Published in final edited form as:

J Expo Sci Environ Epidemiol. 2019 September ; 29(5): 648–654. doi:10.1038/s41370-018-0108-z.

Toenail manganese as biomarker of drinking water exposure: a reliability study from a U.S. pregnancy cohort

Antonio J. Signes-Pastor^{1,*}, Maryse F. Bouchard², Emily Baker³, Brian P. Jackson⁴, Margaret R. Karagas¹

¹Department of Epidemiology, Geisel School of Medicine, Dartmouth College, 1 Medical Center Dr., 7927 Rubin Bldg., Lebanon, NH 03756, USA

²Department of Environmental and Occupational Health, Université de Montréal, Montréal, Québec, Canada. Research Center of the Sainte-Justine University Hospital, Montréal, Québec, Canada

³Dartmouth-Hitchcock Medical Center, 1 Medical Center Dr. Lebanon, NH 03756, USA

⁴Department of Earth Sciences, Dartmouth College, Hanover, NH 03755, USA

Abstract

Manganese (Mn) is an essential nutrient; however, overexposure can be neurotoxic. Recent evidence suggests that exposure to Mn from drinking water could be neurotoxic; however, research is hampered by the lack of consensus on a reliable biomarker of Mn exposure. Naturally high concentrations of Mn can occur in groundwater, particularly for private, unregulated water systems. This study aimed to investigate the association between exposure to Mn from drinking water with a relatively low Mn content (median of 2.9 µg/L; range, 0.0 – 8,340 µg/L) and Mn in toenails from women collected at two time points: during and after pregnancy. Mn concentrations in the paired toenail samples gathered at the second trimester of pregnancy and 2 weeks postpartum were correlated ($r = 0.47$, $p < 0.001$, $n = 596$). Among women consuming drinking water Mn in the highest tertile (i.e., > 9.8 µg/L) significant positive correlations were found between water Mn and toenails Mn ($r = 0.31$ and $r = 0.38$, for toenail samples collected during pregnancy and postpartum, respectively), whereas little to no correlation was observed at lower water concentrations. Overall, our data suggest that maternal toenail samples are a reliable environmental Mn exposure biomarker and reflect exposure from drinking water.

Keywords

Manganese; Biomarker; Drinking water; Toenails

Users may view, print, copy, and download text and data-mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use:http://www.nature.com/authors/editorial_policies/license.html#terms

*Corresponding author: antonio.j.signes-pastor@dartmouth.edu.

Conflict of interest

The authors declare they have no actual or potential competing financial interests.

Introduction

Manganese (Mn) is a naturally occurring abundant element in the environment, and as an essential nutrient plays a vital role in brain growth and development (1,2). Adverse health outcomes have been associated both with Mn deficiency and overexposure with a U shape dose-response relationship (1–3). Diet is the primary source of Mn and while cases of Mn deficiency have only been observed experimentally (4,5), there are data to suggest that subclinical Mn deficiency may be more prevalent than previously thought (6,7). Overexposure to Mn on the other hand is well-documented and the central nervous system has been found to be the most susceptible target of toxicity (8–12). Excessive occupational exposure to airborne Mn induces neuromotor and cognitive impairment, as well as neuropsychiatric symptoms (13,14). In occupational settings, inhalation of airborne particles is generally the main source of Mn exposure and can result in deficits in neurofunctional outcomes such as impaired fine motor skills, hand-eye coordination and reaction time (11,15,16).

The risks from occupational exposure to airborne Mn are recognized, but accumulating evidence suggests that exposure to Mn from drinking water also could be related to neurotoxicity (17–23). Furthermore, *in utero* and early-life exposure to Mn in water may represent vulnerable exposure windows, since exposure during development has been associated with lower intelligence quotient (IQ), impairment of manual dexterity and speed, short-term memory and visual identification among children (17–20,22,23). Naturally high concentrations of Mn are found in groundwater in several regions in the U.S. and throughout the world (24). Concentrations of Mn can vary by several orders of magnitude in a given region, as a result from weathering and leaching of Mn-bearing minerals and rocks into the aquifers (1,2). The U.S. EPA has set a Mn secondary standard of 50 µg/L for aesthetic reasons (color, staining and taste) and a health advisory for lifetime exposure of 300 µg/L in drinking water (25). Canada, based on neurological effects observed in rodents and epidemiologic studies conducted in infants, has proposed a lower level of 100 µg/L along with an aesthetic level of 20 µg/L (11).

