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Variance components of short-term biomarkers of manganese exposure in an inception cohort of welding trainees

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SUMMARY

Various biomarkers of exposure have been explored as a way to quantitatively estimate an internal dose of manganese (Mn) exposure, but given the tight regulation of Mn in the body, inter-individual variability in baseline Mn levels, and variability in timing between exposure and uptake into various biological tissues, identification of a valuable and useful biomarker for Mn exposure has been elusive. Thus, a mixed model estimating variance components using restricted maximum likelihood was used to assess the within- and between-subject variance components in whole blood, plasma, and urine (MnB, MnP, and MnU, respectively) in a group of nine newly-exposed apprentice welders, on whom baseline and subsequent longitudinal samples were taken over a three month period. In MnB, the majority of variance was found to be between subjects (94%), while in MnP and MnU the majority of variance was found to be within subjects (79% and 99%, respectively), even when controlling for timing of sample. While blood seemed to exhibit a homeostatic control of Mn, plasma and urine, with the majority of the variance within subjects, did not. Results presented here demonstrate the importance of repeat measure or longitudinal

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study designs when assessing biomarkers of Mn, and the spurious associations that could result from cross-sectional analyses.

Keywords

Welders; manganese; variability; biomarkers of exposure

INTRODUCTION

Elevated Mn exposures have long been an occupational health concern, as chronic elevated exposures have been implicated in the development of “manganism”, a Parkinson’s-like syndrome [1]. However, even workers with exposures considerably lower than those historically associated with manganism have demonstrated subtle neurological effects, making Mn a relevant occupational and environmental health concern [2–5].

Various biomarkers of exposure have been explored as a way to quantitatively estimate an internal dose of Mn. In a literature-based analysis of the relationship between manganese in whole blood (MnB) and manganese in air (MnA) we hypothesised that there may be a point above which blood begins to act as a biomarker of inhalation exposure to Mn, but this still requires additional time-specific analyses, and knowledge of non-occupational exposure to Mn and its physiological variability in absorption or excretion [6]. For some biological media, notably Mn in plasma (MnP) and Mn in urine (MnU), limits of detection could be problematic. Hoet et al. [7] found 80% of control and 65% of welder urine samples to be below their limit of quantification (0.20 ng/mL) for MnU, making further analysis impossible. The majority of biomarker studies related to occupational inhalation of Mn are cross-sectional, typically only analysing a single biosample. Only a few studies have looked at biomarkers of Mn longitudinally or with repeat measures, [7–11] but none have thoroughly assessed the within-individual or between-individual variability components. Järvisalo et al. [11] briefly discussed the variability in six MnU measurements taken over a 24 hour period in five shielded metal arc welding (SMAW) welders, noting that Mn showed diurnal variation throughout the day, with lower MnU values tending toward the late afternoon or evening, even with increased exposure to Mn over the workday. However, no formal assessment of variability components was carried out in this manuscript.

In proposing a framework for developing and applying biological guidance values, Bevan et al. [12] recommended that inter- and intra-variability in biomarker measurements be considered when developing guidelines for a specific chemical. However, often these data are not available. Similarly, Albertini et al. [13] in a mini-monograph called for research to advance the understanding of biomonitoring to assess human exposure and health risks and specifically study designs to better assess variance components in biomonitoring measurements.

The assessment of variance components and exploration of which external factors may affect them can help to better characterise relationships between exposure, dose, and effects. Thus, the purpose of this manuscript is to investigate the factors associated with variability in MnB, MnP, and MnU in a newly-exposed group of apprentice welders.

MATERIAL AND METHODS

Occupational setting and study population

The focus of this manuscript is nine welder trainees enrolled in a technical college in Washington state, who are a subset of a larger inception cohort study (that is, all subjects joined our cohort prior to any reported occupational exposure to Mn) which we have described elsewhere [6]. All study protocols were reviewed and approved by the University of Washington Institutional Review Board and subjects provided written informed consent. Data collection for the inception cohort study began in April 2011, but this manuscript is restricted to nine welder trainees in the first academic quarter (September 2012—December 2012) of their traineeship, who entered the welding training program with no prior welding experience or reported occupational exposure to Mn.

