Blood and Hair Manganese Concentrations in Pregnant Women from the Infants’ Environmental Health Study (ISA) in Costa Rica

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ABSTRACT: Manganese (Mn), an essential nutrient, is a neurotoxicant at high concentrations. We measured Mn concentrations in repeated blood and hair samples collected from 449 pregnant women living near banana plantations with extensive aerial spraying of Mn-containing fungicide mancozeb in Costa Rica, and examined environmental and lifestyle factors associated with these biomarkers. Mean blood Mn and geometric mean hair Mn concentrations were 24.4 μg/L (8.9−56.3) and 1.8 μg/g (0.05−53.3), respectively. Blood Mn concentrations were positively associated with gestational age at sampling (β = 0.2; 95% CI: 0.1 to 0.2), number of household members (β = 0.4; 95% CI: 0.1 to 0.6), and living in a house made of permeable and difficult-to-clean materials (β = 2.6; 95% CI: 1.3 to 4.0); and inversely related to smoking (β = −3.1; 95% CI: −5.8 to −0.3). Hair Mn concentrations were inversely associated with gestational age at sampling (% change = 0.8; 95% CI: −1.6 to 0.0); and positively associated with living within 50 m of a plantation (% change = 42.1; 95% CI: 14.2 to 76.9) and Mn concentrations in drinking water (% change = 17.5; 95% CI: 12.2 to 22.8). Our findings suggest that pregnant women living near banana plantations aerially sprayed with mancozeb may be environmentally exposed to Mn.

INTRODUCTION

Manganese (Mn) is an essential nutrient that plays an important role in multiple physiological processes such as somatic growth and bone formation.1 Animal and human studies have shown that adverse health effects may result from either deficiency or excess of Mn. For example, epidemiological studies found that both low and high Mn concentrations during pregnancy and early childhood were associated with impaired fetal growth and neurobehavioral deficits in children.3 In addition, animal studies have reported that early exposure to Mn may be related to behavioral and neurological effects later in life, long after cessation of exposure.4,5

Food is the main source of Mn intake for the general population,1 but Mn absorption in the gastrointestinal tract and presystemic elimination by the liver are closely regulated through homeostatic mechanisms.6 Although Mn intake from drinking water is much lower than intake from food,7 ingestion of water from wells with high concentrations of natural Mn or contaminated by industrial waste has been associated with increased blood or hair Mn and negative health effects in human populations.1

Environmental exposure to Mn may also occur through inhalation of the combustion of antiknock additives in gasoline.8

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or inhalation of dust emissions from industrial sources and Mn mines. Airborne Mn can be inhaled and enter the body through the lungs. It may also access the brain directly through the olfactory bulb, bypassing homeostatic regulatory mechanisms.

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

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

#### MATERIALS AND METHODS

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

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

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

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

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

Whole blood Mn was measured in 664 blood samples collected from 418 women. A total of 246 women provided two samples during pregnancy and 172 provided only one. The first blood samples were collected at enrollment or shortly after (mean = 21 weeks, range = 3–38 weeks) and the second samples were collected during the follow-up visits (mean = 29 weeks, range = 14–40 weeks).

**Hair Mn Measurements.** Hair samples (~20–30 strands) were collected from the occipital region, within 2 mm from the scalp, and stored in plastic bags at room temperature until their shipment to the Federal University of Bahia, Brazil. Hair samples were cleaned and analyzed as described elsewhere. Briefly, the one-centimeter closest to the scalp was washed for 15 min in 10 mL of 1% Triton X-100 solution in an ultrasonic bath, rinsed several times with Milli-Q water, dried overnight at 70 °C and ~10 mg of hair were digested in 2 mL of ultrapure concentrated nitric acid at 80 °C for 2 h. Acid-digested samples and reference material from the International Atomic Energy Agency (IAEA-085) were analyzed using electrothermal atomic spectroscopy with Zeeman background correction (GTA-120, Varian Inc.). Reagent blanks were analyzed along with samples in every batch and the intrabatch and batch-to-batch precisions were estimated in 2.4% and 5.9%, respectively. All processed samples and reference materials were analyzed in duplicates and a difference ≤10% was considered acceptable. Accuracy in the concentration
Table 1. Cohort Characteristics and Results from Bivariate Mixed-Effect Models for Blood and Hair Mn Concentrations\textsuperscript{a}