The potential health impact of environmental Mn exposure is an active area of investigation, but research is hampered by the lack of consensus on the most reliable Mn exposure biomarker. Previous studies on Mn-exposed populations have used blood, urine, saliva, and hair. Blood and urine Mn content poorly correlates with long-term Mn exposure because they reflect exposure occurring within the few hours prior (26). Very little data are available for saliva, but it was found to correlate poorly with Mn exposure from drinking water in children (27). The biomarker most reliably correlated with drinking water exposure to Mn is hair, with positive correlations reported in many studies (17,21,23,27–31). However, the potential for external contamination of hair has been raised as an issue affecting the validity of hair Mn as an internal dose measure of exposure (32,33). Hair and nails consist of keratins, which are fibrous proteins that contain disulfide bridges that chelate metal. The slow growth of hair and nails in theory can provide a time-weighted exposure to metals over several months, although the toxicokinetics of Mn incorporation into the hair and nail matrices are not yet fully elucidated (16,27,34). Only a few studies, including in occupational settings and in a study of children from New Brunswick, Canada, have

evaluated toenails as a potential biomarker of Mn exposure (15,16,27,35). Given their relative ease of collection, transport and storage, we investigated the association between exposure to Mn from private drinking water systems and Mn content in toenail samples collected in women during and after pregnancy.

Materials and methods

Study population.

For this study we used data from the ongoing New Hampshire Birth Cohort Study (NHBCS) – a prospective cohort study that enrolled pregnant women from 18 to 45 years of age, living in the same residence since their last menstrual period with no plans to move during pregnancy, and whose primary drinking water source was an unregulated private water system (e.g., private well). Women were enrolled at approximately 24 to 28 weeks of gestation at which time a medical history, a lifestyle, and an occupational history and activities questionnaires were administered. Further details regarding the general design of the NHBCS have been described in detail previously (36,37). The Committee for the Protection of Human Subjects at Dartmouth College approved the protocol of the study, and all participants provided written informed consent in accordance with guidelines from the committee.

Drinking water sampling, measurements, and participant's water consumption.

Participants were provided with a kit to collect a home tap water sample at enrollment using a commercially washed, high-density polyethylene bottle complying with the EPA's standards for water collection. The bottles were kept in clean, sealed bags, and participants were provided with detailed instructions to minimize contamination. Water samples were frozen at -20°C or lower until analysis. Water samples were tested for Mn and other elements at the Trace Element Analysis Core at Dartmouth using a Quadrupole collision cell 7500c Octopole Reaction System Inductively Couple Plasma Mass Spectrometer (ICP-MS) (Agilent). Replicate samples of NIST Natural Water Standard Reference Material 1640a with a total Mn certified value of $40.07 \pm 0.035 \mu\text{g/L}$ (mean $\pm$ SD) and blank samples were included in the analysis as quality control measures. Maternal daily water consumption was estimated as the average consumption reported in a 2-day food diary collected at enrollment. As a reference value, participants were told that 1-cup equals 8 oz.

Toenail collection and measurements.