The welding training program consists of five academic quarters, where students progress through a schedule of different welding processes, though time on each process may vary between subjects. While the nine trainees described here were followed for all five academic quarters, this analysis is restricted to their first academic quarter period of study. During this first quarter of the program, all nine trainees started with oxyacetylene welding (very low Mn exposure) before progressing to SMAW (moderate Mn exposure) after about a months' time. Trainees were in class Monday through Friday, with time split between welding practice and classroom lectures. Each welding booth in the facility has a dedicated adjustable exhaust ventilation hood to control fume, and trainees were given the choice whether or not to wear respiratory protection. No subjects wore respiratory protection while doing oxyacetylene welding, but six of the nine subjects (66.7%) consistently wore respiratory protection during SMAW welding.

Trainees were asked to provide a baseline blood and urine sample on the morning of their first day enrolled in the traineeship (a Tuesday), and at seven additional times over the course of their first quarter: the afternoon of their first day, the morning and afternoon of their first Friday, the morning and afternoon of their last Monday of the quarter, and the morning and afternoon of their last Friday of the quarter. On each biosampling day, subjects were also fitted with a personal air pump for the entire school day to measure airborne exposure to Mn. At the end of each biosampling day, trainees completed a questionnaire to assess their welding activity, use of respiratory protection, and any confounding sources of Mn exposure.

Demographic and work characteristics for these nine trainees are summarized in Table 1. Measured total Mn concentration in the air (total suspended particles) were normalized to eight-hour time weighted averages (TWA) and Mn exposure from SMAW welding was on average about 10 times higher than from oxyacetylene welding. No oxyacetylene or SMAW exposure measurements exceeded the National Institute for Occupational Safety and Health (NIOSH) eight hour TWA recommended exposure limit (REL) of 1 mg/m^3. However, based on self-report, subjects welded on average less than 4 hours each work day (221.2 ± 81.8 minutes).

Blood Mn analysis

At the beginning and end of sampling days, 6 mL of whole blood were collected in plastic Vacutainer® evacuated tubes containing 10.8 mg K_2EDTA anticoagulant (BD, Franklin Lakes, NJ) and transported on ice to University of Washington Environmental Health Laboratory (UW EHL). One mL of whole blood was transferred to a 15 mL Corning CentriStar polypropylene centrifuge tube (Corning, NY) and stored at 4 degrees C until analysis for trace metals. Microwave assisted acid digestion prepared samples for multielement analysis of whole blood by ICP-MS (Agilent 7500 CE) [14]. A control material (ClinChek Level 1 Whole Blood Control, lyophilised for trace elements (Lot 038), Recipe Chemicals, Munich, Germany) was analysed periodically. The results obtained for Mn in ClinChek materials were within the manufacturer's acceptable reference range of 5.6—8.4 ng/mL (found mean test value 7.3 ng/mL, SD: 0.1 ng/mL, *n*=6). The limit of detection for Mn was 0.5 μg/L, and is based on three times the standard deviation of the analysis-batch-specific field blanks. Limit of quantification for Mn in blood (10 times the standard deviation of the blanks) was 1.7 μg/L. No blood samples fell below the limit of detection or limit of quantification for Mn.

Plasma Mn analysis

The remaining whole blood was centrifuged for 20 minutes at 600 CFM to allow for the extraction of 1 mL plasma, which was pipetted into 2.0 mL Eppendorf tubes (Hauppauge, New York). Aliquoted plasma samples were sent on dry ice for analysis at the UK Health and Safety Laboratory (HSL, Harpur Hill, Buxton, UK) where instrumentation allowed for an improved limit of detection compared to the UW EHL. Samples were still frozen upon arriving at HSL, and were stored at 4 degrees until analysis for trace metals. The samples were diluted twenty-fold and analysed using ICP-MS (Thermo X Series 2) in collision cell gas mode. The instrument was calibrated 0.05—20 ng/mL for Mn. Certified reference materials (Seronorm serum CRM Levels 1 (Lot 0903106) and 2 (Lot 0903107), Sero, Billingstad, Norway) and a check standard were analysed periodically throughout the analysis. The results obtained for Mn in Seronorm serum were on average within the manufacturer's acceptable reference range of 14.1—15.9 ng/mL (found mean test value 15.17, SD: 0.52, *n*=6) for Level 1, and on average within the manufacturer's acceptable reference range of 18.8—21.0 ng/mL (found mean test value 20.4, SD: 0.66, *n*=6) for Level 2. Given there are no plasma certified reference materials for trace elements, a blank and a spiked plasma sample were analysed as well as a spiked serum sample at the start and end of the analysis and after every ten samples. Mean spiked plasma recovery for Mn was 113.2% SD: 18.0%. Limit of detection for Mn was 0.005 μg/L, and is three times the standard deviation of the blanks. Limit of quantification for Mn in plasma (10 times the standard deviation of the blanks) was 0.017 μg/L. No plasma samples fell below the limit of detection or limit of quantification for Mn.