| Demographic and Pregnancy-Related Characteristics | overall | blood Mn (µg/L) (n = 664, k = 418) | hair Mn (µg/g) (n = 800, k = 449) |
|-----------------------------------------------|---------|---------------------------------|----------------------------------|
|                                               | N (%)   | mean (95% CI)                  | GM (95% CI)                      |
| age (years)                                   |         |                                |                                  |
| <18                                           | 78 (17.4) | 25.3 (24.0, 26.7)              | 1.8 (1.4, 2.2)                   |
| 18−24                                         | 208 (46.3) | 24.4 (23.6, 25.2)              | 1.7 (1.5, 2.0)                   |
| 25−29                                         | 84 (18.7) | 23.0 (21.7, 24.3)              | 1.7 (1.3, 2.1)                   |
| 30−34                                         | 42 (9.4) | 24.7 (22.8, 26.6)              | 2.0 (1.4, 2.7)                   |
| ≥35                                           | 37 (8.2) | 24.9 (22.9, 27.0)              | 2.1 (1.5, 2.9)                   |
| education level                               |         |                                |                                  |
| ≤6th grade                                    | 235 (52.3) | 24.4 (23.6, 25.2)              | 1.9 (1.6, 2.2)                   |
| >7−11th grade                                 | 201 (44.8) | 24.4 (23.6, 25.2)              | 1.7 (1.5, 2.0)                   |
| completed high school                         | 13 (2.9) | 23.3 (20.1, 26.6)              | 1.2 (0.6, 2.0)                   |
| marital status                                |         |                                |                                  |
| single                                        | 109 (24.3) | 24.6 (23.4, 25.8)              | 1.5 (1.2, 1.8)                   |
| married/living as married                     | 340 (75.7) | 24.3 (23.6, 25.0)              | 1.9 (1.7, 2.1)                   |
| country of birth                              |         |                                |                                  |
| Costa Rica                                    | 364 (81.1) | 24.3 (23.6, 24.9)              | 1.6 (1.5, 1.8)                   |
| other Central American countries              | 85 (18.9) | 24.8 (23.5, 26.1)              | 2.4 (1.9, 2.9)                   |
| family income\textsuperscript{b}              |         |                                |                                  |
| above poverty line                            | 169 (40.6) | 24.1 (23.2, 25.0)              | 1.7 (1.4, 2.0)                   |
| below poverty line and above extreme poverty  | 170 (40.9) | 24.4 (23.5, 25.4)              | 1.8 (1.5, 2.1)                   |
| below extreme poverty line                    | 77 (18.5) | 24.1 (22.7, 25.5)              | 1.9 (1.5, 2.4)                   |
| language at home                              |         |                                |                                  |
| Spanish only                                  | 417 (92.9) | 24.5 (23.9, 25.1)              | 1.8 (1.6, 2.0)                   |
| Spanish and other language                    | 32 (7.1) | 23.2 (21.2, 25.3)              | 1.8 (1.3, 2.6)                   |
| parity\textsuperscript{b}                    |         |                                |                                  |
| 0                                             | 154 (35.6) | 24.3 (23.3, 25.2)              | 1.7 (1.5, 2.0)                   |
| ≥1                                            | 279 (64.4) | 24.4 (23.7, 25.1)              | 1.8 (1.6, 2.0)                   |
| smoking during pregnancy                      |         |                                |                                  |
| no                                            | 431 (96.0) | 24.5 (23.9, 25.1)              | 1.8 (1.6, 2.0)                   |
| yes                                           | 18 (4.0) | 21.4 (18.6, 24.1)              | 1.6 (1.0, 2.6)                   |
| Occupational Characteristics                 |         |                                |                                  |
| agricultural workers in household during pregnancy | 0 | 117 (26.0) | 23.7 (22.6, 24.8)              | 1.5 (1.2, 1.8)                   |
|                                              | 1 | 241 (53.7) | 24.4 (23.6, 25.1)              | 1.8 (1.6, 2.1)                   |
|                                              | ≥2 | 91 (20.3) | 25.3 (24.0, 26.5)              | 2.0 (1.6, 2.5)                   |
| occupation before pregnancy                  |         |                                |                                  |
| none/not agriculture                          | 339 (75.5) | 24.5 (23.8, 25.1)              | 1.6 (1.5, 1.8)                   |
| agriculture                                   | 110 (24.5) | 24.1 (22.9, 25.2)              | 2.3 (1.9, 2.8)                   |
| occupation during pregnancy\textsuperscript{c} |         |                                |                                  |
| none/not agriculture                          | 412 (91.8) | 24.5 (23.9, 25.1)              | 1.7 (1.6, 1.9)                   |
| agriculture                                   | 37 (8.2) | 22.4 (20.5, 24.4)              | 2.2 (1.6, 3.0)                   |
| husband/partner's occupation before pregnancy\textsuperscript{b} |         |                                |                                  |
| none/not agriculture                          | 152 (35.9) | 24.5 (23.5, 25.4)              | 1.4 (1.2, 1.6)                   |
| agriculture                                   | 271 (64.1) | 24.3 (23.6, 25.0)              | 2.0 (1.8, 2.3) <0.001            |
| husband/partner's occupation during pregnancy\textsuperscript{c} |         |                                |                                  |
| none/not agriculture                          | 175 (39.0) | 23.9 (23.0, 24.8)              | 1.5 (1.3, 1.7)                   |
| agriculture                                   | 274 (61.0) | 24.6 (23.9, 25.4)              | 2.0 (1.8, 2.3) 0.001            |
Table 1. continued