Women participants in this study were asked to provide toenail samples twice - at enrollment (at approximately 24 to 28 weeks of gestation) and 2 weeks postpartum. At both time points detailed instructions were provided to collect the toenail clipping samples, which include thoroughly removing any nail polish before sample collection. Before analysis, the samples were first manually washed to remove visible dirt followed by sequential washes in an ultrasonic bath using acetone, Triton X-100 (LabChem, PA, U.S.) and finally 5 rinses with deionized water. Then, the toenails were dried and low-pressure microwave digested. The Mn content in the maternal toenail samples was determined using an ICP-MS at the Trace Element Analysis Core at Dartmouth. In each analysis batch, duplicate analysis of digested toenails samples and spikes of digested samples along with blank and fortified blank digests

were included as quality control measures. There is not an available toenail Mn Certified Reference Material yet. The Core does participate in a proficiency testing program (QEMQAS, Center for Toxicology, Quebec) where hair is one of the sample types; the Core lab results for Mn in hair ($n = 8$) over the duration of the NHBCS analysis was $100 \pm 5\%$ relative to the consensus mean from all the participants of the proficiency testing program.

Statistical analysis.

Drinking water and maternal toenail Mn concentrations were natural log-transformed before statistical analysis due to their skewed distributions. Among women who recorded the amount of household water consumption in the 2-day food diary, their estimated daily-ingested Mn from water was calculated as home water Mn concentrations multiplied by their average reported daily tap water consumption. Among women who reported household water consumption and their daily water intake, Pearson's correlation and Local Polynomial Regression (loess) with 95% confidence intervals were carried out to investigate 1) the association between household drinking water Mn concentrations and toenail samples Mn concentrations collected during pregnancy and postpartum, and 2) the association between estimated women's daily-ingested Mn from household drinking water and Mn content in toenails collected during pregnancy and postpartum. In addition, the association between drinking water and maternal toenail Mn concentrations during and after pregnancy was evaluated within intervals according the tertiles cutoff levels of 0.9 µg/L and 9.8 µg/L calculated using the Mn concentrations in all household water samples analyzed (Figure S1). Pearson's correlation and loess also were used to examine the association between Mn concentrations in paired maternal toenail samples collected during and after pregnancy. The sensitivity analyses included Multivariable Generalized Additive Models (GAM) using toenail Mn as dependent variables and water Mn, water consumption and all the selected characteristics of the study population depicted in Table 1 as independent variables. All statistical analyses were carried out with the R software for statistical computing version v. 3.5.0 (38).

Results

Study population.

Our study included 1033 women recruited between 2009 and 2014. Of the 1033 women recruited in this time frame, 919 provided a water sample from their household tap and confirmed usage of tap water as the main drinking source. Of these, 734 and 717 women provided toenail samples during pregnancy and postpartum, respectively; 596 women provided toenail samples for both these time points. Among them, 598 (81%) and 594 (83%) women gave further details about their daily amount of tap water intake. The women's median age at enrolment in the cohort study was 31 years. The majority of the women were white, married, did not smoke during pregnancy and over half of them had more than one previous live births. The women's median (first - third quartile) daily household water intake, calculated from the average consumption reported in a 2-day food diary, was 1.0 L (0.4 - 1.6 L) (Table 1).

Drinking water and toenail samples Mn content.

The NIST Natural Water Standard Reference Material 1640a Mn average percentage recovery $\pm$ SD was $96.7 \pm 4.7\%$ and the Mn water limit of detection was $0.05 \mu\text{g/L}$. The median household water Mn concentration was $2.9 \mu\text{g/L}$ based on 919 water samples (range, $0.0 - 8,340 \mu\text{g/L}$); 13.2% and 1.9% of them had Mn levels higher than the U.S. EPA guideline of $50 \mu\text{g/L}$ (39) and $300 \mu\text{g/L}$ (25), respectively. The median concentration of Mn in toenail samples collected during pregnancy and postpartum was 0.34 and $0.32 \mu\text{g/g}$ based on 734 and 717 toenail samples, respectively (Table 1).