Urine Mn analysis

At the beginning and end of each sampling day, subjects were asked to provide a sample of urine, collected in a 4 oz. low-density polyethylene wide mouth bottle. (Fisherbrand, Pittsburgh, PA). 5 mL samples were aliquoted into 15 mL Corning CentriStar polypropylene

centrifuge tubes (Corning, NY) and sent on dry ice for analysis at HSL, where instrumentation allowed for an improved limit of detection compared to UW EHL. Samples were still frozen upon arrival at HSL, and remained frozen until analysis. Creatinine measurements were undertaken, and the samples were diluted twenty-fold and analysed using ICP-MS (Thermo X Series 2) in collision cell gas mode. The instrument was calibrated 0.05—10 ng/mL for Mn. Control materials (ClinChek Levels 1 and 2 Lot: 122, Recipe, Germany) and a check standard were analysed throughout the analysis. The results obtained for Mn in ClinChek were within the manufacturer's acceptable reference range of 3.34—5.02 ng/mL (found mean test value 4.09, SD: 0.07, *n*=5) for Level 1, and within the manufacturer's acceptable reference range of 15.8—23.6 ng/mL (found mean test value 19.3, SD: 0.42, *n*=5) for Level 2. The limit of detection for Mn was 0.038 μg/L and is based on three times the standard deviation of the blanks. Eighteen of the 63 urine samples (28.6%) fell below the limit of detection. The limit of quantification for Mn in urine (10 times the standard deviation of the blanks) was 0.13 μg/L. Forty-four of the 63 urine samples (69.8%) fell below the limit of quantification. For all subsequent analyses, urine samples were creatinine corrected.

Statistical methods

Plasma and blood data were determined to be log normally distributed, and thus were log-transformed for analysis. In all subsequent analyses, the urine samples that fell below the LOD were treated two different ways in mixed models to assess variability components. First, they were replaced with the LOD/$\sqrt{2}$ [15] and normalized to a creatinine concentration of 1 g/L by dividing the measured Mn concentration (in μg/L) by its measured creatinine concentration (in mg/mL). Resultant units of the creatinine corrected urine values are thus μg Mn/g creatinine, which were then log transformed yielding the units of ln(μg Mn/g creatinine). Secondly, values that fell below the LOD were imputed using multiple imputation interval regression. This form of multiple imputation can be used with censored data, and imputes values in a specified interval (in our case, below the LOD but greater than 0) assuming the distribution of the censored data is similar to the distribution of the uncensored data [16]. Multiple imputation can be a strong tool for missing data (or in our case, data below the LOD) as it maintains features of the whole data set, such as variances, means, or correlations between variables used in the imputation model [17].

Uncorrected urine values below the LOD of 0.038 μg/L were flagged as left censored. A multiple imputation interval regression specifying an upper limit of 0.038 μg/L and lower limit of 0 was used to generate 20 imputed data sets, each with 45 original values and 18 imputed values below the LOD. The multiple imputation interval regression included subject, respirator use (yes or no) and temporal characteristics of the urine sample (morning vs. afternoon, beginning of quarter vs. end of quarter, and beginning of week vs. end of week) in the model. Welding type was not included in the model, as all subjects were doing the same type of welding on each sampling day—oxyacetylene on both days at the beginning of the quarter, and SMAW on both days at the end of the quarter. These 20 data sets with imputed values were then creatinine corrected and log-transformed prior to additional analyses. For descriptive purposes, the mean of all 20 log transformed and creatinine corrected imputed values is what is considered for each value that was originally

below the LOD, but for the restricted maximum likelihood regression (REML) analyses to assess variability components, all 20 imputed data sets were considered, with individual point estimates combined, and standard errors estimated based on Rubin's Rules for multiple imputation [18].