| Environmental Characteristics | Overall | Blood Mn (μg/L) (n = 664, k = 418) | Hair Mn (μg/g) (n = 800, k = 449) |
|-------------------------------|---------|----------------------------------|----------------------------------|
|                               | N (%)   | mean (95% CI)        | ME GM (95% CI)        | pME       |
| Source of drinking water in the home |        |                    |                        |            |
| Aqueduct                      | 548 (71.3) | 24.6 (24.0, 25.4) | 1.8 (1.5, 2.2) | 0.02 |
| Well (banana company or private) | 72 (9.4) | 23.3 (21.9, 24.7) | 3.7 (3.0, 4.7) | <0.001 |
| Other source (river, rain, tank, bottled water) | 50 (6.4) | 23.9 (22.5, 25.4) | 2.3 (1.5, 3.3) | 0.04 |
| Drinking water Mn concentrations (μg/L)\(^b\) |        |                    |                        |            |
| First quartile: 0.0–0.5        | 36 (26.1) | 25.0 (23.2, 26.9) | 0.7 (0.5, 1.0) | - |
| Second quartile: 0.6–9.8       | 33 (23.9) | 23.7 (21.7, 25.7) | 1.5 (1.1, 2.1) | 0.002 |
| Third quartile: 9.9–157.7      | 35 (25.4) | 25.7 (23.7, 27.6) | 2.0 (1.5, 2.8) | <0.001 |
| Fourth quartile: 157.8–1600.0  | 34 (24.6) | 24.2 (22.3, 26.1) | 3.6 (2.6, 5.0) | <0.001 |
| Wall and floor materials of the house\(^b\) |        |                    |                        |            |
| Both made of nonpermeable and easy-to-clean materials | 119 (40.5) | 22.5 (21.4, 23.6) | 1.4 (1.2, 1.8) | - |
| One or both made of permeable and difficult-to-clean materials | 175 (59.5) | 25.4 (24.5, 26.3) | <0.001 | 1.5 (1.3, 1.8) | 0.61 |
| Reported aerial spraying near the home\(^c\) |        |                    |                        |            |
| Day before sample collection |        |                    |                        |            |
| No                            | 333 (74.2) | 24.5 (23.9, 25.2) | 1.7 (1.5, 1.9) | - |
| Yes                           | 116 (25.8) | 23.6 (22.6, 24.6) | 1.9 (1.6, 2.3) | 0.15 |
| Day of sample collection |        |                    |                        |            |
| No                            | 334 (74.4) | 24.4 (23.8, 25.0) | 1.7 (1.5, 1.9) | - |
| Yes                           | 115 (25.6) | 24.2 (23.1, 25.3) | 2.1 (1.8, 2.5) | 0.01 |
| Reported washing work clothes of agricultural workers\(^d\) |        |                    |                        |            |
| Day before sample collection |        |                    |                        |            |
| No                            | 335 (80.2) | 24.3 (23.7, 25.0) | 1.7 (1.6, 1.9) | - |
| Yes                           | 83 (19.8) | 24.3 (23.2, 25.5) | 2.0 (1.7, 2.4) | 0.16 |
| Day of sample collection |        |                    |                        |            |
| No                            | 360 (80.1) | 24.6 (23.9, 25.2) | 1.7 (1.5, 1.9) | - |
| Yes                           | 89 (19.9) | 23.6 (22.4, 24.7) | 2.2 (1.8, 2.6) | 0.01 |

\(^a\)Abbreviations: \(n\), number of samples; \(k\), number of women; CI, confidence interval; ME, mixed-effect models; GM, geometric mean. \(^b\)Information was missing for several women with at least one blood sample (\(k = 31\) for family income, \(k = 15\) for parity, \(k = 24\) for husband/partner’s occupation before pregnancy, \(k = 287\) for drinking water Mn concentrations, \(k = 143\) for wall and floor materials of the house, and \(k = 26\) for washing work clothes the day before sample collection) and for several women with at least one hair sample (\(k = 33\) for family income, \(k = 16\) for parity, \(k = 26\) for husband/partner’s occupation before pregnancy, \(k = 311\) for drinking water Mn concentrations, \(k = 155\) for wall and floor materials of the house, and \(k = 31\) for washing work clothes the day before sample collection). \(^c\)Variables were analyzed as time-varying characteristics in the bivariate and multivariate linear mixed-effects models.

Range of 8.3–9.3 μg/g was 102.6% and the analytical LOD for Mn in hair was 0.1 μg/g. Hair samples with Mn concentrations below LOD (\(n = 3\)) were set at LOD/2.

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

**Drinking Water Mn Measurements.** A convenience sample of 138 participants’ houses distributed in 37 villages was selected for drinking water sampling. In houses with water supply (\(n = 92\)), tap water samples of 10 mL were collected into polypropylene tubes (PerformR Centrifuge tubes, Labcon, Petaluma, CA) using the following standardized procedure (adapted from van der Hoven and Slaats):24 open the tap for three minutes, reduce flow, and collect sample. In houses with no water supply (\(n = 46\)), women collected drinking water from wells, community taps, rivers, or bottled water and stored it in large plastic containers. Water samples of 10 mL were collected directly from the plastic containers in these houses. Drinking water samples were stored at −20 °C until their shipment to Lund University Hospital, Sweden. Water Mn concentrations were measured in acidified samples (2% HNO\(_3\)) using ICP-MS (Thermo X7, Thermo Elemental, Winsford, UK). Analytical accuracy was estimated based on reference material (SLRS-2, Riverine Water Reference Material for Trace Metals, Otawa, CA). The results (mean ± standard deviation (SD)) obtained
were 8.9 ± 0.3 (n = 14) vs recommended 10.1 ± 0.3 μg/L. All samples were analyzed in duplicate and the method imprecision (calculated as the coefficient of variation for duplicate preparations measurements) was 2.5%. The analytical detection limit was defined as three times the SD of the blank samples and was estimated as 0.05 μg/L.