A positive correlation was found between Mn content in toenails collected during pregnancy and that in postpartum toenail samples ($r = 0.47$, $p < 0.001$, $n = 596$) (Figure 1), which was consistent for the low ($r = 0.48$), medium ($r = 0.43$) and high ($r = 0.43$) water Mn tertiles. Additionally, a non-linear positive association was found between drinking water Mn concentrations and maternal toenail Mn content ($r = 0.19$, $p < 0.001$, $n = 734$ and $r = 0.23$, $p < 0.001$, $n = 717$, during pregnancy and postpartum, respectively) (Figure 2) with a stronger association observed at higher concentrations of water Mn. The correlation coefficient was statistically significant among women consuming water with Mn concentrations higher than the second tertile cutoff point of $9.8 \mu\text{g/L}$ ($r = 0.31$, $p < 0.001$, $n = 234$ and $r = 0.38$, $p < 0.001$, $n = 239$, during pregnancy and postpartum, respectively) and closer to null among those consuming water with a Mn concentration lower than or equal to $9.8 \mu\text{g/L}$ ($r = 0.04$, $p = 0.49$ and $r = 0.08$, $p = 0.23$ during pregnancy and postpartum, respectively) (Table 2). The overall correlation coefficients and shape of the associations were consistent with those observed between estimated maternal daily-ingested Mn from household water consumption and toenail Mn concentrations ($r = 0.22$, $p < 0.001$, $n = 598$, and $r = 0.23$, $p < 0.001$, $n = 594$, during pregnancy and postpartum, respectively) (Figure 3).

The sensitivity analyses showed that none of the variables of the selected characteristics of the study population were associated with toenail Mn in the postpartum samples, whereas an association was observed between toenail Mn in samples collected during pregnancy and smoking and parity; however, the inclusion of these variables did not appreciably influence our results (data not shown).

Discussion

In the present study, we evaluated the reliability of maternal toenail samples collected during the second trimester of pregnancy and two weeks postpartum and compared toenail Mn concentrations with Mn content in home tap water. Among women that provided toenail samples (i.e., during pregnancy or postpartum) only 12.3% and 1.9% of them were exposed to water Mn concentrations higher than the U.S. EPA standard of $50 \mu\text{g/L}$ and $300 \mu\text{g/L}$, respectively. Despite the generally low level of exposure from water intake, we found that toenail Mn concentrations and water Mn content followed a nonlinear positive association with an increasing trend at higher levels of water Mn (i.e., above $10 \mu\text{g/L}$). At these levels, water concentrations explained between 10% and 14% of the Mn variability in toenail samples collected during and after pregnancy, respectively. The lack of correlation at the lower levels of water Mn intake could have been due to misclassification of exposure due to dietary sources of Mn intake. Alternatively, it is conceivable that a greater increase of toenail

Mn content at higher levels of water Mn exposure is due to Mn homeostasis becoming overwhelmed at levels above a certain threshold according to the reported Mn U shape dose-response relationship (1,3). Understanding these possibilities will require further investigation.

To our knowledge this is one of the few population studies to evaluate toenails as an exposure biomarker to ingested Mn from water consumption. A prior study carried out in Canada on school age children (6 - 13 years) consuming well water reported that toenails Mn concentration was positively correlated with Mn concentration in drinking water ($r = 0.29$). In this study, the geometric mean of Mn concentrations was $1.95 \mu\text{g/g}$ in children toenails (range, 0.11 to $32.0 \mu\text{g/g}$) and $5.7 \mu\text{g/L}$ in drinking water (range, 0.1 to $1,046 \mu\text{g/L}$) (27). A few studies reported on toenail as a biomarker of exposure in occupational settings, where concerns arise from inhalation of airborne particles. One study conducted in welders reported that toenail Mn concentrations correlated most strongly with cumulative Mn exposure during the period 7 - 12 months before toenail collection, suggesting that toenails may reflect Mn exposure within that window of toenail growth (15). It is noteworthy that toenail Mn did not correlate with dietary Mn intake in the latter study. Another study reported that toenail Mn concentrations were higher among welders compared to control subjects (mean of 6.87 and $2.70 \mu\text{g/g}$, respectively) (16). In our study, we found much lower mean toenail Mn concentrations (median of $0.34 - 0.32 \mu\text{g/g}$) compared these studies in occupational settings (15,16) or the study on children consuming well water in Canada (26).