A mixed model estimating variance components using REML was used to assess the variance components in MnB, MnP, and MnU, including subject as a random effect and time of the sample (morning vs. afternoon, beginning of quarter vs. end of quarter, and beginning of week vs. end of week) and use of respiratory protection (yes or no) as fixed effects. For temporal variables, the beginning of the day, beginning of the week, and beginning of the quarter were the reference groups (coded as 0). Temporal measures were included in the model as surrogate measures of exposure, since exposures are assumed to increase over the course of a day, week, and quarter. Moreover, including time of day in the model also accounts for dirurnal variation in Mn at the physiological level, such as that seen by Järvisalo et al. [11]. All statistical analyses were done in Stata 12 (College Park, TX). While we do have actual measured Mn exposure, analysis of the relationship between Mn in air and Mn in blood for the full cohort will be presented in a future manuscript.

RESULTS

Descriptive statistics for MnB, MnP, and MnU are presented in Table 2, stratified by the beginning and end of each temporal period in question: day, week, and quarter. Over the course of a week, geometric mean MnP shows a significant decrease (p=0.006), and while MnP also tends to decrease over the course of a day, congruent with what Järvisalo saw in MnU, this decrease is not significant. Geometric mean MnB showed no significant changes over a day, week, or quarter, nor did geometric mean MnU both when values below the LOD were replaced with LOD/√2 and imputed using interval regression. Imputing MnU data (and then taking the mean of the data sets for the descriptives) resulted in lower geometric mean MnU for all time points compared to the MnU data in which values under the LOD were replaced with LOD/√2. This is logical since the lower limit of the imputed values was allowed to approach zero prior to adjusting for creatinine. Similarly, the imputed MnU data has larger geometric standard deviations than the replaced MnU data, which can likely be attributed to the increased range (on the lower end) and spread of the imputed data compared to the replaced data.

Table 3 shows the distribution of urine samples that fell below the LOD, stratified by time of sample, comparing AM samples to PM samples, start of the week samples to end of the week samples, and start of quarter samples to end of quarter samples. Using a Pearson's chi-squared test, no significant difference in the proportion of samples that fell below the LOD was observed when considering changes over the various exposure periods; though when considering the proportion under the LOD at the start of the quarter vs. the end of the quarter, there was a marginally significant difference (p=0.056). However, when stratifying MnU values by beginning of quarter and end of quarter, there was no significant difference between the geometric means, using a two-tailed t test.

Table 4 shows the within-individual and between-individual variance components from the mixed model (also shown graphically in Figure 1 for MnB, MnP, and MnU with censored data treated both ways), in addition to coefficients for the fixed effects included in the model. Temporal elements (time of day, week, quarter) and respiratory protection were included as fixed effects in the mixed models, with subject being included as a random effect. In the mixed models using REML to estimate variance components for MnP, time of week (beginning of week vs. end of week) was a significant contributor to the model (p=0.003), suggesting a 19.3% (95% CI: 6.36%, 33.8%) higher geometric mean in samples taken at the beginning of the week compared to samples taken at the end of the week. This is consistent with the descriptive statistics presented in Table 2. For MnB, none of the temporal variables or the use of respiratory protection were significant contributors to the mixed model. Similarly, when considering MnU both imputed and replaced with LOD/√2, none of the temporal variables or the use of respiratory protection were significant contributors to the mixed model.

When considering creatinine corrected urine values below the LOD as 0.038 μg/L $\sqrt{2}$, nearly all of the variance in MnU is within the individual (>99.99%) with only a fraction of a percent (<0.01%) being due to differences between individuals. When using interval regression multiple imputation to predict creatinine corrected MnU values below the LOD, the total variance in the data increased to 2.14 from 1.34, but was still nearly all within the individual. The increase in total variance can, again, likely be attributed to the increased range and spread of the data, due to enough values below the LOD being imputed to a range bounded by zero and LOD prior to being creatinine corrected, instead of all being given the same value prior to creatinine correction. Like MnU, MnP also exhibits more variability within the individual (78.79%) than between individuals (21.21%); however not as starkly contrasted as in urine. Considering MnB, nearly all of the variability (93.69%) is between individual, with only 6.31% being within individual, making blood the only biological component considered with the majority of variance being between individuals as opposed to within the individual.