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

**Statistical Analysis.** Blood Mn concentrations were normally distributed, whereas hair and water Mn concentrations were skewed. Thus, we transformed hair and water Mn concentrations to the log₁₀ scale to normalize the residuals. To assess the within- and between-woman variability and reproducibility of blood Mn and hair Mn concentrations, we calculated the intraclass correlation coefficient (ICC) using mixed-effects models.²⁵

We ran bivariate and multivariate analyses for blood and hair Mn separately. First, we used linear mixed-effects models with random intercepts to assess the bivariate associations between potential explanatory variables and blood or hair Mn concentrations and to estimate the amount of variability in measured levels explained by the model while accounting for the correlation among repeated measures.²⁶ We also explored potential nonlinear associations between continuous explanatory variables and blood or hair Mn by examining penalized splines in generalized additive models (these did not account for repeated measures). We included potential nonlinear associations between continuous explanatory variables and blood or hair Mn concentrations as additional variables (e.g., drinking water Mn concentrations (k = 138) and housing materials (k = 275)) in the subset of women with both blood and hair concentrations (k = 411).

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

## RESULTS

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

Table 2 presents descriptive statistics for blood and hair Mn concentrations. Blood Mn concentrations ranged from 8.9 to 56.3 μg/L (mean ± SD = 24.4 ± 6.6 μg/L) and hair Mn from 0.05 to 53.3 μg/g (geometric mean, GM (geometric standard deviation, GSD) = 1.8 (3.2) μg/g). Blood Mn concentrations increased significantly with gestational age (β = 0.2, 95% CI: 0.1

| biomarkers     | n  | k  | mean (SD) | GM (GSD) | min | 25th | 50th | 75th | max | σ² btw | σ² within | ICC |
|----------------|----|----|-----------|----------|-----|------|------|------|-----|--------|-----------|-----|
| blood manganese (μg/L) | 664| 418| 24.4 (6.6) | 23.5 (1.3) | 8.9 | 20.2 | 24.0 | 28.4 | 56.3 | 18.49 | 23.78 | 0.44 |
| hair manganese (μg/g) | 800| 449| 3.6 (5.9)  | 1.8 (3.2)  | 0.05| 0.8  | 1.6  | 3.7  | 53.3 | 0.14  | 0.10  | 0.58 |

Abbreviations: n, number of samples; k, number of women; SD, standard deviation; GM, geometric mean; GSD, geometric standard deviation; ICC, intraclass correlation coefficient. Calculations of the variance between- and within-woman and ICC for blood and hair Mn concentrations were adjusted for gestational age.

“Variances between- and within-woman and ICC were calculated and reported for log₁₀-transformed hair Mn concentrations.”
to 0.2; SI Figure S1A), while hair Mn concentrations showed a small but statistically significant decrease ($\beta = -0.99, 95\% CI: -0.98 to -1.0; SI Figure S1B). Blood Mn was not correlated with hair Mn concentrations ($r_c = -0.04, p = 0.27, n = 645$).

An ICC value of 0.44 was observed for blood Mn suggesting greater within- than between-woman variability in concentrations (Table 2). In contrast, an ICC value of 0.58 for log_{10} transformed hair Mn concentrations indicated that only 42% of the variability of hair Mn was due to intraindividual variability.

Factors Associated with Blood Mn. In the bivariate analyses, blood Mn concentrations were lower among women who smoked, had a prepregnancy BMI <18.5 kg/m², worked in agriculture during pregnancy, lived <50 m from a banana plantation, and somewhat lower in women who drank water from a well during pregnancy and who reported aerial spraying near their home the day before the blood specimen was collected (Table 1), but higher in women who lived with ≥2 agricultural workers during pregnancy, reported gestational anemia, and took iron supplementation during pregnancy (data not shown). Blood Mn concentrations were not associated with frequency of fruit, vegetable, grain, and tea consumption, or with hemoglobin levels (data not shown). In the subset of women for whom information on house materials was available, blood Mn concentrations were higher among those who lived in houses with walls and/or floors made of permeable and difficult-to-clean materials such as wood and plastic (Table 1).

Figure 1 summarizes the results from the multivariate mixed-effects model for blood Mn (SI Table S1, Model 1). Blood Mn concentrations were positively and significantly associated with hair Mn concentrations, with a 0.8% (95% CI: −1.6 to 0.0) decrease in hair Mn per one-week increase in gestational age. Hair Mn concentrations were 29.6% (95% CI: 3.2 to 62.8) higher among women born outside of Costa Rica compared to women born in the country, 25.1% (95% CI: 1.6 to 54.0) higher among pregnant women who worked in agriculture before pregnancy compared to women who did not, 128.6% (95% CI: 78.9 to 192.0) higher in women who drank water from a well compared to women who drank water from an aqueduct, and 23.9% (95% CI: 5.0 to 46.2) higher in women who reported aerial spraying near their home on the day of the hair sample collection compared to women who did not. Hair Mn concentrations were also positively associated with the number of people living in the house during pregnancy, increasing 4.8% with each additional household member (95% CI: 0.4 to 9.4). In addition, Mn concentrations in hair were 42.1% (95% CI: 14.2 to 76.9) higher in women who lived <50 m from a banana plantation compared to women who lived 50 to 600 m away from a plantation.

When we restricted our analysis to the subset of women for whom we had information on drinking water Mn concentrations (k = 138), hair Mn concentrations were no longer associated with occupation before pregnancy (% change = 11.1, 95% CI: −26.3 to 67.4), number of household members (% change = 2.6, 95% CI: −1.4 to 11.0), and aerial spraying near the home the day of the hair sample collection (% change = 12.0, 95% CI: −17.5 to 52.1; data not shown). Then, when we included drinking water Mn concentrations in the multivariate model and excluded source of drinking water (because they were strongly associated: p < 0.001; SI Table S2, Model 2), we found a 17.5% (95% CI: 12.2 to 22.8) increase in hair Mn concentrations per μg/L-increase in drinking water Mn concentrations.