Our study has notable strengths and limitations. We had a relatively large dataset including replicate maternal toenail samples collected during and after pregnancy in addition to detailed information about Mn concentrations in household water. However, we did not estimate Mn maternal exposure from other sources outside the home tap drinking water that may have contributed to the Mn body burden such as diet, which could include prenatal vitamins for pregnant women. Furthermore, we estimated the level of water consumption using reported information on a 2-day food diary, which may not accurately capture the long-term average water consumption patterns. Interesting, the correlation based on tap water concentrations was similar to that based on the estimated intake that considered also the amount of water consumed, suggesting that the addition of the consumption variable did not appreciably alter our findings. Moreover, lack of accurate water intake information would likely have biased our findings toward the null. We were not able to account for temporal variability in household water Mn since the concentrations were analyzed only once; however, Mn content in water of private wells are likely to be more stable than public sources (40).

High levels of Mn exposure have been associated with adverse neurodevelopmental outcomes, including exposure during pregnancy and early-life (3,16–20,22,41,42). Our findings were based on toenail samples from women with no occupational exposure to Mn, identified from the information reported on the occupational history and activities questionnaire, and consuming household drinking water with generally lower Mn concentrations than in previous studies (17–23). They indicate that toenail samples may be a reliable biomarker of environmental Mn exposure and specifically reflect drinking water exposure, especially at higher Mn water concentrations. In addition, our findings on the

correlation between Mn concentrations in paired toenail samples suggests that a single sample may be a reasonable measure of chronic environmental Mn exposure, particularly to estimate exposure from water consumption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

This study was funded in part by the U.S. National Institutes of Environmental Health Sciences grant P01ES022832 and P42ES007373 and the RD83544201 from the U.S. EPA.

References

1. ATSDR. Toxicological profile for manganese. 2012; Available from: <https://www.atsdr.cdc.gov/toxprofiles/tp151.pdf>
2. Freeland-Graves JH, Mousa TY, Kim S. International variability in diet and requirements of manganese: Causes and consequences. *J Trace Elem Med Biol* 2016;38:24–32. [PubMed: 27264059]
3. Chung SE, Cheong H-KK, Ha E-HH, Kim B-NN, Ha M, Kim Y, et al. Maternal blood manganese and early neurodevelopment: The mothers and children's environmental health (MOCEH) study. *Environ Health Perspect* 2015;123(7):717–22. [PubMed: 25734517]
4. Keen CL, Ensunsa JL, Watson MH, Baly DL, Donovan SM, Monaco MH, et al. Nutritional aspects of manganese from experimental studies. Vol. 20, *NeuroToxicology*. 1999 p. 213–23. [PubMed: 10385885]
5. 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. [PubMed: 15147916]
6. Claus Henn B, Austin C, Coull BA, Schnaas L, Gennings C, Horton MK, et al. Uncovering neurodevelopmental windows of susceptibility to manganese exposure using dentine microspatial analyses. *Environ Res* 2018;161:588–98. [PubMed: 29247915]
7. Henn BC, Ettinger AS, Schwartz J, Téllez-Rojo MM, Lamadrid-Figueroa H, Hernández-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. *Epidemiology*. 2010;21(4):433–9. [PubMed: 20549838]
8. Takeda A Manganese action in brain function. *Brain Res Brain Res Rev* 2003;41(1):79–87. [PubMed: 12505649]
9. Aschner JL, Aschner M. Nutritional aspects of manganese homeostasis. *Mol Aspects Med* 2005;26:353–62. [PubMed: 16099026]
10. Chen P, Bornhorst J, Aschner M. Manganese metabolism in humans. *Front Biosci* 2018;23:1655–79.
11. Federal-Provincial-Territorial Committee on Drinking water. Manganese in drinking water [Internet]. 2016 Available from: http://publications.gc.ca/collections/collection_2017/sc-hc/H144-44-2016-eng.pdf
12. Chang Y, Kim Y, Woo S-T, Song H-J, Kim SH, Lee H, et al. High signal intensity on magnetic resonance imaging is a better predictor of neurobehavioral performances than blood manganese in asymptomatic welders. *Neurotoxicology*. 2009;30(4):555–63. [PubMed: 19376157]
13. Bouchard M, Mergler D, Baldwin M, Panisset M, Roels HA. Neuropsychiatric symptoms and past manganese exposure in a ferro-alloy plant. *Neurotoxicology*. 2007;28(2):290–7. [PubMed: 16962176]
14. Olanow CW. Manganese-induced parkinsonism and Parkinson's disease. *Ann N Y Acad Sci* 2004;1012:209–23. [PubMed: 15105268]