DISCUSSION

This is the first manuscript to address within and between subject variance components of MnB, MnP, and MnU in a well-characterized longitudinal cohort. From a mixed-model using REML to estimate variances, we observed different partitioning of variability components in different biological media. This would influence which biological media to use as a biomarker of exposure, how to interpret the data, and methods to best collect the specimen. The trainees' eight hour TWA exposure levels for Mn (even when SMAW welding) were on the low end compared to reported eight hour TWA exposures for welders in industry settings [19–24], and when compared to workers employed in other Mn-utilizing industries such as smelting [25, 26], or ferroalloy production [27, 28]. However, despite their comparatively low exposures, the trainees' exposures did exceed environmental background levels, and thus we considered temporal elements as a surrogate measure of exposure in all mixed-models, assuming exposures would increase over the course of a day, week, and quarter. Therefore, any variance between subjects would be due less to

differences in exposures, and more to unmeasured subject-specific variables which were not accounted for in these analyses.

In blood, we found nearly all the variability in Mn to be between subjects, meaning that repeat measurements on a given subject tended to be fairly similar to the baseline measurement. Thus, for subjects in our study, a single blood measurement gave a reliable measure of the subject's MnB at other time points, which could be indicative of a homeostatic regulation of Mn in the blood, at least at the low Mn exposure levels our subjects experienced in this study. The baseline MnB for our nine subjects ranged from 4.95 μg/mL to 13.40 μg/mL, a range of 8.45 μg/mL. While between-subject differences in dietary exposure to Mn may provide a possible explanation for the observed subject-specific differences in average MnB, dietary intake of Mn is likely to vary substantially over time. Thus, it seems unlikely that this explanation would simultaneously account for both the low-within subject variability in MnB, and the unique subject-specific average MnB values that we observe. The low within subject variability of MnB, in combination with the high within-subject variability in MnU and MnB suggest tight homeostatic control of MnB. Given that all subjects entered our study with no previous reported exposure to occupational Mn, it is possible that a yet-to-be-determined physiologic or metabolic difference between the subjects that is driving the observation that each subject has a unique average MnB.

Given our low levels of exposure, it is interesting to hypothesise how our results could be extrapolated to workers with higher exposures to Mn. Workers with higher average exposures, especially those working in traditional settings as opposed to training classrooms, are likely to have greater between-subject variability in exposure as compared to our trainees, as they would be doing tasks different from each other, but similar day-to-day. This could reinforce the between-subject component for variability in MnB. However, if Mn exposure became high enough, the homeostatic regulation of MnB we observed in our inception cohort could be overcome, and thus the within-person variability would increase. However, it is as-yet unknown if the point where homeostatic control is overcome would be similar for all people, or if subjects with higher baseline Mn would reach that point before subjects with a lower baseline Mn. If so, baseline Mn could play a role in predicting workers more susceptible to adverse health outcomes associated with Mn exposure.

Plasma was the only biological media to show a significant change in Mn concentration with any of our measures of temporality, with plasma samples taken at the end of a week (Friday) being significantly lower than those taken at the beginning of the week. This observation runs counter to our *a priori* hypothesis, since the welders would presumably experience an increase in cumulative Mn exposure over the course of a work week. Diurnal variations are common in many human physiological processes, but in our sample we did not see a significant decrease over the course of a day in MnP or MnU like those observed by Järvisalo [11].

Both MnP and MnU showed the majority of variability to be within subjects as opposed to between subjects. This partitioning of variance can be interpreted to mean that multiple measurements on a single subject are apt to show a large degree of variation, and a single sample isn't necessarily representative of an individual's MnU or MnP concentration at any

other time point, even when controlling for exposure. Given the majority of the variance in these media is within a subject, comparing a single MnP or MnU measurement to a single external exposure value, as is common in the largely cross-sectional studies of biomarkers of Mn reported in the literature, could be misleading. Additionally, unlike MnB, it could be difficult to calculate a meaningful change in MnP or MnU values even if a baseline measurement is available, since it would be hard to determine if a change is related to actual changes in exposure, internal physiologic or metabolic factors, or periodicity. For these reasons, in addition to the LOD factors present with MnU, interpretation of urine and plasma as biomarkers of Mn remains challenging.