**Figure 1.** Change in blood Mn concentrations (μg/L) for explanatory variables from multivariate linear mixed-effects model (n = 664, k = 418). Beta coefficients and 95% confidence intervals.
Sensitivity analyses using only the subset of women for whom we had both hair and blood Mn concentrations (k = 411), did not show substantial differences in the associations between potential explanatory variables and blood Mn. However, hair Mn concentrations were no longer significantly associated with maternal country of birth (% change = 20.2, 95% CI: −6.4 to 54.5) or maternal occupation before pregnancy (% change = 20.7, 95% CI: −3.9 to 51.6; data not shown).

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

Discussion

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

Blood Mn concentrations in this population were higher than those found in our study population (GM = 3.5 and 1.8 μg/g, respectively).9 While several studies have shown associations between hair Mn concentrations and environmental Mn exposures,23,38 the susceptibility of hair to external contamination remains an issue of concern that may impact its utility as biomarker of exposure.3,38 Currently, there is no definitive method for distinguishing between exogenously derived Mn contamination versus metabolically incorporated Mn in hair.38,39 Therefore, it remains possible that hair Mn concentrations in this population reflect Mn body burden, external contamination from airborne or waterborne Mn, or a mixture of both.

Blood Mn concentrations measured in repeated samples collected during pregnancy showed only a moderate level of within-subject correlation (gestational age-adjusted ICC = 0.44), indicating that one hair measurement does not accurately represent Mn body burden during gestation. Conversely, we found a relatively high within-person correlation for repeated measures of hair Mn concentrations (gestational age-adjusted ICC = 0.58), suggesting that one hair measurement may represent Mn body burden during pregnancy better than one blood measurement. This is not unexpected, given that a one-centimeter section of hair, as analyzed in this study, would integrate circulating Mn concentrations over the one-month duration of hair growth, while a single blood measurement would reflect body Mn dynamics on the order of days.22

Consistent with previous studies in pregnant women (SI Figure S3),13,35,37,40,41 we observed a significant increase in blood Mn concentrations with increasing gestational age, specifically between the first and third trimesters of gestation. This increase in blood Mn during pregnancy could be related to an increase in Mn intestinal absorption41–43 or changes in Mn metabolism.41 It could also result from physiological factors such as differences in tissue Mn mobilization due to increased estrogen and progesterone concentrations during pregnancy.13,44

In contrast to blood Mn, we found a small but significant decrease in hair Mn concentrations between the first and third trimesters of gestation (GM = 1.9 and 1.7 μg/g, respectively). A similar finding was reported in a study of 20 pregnant women in Colorado (means at II and III trimester = 0.2 and 0.1 μg/g, respectively).37 We can only speculate as to the reason why hair Mn concentrations decrease as blood Mn concentrations increase over gestation. One hypothesis is that fetal Mn requirements increase during pregnancy, resulting in a decrease in Mn excretion from the body and into hair (similar findings have been reported in relation to zinc).43 Another hypothesis is that hair dynamics change over the course of pregnancy (e.g., increased hair growth due to higher estrogen concentrations).46

Blood Mn concentrations did not show consistent associations with environmental and occupational factors: some characteristics were positively associated with blood Mn concentrations (e.g., number of household members and living in a house made of permeable and harder-to-clean walls and/or floors), but others were negatively associated (e.g., occupation in agriculture during pregnancy and aerial spraying near the home the day before the blood specimen collection). These inconsistent findings could be due to the homeostatic mechanisms that closely regulate blood Mn concentrations,46 or due to changes in Mn mobilization from brain or bone deposits due to increased fetal Mn requirements.47 Variations in iron metabolism, liver function, and bone metabolism could also explain the observed results.48–53

We observed significantly lower blood Mn concentrations in the few women who smoked during pregnancy compared to those who did not smoke. Similar findings have been previously
reported in pregnant women from a study in Canada.\textsuperscript{13,44} and in adult smokers from a national study in Korea.\textsuperscript{54} This negative association could result from the decrease in estrogen levels linked to cigarette smoking and its consequent effect on Mn mobilization.\textsuperscript{4,23,38} Further studies are required to determine the mechanisms by which smoking affects blood Mn concentrations.

Hair Mn concentrations were associated with several environmental characteristics such as residential distance from banana plantations, agricultural field treated with Mn-containing fungicides during pregnancy, and drinking water Mn concentrations. Studies of children exposed to Mn have reported higher hair and teeth Mn concentrations in relation to sources of environmental airborne exposure.\textsuperscript{4,23,38}

For example, children who lived near or directly downwind from a ferromanganese alloy plant in Brazil had higher hair Mn concentrations compared to children who lived farther away.\textsuperscript{23} Similarly, Italian children living near an active ferroalloy plant had significantly higher hair Mn concentrations compared to children living near a historic but currently inactive plant.\textsuperscript{38} More recently, California children whose mothers lived within three km from an agricultural field treated with Mn-containing fungicides during pregnancy had higher teeth Mn concentrations compared to children whose mothers lived farther away.\textsuperscript{14} These findings suggest that higher hair Mn concentrations observed in women living <50 m from a banana plantation compared to women who lived farther away could be due to drift associated with applications of mancozeb, and this drift could result in higher Mn body burden and/or external contamination from airborne Mn.