15. Laohaudomchok W, Lin X, Herrick RF, Fang SC, Cavallari JM, Christiani DC, et al. Toenail, Blood, and Urine as Biomarkers of Manganese Exposure. *J Occup Environ Med* 2011;53(5):506–10. [PubMed: 21494156]
16. Ward EJ, Edmondson DA, Nour MM, Snyder S, Rosenthal FS, Dydak U. Toenail Manganese: A Sensitive and Specific Biomarker of Exposure to Manganese in Career Welders. *Ann Work Expo Heal*. 2017;62(1): 101–11.
17. Bouchard MF, Sauvé S, Barbeau B, Legrand M, Brodeur M-ÈÈ, Bouffard T, et al. Intellectual impairment in school-age children exposed to manganese from drinking water. *Environ Health Perspect* 2011; 119(1): 138–43. [PubMed: 20855239]
18. Khan K, Factor-Litvak P, Wasserman GA, Liu X, Ahmed E, Parvez F, et al. Manganese Exposure from Drinking Water and Children's Classroom Behavior in Bangladesh. *Environ Health Perspect* 2011;119(10): 1501–6. [PubMed: 21493178]
19. Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, et al. Manganese exposure from drinking water and children's academic achievement. *Neurotoxicology*. 2012;33(1):91–7. [PubMed: 22182530]
20. Oulhote Y, Mergler D, Barbeau B, Bellinger DC, Bouffard T, Brodeur M-E, et al. Neurobehavioral Function in School-Age Children Exposed to Manganese in Drinking Water. *Environ Health Perspect* 2014;122(12):1343–50. [PubMed: 25260096]
21. Kondakis XG, Makris N, Leotsinidis M, Prinou M, Papapetropoulos T. Possible Health Effects of High Manganese Concentration in Drinking Water. *Arch Environ Heal An Int J*. 1989;44(3):175–8.
22. Wasserman GA, Liu X, Parvez F, Ahsan H, Levy D, Factor-Litvak P, et al. Water manganese exposure and children's intellectual function in Araihaazar, Bangladesh. *Environ Health Perspect* 2006; 114(1): 124–9. [PubMed: 16393669]
23. Bouchard M, Laforest 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(1):122–7.
24. Frisbie SH, Mitchell EJ, Dustin H, Maynard DM, Sarkar B. World health organization discontinues its drinking-water guideline for manganese. Vol. 120, *Environmental Health Perspectives*. 2012 p. 775–8. [PubMed: 22334150]
25. EPA. 2012 Edition of the Drinking Water Standards and Health Advisories (EPA 822-S-12-001). Available from: <https://www.epa.gov/sites/production/files/2015-09/documents/dwstandards2012.pdf>
26. Smith D, Gwiazda R, Bowler R, Roels H, Park R, Taicher C, et al. Biomarkers of Mn exposure in humans. *Am J Ind Med* 2007;50(11):801–11. [PubMed: 17924418]
27. Ntiabose R, Surette C, Foucher D, Clarisse O, Bouchard MF. Assessment of saliva, hair and toenails as biomarkers of low level exposure to manganese from drinking water in children. *Neurotoxicology*. 2017;In Press.
28. Agusa T, Kunito T, Fujihara J, Kubota R, Minh TB, Kim Trang PT, et al. Contamination by arsenic and other trace elements in tube-well water and its risk assessment to humans in Hanoi, Vietnam. *Environ Pollut* 2006;139(1):95–106. [PubMed: 16009476]
29. Bader M, Dietz MC, Ihrig A, Triebig G. Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. *Int Arch Occup Environ Health*. 1999;72(8):521–7. [PubMed: 10592004]
30. He P, Liu DH, Zhang GQ. Effects of high-level-manganese sewage irrigation on children's neurobehavior. *Zhonghua Yu Fang Yi Xue Za Zhi*. 1994;28(4):216–8. [PubMed: 7842882]
31. Coetzee DJ, McGovern PM, Rao R, Harnack LJ, Georgieff MK, Stepanov I. Measuring the impact of manganese exposure on children's neurodevelopment: Advances and research gaps in biomarker-based approaches. *Environ Heal A Glob Access Sci Source*. 2016; 15(1): 1–20.
32. Esteban M, Castaño A. Non-invasive matrices in human biomonitoring: A review. *Environ Int* 2009;35(2):438–49. [PubMed: 18951632]
33. Michalke B, Fernsebner K. New insights into manganese toxicity and speciation. *J Trace Elem Med Biol* 2014;28(2):106–16. [PubMed: 24200516]