It is physiologically unclear why MnB exhibits the majority of variance between subjects and MnP and MnU exhibit the majority of variance within subjects. One possibility could be the apparent homeostatic regulation of MnB. When additional inhaled or ingested Mn is deposited in the blood may be quickly removed and transported via the plasma into the urine (perhaps as a negative feedback mechanism), thus making MnB levels more stable and MnP and MnU levels more variable within a subject. Mn is known to complex with other substances in the plasma for transport [29, 30]. Animal studies have found that these plasma transporters used by Mn are also used by other metals such as iron, copper, or zinc [31–33]. Thus, co-exposures to these metals could explain some of the within-subject variation in MnP levels due to the competition for transporter binding. In a cross-sectional study of Mn-exposed ferroalloy smelter workers, Cowan et al. found that an increase in Mn exposure yielded a general decrease in iron levels in various blood compartments, hypothesizing that this inverse relationship may be due to increased competition for binding sites [26]. Further, these authors found that calculating a Mn to iron ratio in both plasma and erythrocytes was a useful biomarker to distinguish Mn-exposed workers from unexposed referents. However, even in the unexposed controls, many subjects in the Cowan et al. study had MnP levels on the order of 10 to 100 times higher than those presented in this manuscript, and the cross-sectional nature of the study did not allow Cowan et al. to calculate within-subject variation in any of the myriad biomarkers they measured.

Additionally, the increased within-subject variability in plasma and especially urine could be due to analytic variability, since Mn concentrations are much lower and closer to the LOD in these media than in blood. Similarly, our data exhibited a high MnB to MnP ratio, so hydrolysis of even a small fraction of the red blood cells during the blood draw or laboratory separation could dramatically increase MnP. Circadian and seasonal rhythms could also play a role in MnP levels to a greater extent than MnB levels, and our measures of temporality were too crude to address these measures of variability. Touitou et al. [34] found that proteins in plasma followed circadian and seasonal variations, even when controlling for changes in the whole blood such as red blood cell count, haematocrit, and haemoglobin. Because Mn may be protein-bound in the plasma, variations in levels of the proteins that bind Mn in the plasma could logically lead to variations in the levels of Mn in the plasma, as well.

Our analysis has several limitations which could be improved upon in future studies. First, our subjects were exposed to fairly low levels of Mn in welding fume over the three month study period, making inference from our results to typical occupationally exposed

populations difficult. In a previous manuscript we provided evidence that there is a threshold above which inhalation exposures are high enough to overcome internal regulation or dietary background, and a relationship between Mn exposure and MnB is apparent [6]. This threshold was around 10μg/m^3—a Mn exposure level higher than what our welder trainees were exposed to during oxyacetylene welding but generally lower than what they were exposed to during SMAW. If this is the case, then perhaps with sustained exposure above this threshold the subject-specific mean MnB would increase. The within-subject variance component of MnB may also increase, due to either the greater exposure variability, or the higher Mn exposure overcoming the body's homeostatic control of MnB.

In the urine data, the nearly 30% of values that fell below the LOD were (1) replaced with the LOD/√2 or (2) imputed using interval regression. Replacing nearly 30% of the data with the same value could artificially decrease total variance in the sample, while imputing values to zero could artificially increase total variance in the sample compared to what the actual values would yield. However, barring improvements to analytical equipment or assay procedures, the handling of values below the LOD with either replacement or imputation is likely unavoidable in studies dealing with low levels of MnU as observed in this study. Using replacement vs. imputation with our data did little to change point estimates or estimates of variance partitioning, though imputation did increase total variation in the sample, as would be expected. Knowing the variance components in MnB, MnU, and MnP in a study of only environmentally exposed persons would also help inform this analysis, however, that has not been explored in the literature.

CONCLUSIONS

This study represents the first time that variance components of MnU, MnP, and MnB have been explored in the literature. Our findings show that Mn seems to be fairly tightly regulated in the blood, at least at the low and short term exposures experienced in this study, which does not appear to be the case when considering Mn in plasma or urine. Both MnU and MnP exhibited the majority of variance within the individual, meaning a single sample of these biofluids may not be representative of a person's MnU or MnP concentration at any other time point, making comparisons to occupational exposures or a health outcome challenging and uninformative. Under the conditions experienced in our study (that is, low exposure, short duration) a single blood measurement adequately reflects MnB in a given subject. Thus, going forward, we need to better elucidate what factors may influence the as yet unexplained subject-specific differences observed in baseline MnB, and what factors may influence the variable MnP and MnU measurements observed in a subject over short time periods.