We observed a positive and significant relationship between hair Mn and drinking water Mn concentrations in this population, consistent with other studies.\textsuperscript{56–60} Mn concentrations in well water were significantly higher than in water supplied by the national water system or local aqueduct and, although relatively few women drank water from wells (n = 72), their hair Mn concentrations were significantly higher than those of women who drank water from other sources. Mn concentrations in well water could be naturally occurring or reflect residues from mancozeb spraying.\textsuperscript{47} It is possible that women who drank water with high Mn concentrations also washed their hair with this water and, consequently, contaminated their hair with waterborne Mn.\textsuperscript{38}

It is noteworthy that women born outside of Costa Rica had significantly higher hair Mn concentrations compared to those born in the country. These differences are partially explained by the strong association between country of birth and residential distance from plantations, husband/partner’s occupation before and during pregnancy, and agricultural spraying near the home the day before or the day of the hair sample collection.\textsuperscript{20} However, country of birth may also be a proxy for unmeasured explanatory variables, since it remained significant in the multivariate model, even after adjusting for other related variables. Occupation in agriculture before pregnancy, but not during pregnancy, was also associated with increased hair Mn concentrations. This association could be due to the fact that hair integrates exposures over longer time frames rather than recent exposures.\textsuperscript{22,21}

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

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

## ASSOCIATED CONTENT

### Supporting Information

Map of residential locations and banana plantations in Matina County (Figure S1); distribution of blood and hair Mn concentrations by gestational age at sampling (Figure S2); final multivariate linear mixed-effects models for blood Mn concentrations (Table S1); final multivariate linear mixed-effects models for hair Mn concentrations (Table S2); comparison of blood Mn concentrations in pregnant women by study site (Table S3); and variability in median blood Mn concentrations during pregnancy by study site (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

The authors declare no competing financial interest.

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## ABBREVIATIONS

- **BMI:** body mass index
- **CARTA:** Costa Rica Airborne Research and Technology Applications
- **CI:** confidence interval
- **EBDC:** ethylene bisdithiocarbamates
- **ETU:** ethylenthioiurea
- **GM:** geometric mean
- **GPS:** geographic positioning system
GSD: geometric standard deviation
IAEA: International Atomic Energy Agency
ICC: intraclass correlation coefficient
ICP-MS: inductively coupled plasma mass spectrometry
ISA: Infants’ Environmental Health study ("Infantes y Salud Ambiental" in Spanish)
LOD: limit of detection
Mn: manganese
NIST: National Institute of Standards and Technology
SD: standard deviation
SRM: standard reference materials