34. Viana GF de S, Carvalho CF de, Nunes LS, Rodrigues JLG, Ribeiro NS, Almeida DA de, et al. Noninvasive biomarkers of manganese exposure and neuropsychological effects in environmentally exposed adults in Brazil. *Toxicol Lett* 2014;231(2):169–78. [PubMed: 24992226]
35. Sriram K, Lin GX, Jefferson AM, Roberts JR, Andrews RN, Kashon ML, et al. Manganese accumulation in nail clippings as a biomarker of welding fume exposure and neurotoxicity. *Toxicology*. 2012;291(1–3):73–82. [PubMed: 22085607]
36. Farzan SF, Korrick S, Li Z, Enelow R, Gandolfi AJ, Madan J, et al. In utero arsenic exposure and infant infection in a United States cohort: A prospective study. *Environ Res* 2013;126:24–30. [PubMed: 23769261]
37. Gilbert-Diamond D, Cottingham KL, Gruber JF, Punshon T, Sayarath V, Gandolfi AJ, et al. Rice consumption contributes to arsenic exposure in US women. *Proc Natl Acad Sci U S A*. 2011;108(51):20656–60. [PubMed: 22143778]
38. R Core Team. R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing Vienna; 2014.
39. EPA US. Drinking Water Regulations and Contaminant [Internet]. Available from: <https://www.epa.gov/dwregdev/drinking-water-regulations-and-contaminants>
40. Ayotte JD, Belaval M, Olson SA, Burow KR, Flanagan SM, Hinkle SR, et al. Factors affecting temporal variability of arsenic in groundwater used for drinking water supply in the United States. *Sci Total Environ* [Internet]. 2015;505:1370–9. Available from: 10.1016/j.scitotenv.2014.02.057
41. Claus Henn B, Schnaas L, Ettinger AS, Schwartz J, Lamadrid-Figueroa H, Hernández-Avila M, et al. Associations of Early Childhood Manganese and Lead Coexposure with Neurodevelopment. *Environ Health Perspect* 2011; 120(1): 126–31. [PubMed: 21885384]
42. 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):667–74. [PubMed: 12900080]

Figure 1:
Pearson's correlation and Local Polynomial Regression (loess) with shaded 95% confidence intervals between natural logarithm Mn concentrations in paired toenails from women collected during and after pregnancy.

Figure 2: Pearson's correlation and Local Polynomial Regression (loess) with shaded 95% confidence intervals between natural logarithm Mn concentrations in drinking water and natural logarithm Mn concentrations in toenails from women collected during and after pregnancy.

Figure 3:
 Pearson's correlation and Local Polynomial Regression (loess) with shaded 95% confidence intervals between natural logarithm of estimated daily-ingested Mn from drinking water consumption and natural logarithm Mn concentration in toenails from women collected during and after pregnancy.