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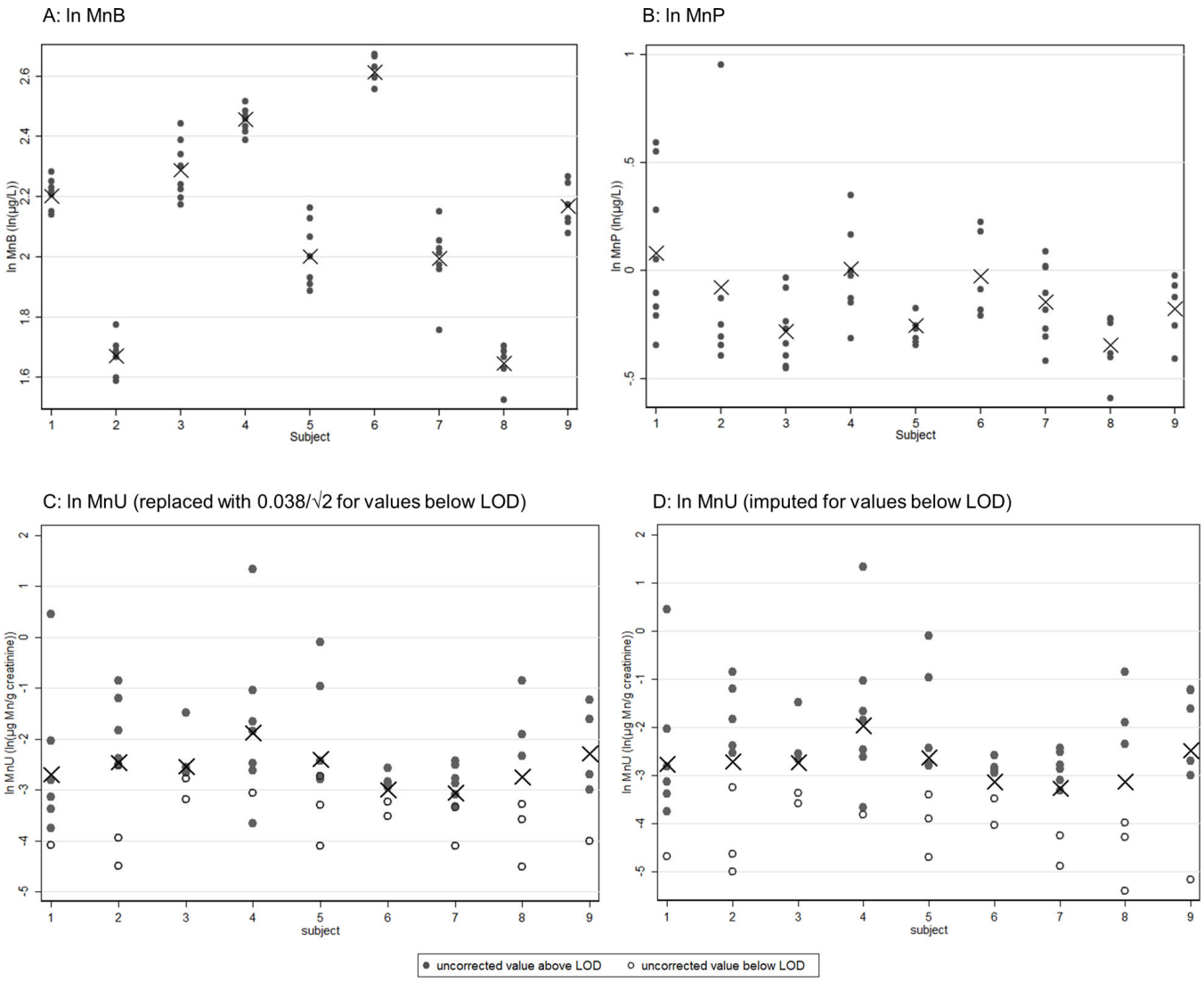

Figure 1. Biological measures of Mn exposure, by subject

Log-transformed biological measures of Mn exposure, by subject for (A) ln MnB (µg/L), (B) ln MnP (µg/L), (C) ln MnU with values below the LOD replaced with 0.038/√2 (µg Mn/g creatinine), and (D) ln MnU with values below the LOD imputed using interval regression multiple imputation (µg Mn/g creatinine).