REFERENCES

(1) Toxicological Profile for Manganese; Agency for Toxic Substances and Disease Registry: Atlanta, GA, 2012.
(2) Zota, A. R.; Ettinger, A. S.; Bouchard, M.; Amarasingirawdane, C. J.; Schwartz, J.; Hu, H.; Wright, R. O. Maternal blood manganese levels and infant birth weight. *Epidemiology* 2009, 20, 367–373.
(3) Claus Henn, B.; Ettinger, A. S.; Schwartz, J.; Tellez-Rojo, M. M.; Lamadrid-Figueroa, H.; Hernandez-Avila, M.; Schnaas, L.; Amarasingirawdane, C.; Bellinger, D. C.; Hu, H.; Wright, R. O. Early postnatal blood manganese levels and children’s neurodevelopment. *Epidemiology* 2010, 21, 433–439.
(4) Kern, C. H.; Smith, D. R. Preweaning Mn exposure leads to prolonged astrocyte activation and lasting effects on the dopaminergic system in adult male rats. *Synapse* 2011, 65, 532–544.
(5) Beaudin, S. A.; Nisam, S.; Smith, D. R. Early life versus lifelong oral manganese exposure differently impairs skilled forelimb performance in adult rats. *Neurotoxicol. Teratol.* 2013, 38, 36–45.
(6) Roth, J. A. Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biol. Res.* 2006, 39, 45–57.
(7) Manganese in Drinking Water: Background Document for Development of WHO Guidelines for Drinking-Water Quality; World Health Organization: Geneva, 2001.
(8) Zayed, J.; Vyskočil, A.; Kennedy, G. Environmental contamination and human exposure to manganese—Contribution of methycyclopentadienyl manganese tricarbonyl in unleaded gasoline. *Int. Arch. Occup. Environ. Health* 1999, 72, 7–13.
(9) Menezes-Filho, J. A.; Novaes Cde, O.; Moreira, J. C.; Sarcinelli, P. N.; Mergler, D. Elevated manganese and cognitive performance in children living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxico*logy 2009, 30, 1207–1213.
(10) van den Hoven, T.; Slats, N. Lead monitoring. In *Analytical Methods for Drinking Water, Advances in Sampling and Analysis*; Quevaullier, P., Thompson, K. C., Eds.; Wiley: Hoboken, NJ, 2006.
(11) Rosner, B. Fundamentals of Biostatistics, 6th ed.; Duxbury Press: Pacific Grove, CA, 2006.
(12) Peretz, C.; Goren, A.; Smid, T.; Kromhout, H. Application of mixed-effects models for exposure assessment. *Ann. Occup. Hyg.* 2002, 46, 69–77.
(13) Kopp, R. S.; Kumbartski, M.; Harth, V.; Bruning, T.; Kaffeerlein, 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, 1571–1581.
(14) Yu, X.; Cao, L. Elevated cord serum manganese level is associated with a neonatal high ponderal index. *Environ. Res.* 2013, 121, 79–83.
(15) Claus Henn, B.; Kim, J.; Wessling-Resnick, M.; Tellez-Rojo, M. M.; Jayawardene, I.; Ettinger, A. S.; Hernandez-Avila, M.; Schwartz, J.; Christiansen, 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, 97.
(16) Canossa, E.; Angiuli, G.; Garasto, G.; Buzzoni, A.; De Rosa, E. Dosage indicators in farm workers exposed to mancozeb. *Med. Lav.* 1993, 84, 42–50.
(17) Geissen, V.; Ramos, F. Q.; de, J.; Bastidas-Bastidas, P.; Diaz-Gonzalez, G.; Bello-Mendoza, R.; Huerta-Lwanga, E.; Ruiz-Suarez, L. E. Soil and water pollution in a banana production region in tropical Mexico. *Bull. Environ. Contam. Toxicol.* 2010, 85, 407–413.
(18) Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates; U.S. Environmental Protection Agency: Washington, DC, 2011.
(19) Ramirez, F. Pesticides imports in Costa Rica 2007–2009: Report for the REPCar Project. Central American Institute for Studies on Toxic Substances; Universidad Nacional de Costa Rica, 2010.
(20) van Wendel de Joode, B.; Mora, A. M.; Cordoba, L.; Cano, C.; Quesada, R.; Skyyt, A.; Wesseling, C.; Ruepert, C.; Oberg, M.; Eskenazi, B.; Mergler, D.; Lindh, C. H. Mancozeb applications with light aircrafts are associated with increased ethylenethiourea (ETU) concentrations in pregnant women: Results from the Infants’ Environmental Health Study (ISA), submitted.
(21) Costa Rican Institute of Statistics and Census (INEC) 2011 Census; Costa Rican Institute of Statistics and Census: San Jose, Costa Rica, 2011.
(22) Smith, D. H.; Gwiazda, R.; Bowler, R.; Roeis, H.; Park, R.; Taicher, C.; Lucchini, R. Biomarkers of Mn exposure in humans. *Am. J. Ind. Med.* 2007, 50, 801–811.
(23) Menezes-Filho, J. A.; Paes, C. R.; Pontes, A. M.; Moreira, J. C.; Sarcinelli, P. N.; Mergler, D. High levels of hair manganese in children living in the vicinity of a ferro-manganese alloy production plant. *Neurotoxicology* 2009, 30, 1207–1213.
(24) van den Hoven, T.; Slats, N. Lead monitoring. In *Analytical Methods for Drinking Water, Advances in Sampling and Analysis*; Quevaullier, P., Thompson, K. C., Eds.; Wiley: Hoboken, NJ, 2006.
(25) Rosner, B. Fundamentals of Biostatistics, 6th ed.; Duxbury Press: Pacific Grove, CA, 2006.
(26) Peretz, C.; Goren, A.; Smid, T.; Kromhout, H. Application of mixed-effects models for exposure assessment. *Ann. Occup. Hyg.* 2002, 46, 69–77.
(27) Kopp, R. S.; Kumbartski, M.; Harth, V.; Bruning, T.; Kaffeerlein, 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, 1571–1581.
(28) Yu, X.; Cao, L. Elevated cord serum manganese level is associated with a neonatal high ponderal index. *Environ. Res.* 2013, 121, 79–83.
(29) Claus Henn, B.; Kim, J.; Wessling-Resnick, M.; Tellez-Rojo, M. M.; Jayawardene, I.; Ettinger, A. S.; Hernandez-Avila, M.; Schwartz, J.; Christiansen, 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, 97.
(30) Rudge, C. V.; Rollin, H. B.; Nogueira, C. M.; Thomassen, Y.; Rudge, M. C.; Olland, J. O. The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women. *J. Environ. Monit.* 2009, 11, 1322–1330.
(31) Abdelouahab, N.; Huel, G.; Suvorov, A.; Foliguet, B.; Goua, V.; Debatte, G.; Sahuquillo, J.; Charles, M. A.; Takser, L. Monoamine oxidase activity in placenta in relation to manganese, cadmium, lead, and mercury at delivery. *Neurotoxicol. Teratol.* 2010, 32, 256–261.
(32) Lin, C. M.; Doyle, P.; Wang, D.; Hwang, Y. H.; Chen, P. C. The role of essential metals in the placental transfer of lead from mother to child. *Reprod. Toxicol.* 2010, 29, 443–446.
(33) Ljung, K. S.; Kippler, M. J.; Goessler, W.; Grandr, G. M.; Nermell, B. M.; Vahter, M. E. Maternal and early life exposure to manganese in rural Bangladesh. *Environ. Sci. Technol.* 2009, 43, 2595–2601.
(34) Rollin, H. B.; Rudge, C. V.; Thomassen, Y.; Mathee, A.; Olland, J. O. Levels of toxic and essential metals in maternal and umbilical cord blood from selected areas of South Africa—results of a pilot study. *J. Environ. Monit.* 2009, 11, 618–627.
(35) Bradman, A.; Gunier, R. B.; Eskenazi, B.; Smith, D. R. Biomarkers of manganese exposure in pregnant women living in an agricultural community in the Salinas Valley, California. Unpublished results.

(36) Takser, L.; Mergler, D.; Hellier, G.; Sahuquillo, J.; Huel, G. Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology 2003, 24, 667–674.

