increased whole blood manganese concentrations observed in children with iron deficiency anaemia. *J. Trace Elem. Med. Biol.* **2013**, *27* (1), 65–9.
- (55) Claus-Henn, B. C.; Kim, J.; Wessling-Resnick, M.; Tellez-Rojo, M. M.; Jayawardene, I.; Ettinger, A. S.; Hernandez-Avila, M.; Schwartz, J.; Christiani, D. C.; Hu, H.; Wright, R. O., Associations of iron metabolism genes with blood manganese levels: A population-based study with validation data from animal models. *Environ. Health* **2011**, *10*.