Table 1:

Summary statistic of selected characteristics of the study population (median (first – third quartile) for continuous variables and n (%) for categorical samples).

Datasets:	Complete ¹	During pregnancy ²	Postpartum ³
<i>n</i>	919	734	717
Maternal highest attained level of education			
< 11 th grade	9 (1)	7 (1)	7 (1)
High school graduate	90 (10)	69 (9)	61 (9)
Junior college	185 (20)	148 (20)	137 (19)
College graduate	340 (37)	276 (38)	282 (39)
Postgraduate schooling	238 (26)	198 (27)	211 (29)
NA	57 (6)	36 (5)	19 (3)
Maternal ethnicity			
White	903 (98)	723 (99)	705 (98)
Other	16 (2)	11 (1)	12 (2)
NA	-	-	-
Marital status			
Married	745 (81)	603 (82)	622 (87)
Single	87 (10)	71 (10)	58 (8)
Divorced	30 (3)	24 (3)	18 (3)
NA	57 (6)	36 (5)	19 (2)
Smoke during pregnancy			
No	829 (90)	675 (92)	675 (94)
Yes	55 (6)	40 (5)	39 (5)
NA	35 (4)	19 (3)	3 (1)
Parity			
0	372 (40)	299 (41)	286 (40)
1	544 (59)	435 (59)	431 (60)
NA	3 (1)	-	-
Maternal age of enrolment (years)	30.9 (27.9 - 30.1)	30.9 (28.1 - 34.1)	31.1 (28.3 - 34.6)
NA	-	-	-
Maternal BMI (kg/m ²)	23.8 (21.5 - 27.4)	23.9 (21.4 - 27.9)	23.8 (21.4 - 27.4)
NA	48	22	12
Mn water content (µg/L)	2.9 (0.5 - 21.0)	2.3 (0.4 - 18.6)	2.5 (0.4 - 19.3)
NA	-	-	-
Maternal water consumption (L/day)	1.06 (0.47 - 1.65)	1.06 (0.59 - 1.65)	1.06 (0.59 - 1.65)
NA	145	136	123
Maternal toenail Mn content (µg/g) during pregnancy	0.34 (0.17 - 0.72)	0.34 (0.17 - 0.72)	-
NA	185	-	-

Datasets:	Complete ¹	During pregnancy ²	Postpartum ³
Maternal postpartum toenail Mn content (µg/g)	0.32 (0.16 - 0.62)	-	0.32 (0.16 -0.62)
NA	202	-	-

¹The "Complete" dataset includes women that provided water samples from their household tap and confirmed usage of home tap water as the main drinking source.

²This dataset include women from the "Complete" dataset that provided toenail samples collected during pregnancy.

³This dataset include women from the "Complete" dataset that provided toenail samples postpartum.

NA means not available.

Table 2:

Pearson's correlation coefficients with 95% confidence interval (CI) between natural logarithm Mn concentrations in drinking water and natural logarithm Mn concentrations in toenails samples from women collected during and after pregnancy within intervals according to the water Mn tertiles cutoff levels.

Household water Mn tertiles cutoff points ($\mu\text{g/L}$) ^I	During pregnancy			Postpartum		
	<i>n</i>	<i>r</i> (95% CI)	<i>p</i> -value	<i>n</i>	<i>r</i> (95% CI)	<i>p</i> -value
<0.9	254	0.04 (−0.08 - 0.16)	0.51	240	0.08 (−0.04 - 0.20)	0.23
[0.9 - 9.8]	246	0.04 (−0.08 - 0.17)	0.49	238	0.06 (−0.06 - 0.19)	0.30
>9.8	234	0.31 (0.18 - 0.42)	<0.001	239	0.38 (0.26 - 0.48)	<0.001

^IThe drinking water Mn cutoff points have been calculated using the Mn concentrations in all household water samples analyzed (*n* = 919).