(37) Hambridge, K. M.; Droegemueller, W. Changes in plasma and hair concentrations of zinc, copper, chromium, and manganese during pregnancy. Obstet. Gynecol. 1974, 44, 666–672.

(38) 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, 1629–1637.

(39) Stauber, J. L.; Florence, T. M. Manganese in scalp hair: Problems of exogenous manganese and implications for manganese monitoring in Groote Eylandt Aborigines. Sci. Total Environ. 1989, 83, 85–98.

(40) Spencer, A. Whole blood manganese levels in pregnancy and the neonate. Nutrition 1999, 15, 731–734.

(41) 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, 13–17.

(42) Kaludin, I.; Ganovski, K. [Manganese and zinc absorption in juvenile, sexually mature and pregnant animals]. Vet. Med. Nauki. 1981, 18, 64–69.

(43) Kirchgeessner, M.; Sherif, Y. S.; Schwarz, F. J. [Changes in absorption of manganese during gravidity and lactation]. Ann. Nutr. Metab. 1982, 26, 83–89.

(44) Das, K.; Chowdhury, A. R. Metallic ion concentration during menstrual cycle in normally menstruating women. Indian J. Med. Sci. 1997, 51, 52–54.

(45) Carbone, P.; Sobreviela, M.; Jimenez, D.; Martinez, C.; Pocovi, M. Hair zinc and dietary zinc intake during pregnancy and puerperium. Eur. J. Obstet. Gynecol. Reprod. Biol. 1992, 47, 103–108.

(46) Gizlenti, S.; Ekmekci, T. R. The changes in the hair cycle during gestation and the post-partum period. J. Eur. Acad. Dermatol. Venereol. 2013, DOI: 10.1111/jdv.12188.

(47) Melgar, C.; Geissen, V.; Cram, S.; Sokolov, M.; Bastidas, P.; Ruiz-Suárez, L. E.; Que-Ramos, F. J.; Jarquin-Sanchez, A. Pollutants in drainage channels following long-term application of Mancozeb to banana plantations in southeastern Mexico. J. Plant Nutr. Soil Sci. 2008, 171, 597–604.

(48) Jarvinen, R.; Ahlstrom, A. Effect of the dietary manganese level on tissue manganese, iron, copper and zinc concentrations in female rats and their fetuses. Med. Biol. 1975, 53, 93–99.

(49) Layrargues, G. P.; Rose, C.; Spahr, L.; Zayed, J.; Normandin, L.; Butterworth, R. F. Role of manganese in the pathogenesis of portal-systemic encephalopathy. Metab. Brain Dis. 1998, 13, 311–317.

(50) Mené, I.; Horúchi, K.; Burke, K.; Cotizas, G. C. Chronic manganese poisoning. Individual susceptibility and absorption of iron. Neurology 1969, 19, 1000–1006.

(51) Spahr, L.; Butterworth, R. F.; Fontaine, S.; Bui, L.; Therrien, G.; Millette, P. C.; Lebrun, L. H.; Zayed, J.; Leblanc, A.; Pomier-Layrargues, G. Increased blood manganese in cirrhotic patients: Relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. Hepatology 1996, 24, 1116–1120.

(52) Hauser, R. A.; Zesiewicz, T. A.; Rosemurgy, A. S.; Martinez, C.; Olanow, C. W. Manganese intoxication and chronic liver failure. Ann. Neurol. 1994, 36, 871–875.

(53) Davis, C. D.; Zech, L.; Greger, J. L. Manganese metabolism in rats: An improved methodology for assessing gut endogenous losses. Proc. Soc. Exp. Biol. Med. 1993, 202, 103–108.

(54) Lee, B. K.; Kim, Y. Relationship between blood manganese and blood pressure in the Korean general population according to KNHANES 2008. Environ. Res. 2011, 111, 797–803.

(55) Bernstein, L.; Pike, M. C.; Lobo, R. A.; Depue, R. H.; Ross, R. K.; Henderson, B. E. Cigarette smoking in pregnancy results in marked decrease in maternal hCG and oestradiol levels. Br. J. Obstet. Gynaecol. 1989, 96, 92–96.

(56) Agusa, T.; Kunito, T.; Fujihara, J.; Kubota, R.; Minh, T. B.; Kim Trang, P. T.; Iwata, H.; Subramanian, A.; Viet, P. H.; Tanabe, S. Contamination by arsenic and other trace elements in tube-well water and its risk assessment to humans in Hanoi, Vietnam. Environ. Pollut. 2006, 139, 95–106.

(57) Bouchard, M.; Laforet, F.; Vandelac, 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, 122–127.

(58) 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, 138–143.

(59) He, P.; Liu, D. H.; Zhang, G. Q. Effects of high-level-manganese sewage irrigation on children’s neurobehavior. Zhonghua Yufang Yixue ZaZhi 1994, 28, 216–218.

(60) Kondakis, X. G.; Makris, N.; Leotsinidis, M.; Prinou, M.; Papapetropoulos, T. Possible health effects of high manganese concentration in drinking water. Arch. Environ. Health 1989, 44, 175–178.

(61) Schroeter, J. D.; Nong, A.; Yoon, M.; Taylor, M. D.; Dorman, D. C.; Andersen, M. E.; Clewell, H. J. 3rd. Analysis of manganese tracer kinetics and target tissue dosimetry in monkeys and humans with multi-route physiologically based pharmacokinetic models. Toxicol. Sci. 2011, 120, 481–498.