Table 1

Subject demographic and exposure characteristics

n	9
Age (years)	22.1 ± 2.9
Male, *n* (%)	9 (100)
Current Smoker, *n* (%)	1 (11.1)
Respirator User, *n* (%)	6 (66.7)
Time welding/day (mins)	221 ± 82
Oxy welding 8 hr TWA Mn	3.6 ± 3.5 μg/m^3
SMAW welding 8 hr TWA Mn	39 ± 46 μg/m^3
n blood samples/subject	7.1 ± 1.1
n plasma samples/subject	6.9 ± 1.4
n urine samples/subject	7.0 ± 1.2

Table 2

Descriptive statistics for MnB, MnP, and MnU

		Beginning		End		
		n	**GM (GSD)**	**n**	**GM (GSD)**	***p*‡**
Blood (μg/L)	*Over Day*	32	8.47 (1.36)	32	8.32 (1.36)	0.82
	Over Week	32	8.27 (1.34)	32	8.52 (1.37)	0.70
	Over Quarter	34	8.52 (1.38)	30	8.25 (1.33)	0.68
Plasma (μg/L)	*Over Day*	31	0.894 (1.26)	32	0.861 (1.36)	0.58
	Over Week	31	0.963 (1.37)	32	0.801 (1.20)	**0.01**
	Over Quarter	34	0.841 (1.26)	29	0.921 (1.36)	0.19
Urine (μg Mn/g creatinine)*	*Over Day*	31	0.067 (2.95)	32	0.089 (3.26)	0.33
	Over Week	30	0.083 (2.96)	33	0.074 (3.29)	0.69
	Over Quarter	33	0.073 (2.54)	30	0.083 (3.80)	0.66
Urine (μg Mn/g creatinine)†	*Over Day*	31	0.052 (3.77)	32	0.079 (3.80)	0.22
	Over Week	30	0.068 (3.64)	33	0.061 (4.03)	0.74
	Over Quarter	33	0.065 (2.93)	30	0.063 (4.93)	0.91

*values below the LOD replaced with 0.038/√2, then creatinine corrected

†values below the LOD imputed using interval regression multiple imputation then creatinine corrected multiple imputation

‡two-tailed t test

Table 3

MnU measurements below the LOD

	# below LOD	*p**
AM	11 (35.5%)	0.23
PM	7 (21.9%)	
Start Week	8 (26.7%)	0.75
End Week	10 (30.3%)	
Start quarter	6 (18.2%)	0.06
End quarter	12 (40.0%)	

*Pearson's chi-squared

Table 4

Fixed effects and variance components from mixed models using REML to estimate variances

	ln MnB		ln MnP		ln MnU*		ln MnU†	
Fixed Effects	Coefficient (SE)	*p*	Coefficient (SE)	*p*	Coefficient (SE)	*p*	Coefficient (SE)	*p*
Change over day	−0.017 (0.020)	0.40	−0.042 (0.091)	0.47	0.284 (0.291)	0.33	0.417 (0.341)	0.22
Change over week	0.006 (0.021)	0.79	−0.176 (0.058)	**0.003**	−0.103 (0.294)	0.73	−0.080 (0.344)	0.82
Change over quarter	0.016 (0.027)	0.55	0.112 (0.072)	0.12	0.220 (0.330)	0.50	0.135 (0.386)	0.73
Respirator use	0.029 (0.037)	0.43	0.035 (0.091)	0.70	0.211 (0.381)	0.58	0.401 (0.446)	0.37
Variance Components	Coefficient (SE)	%	Coefficient (SE)	%	Coefficient (SE)	%	Coefficient (SE)	%
Within-subject variance	0.007 (0.001)	6.31%	0.052 (0.010)	78.79%	1.34 (0.248)	>99.9%	2.13 (0.888)	99.53%
Between-subject variance	0.105 (0.053)	93.69%	0.014 (0.011)	21.21%	<0.001 (<0.001)	<0.01%	0.011 (0.170)	0.47%
Total variance	0.111 (0.053)	--	0.111 (0.053)	--	1.34 (0.248)	--	2.14 (0.904)	--

*values below the LOD replaced with 0.038/√2, then creatinine corrected

†values below the LOD imputed using interval regression multiple imputation then creatinine corrected