

Manganese Exposure Estimation and its Association with Blood Manganese

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Abstract

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Despite evidence of adverse health effects resulting from exposure to welding fume and in particular to manganese (Mn), biomarkers of exposure are poorly understood and have not been thoroughly evaluated in a longitudinal design. This study evaluates the relationship between well-characterized inhaled Mn exposure and whole blood Mn concentrations. A cohort of 34 apprentice welders was observed for three academic quarters during which subjects were exposed to welding fume. Personal airborne exposures to Mn and total particulate mass were measured five times each quarter and blood samples were taken twice on four days in each quarter for each subject. Individual characteristics with the potential to influence the relationship between air and biomarker concentrations, including type of welding and respirator use, were assessed in all subjects via questionnaire. Using these data, a mixed effects predictive model was developed to quantitatively estimate air Mn exposures on non-sampling days, allowing for both fixed and random effects. Air exposure results demonstrated that different welding types were significantly associated with air Mn exposure. The relationship between air exposure levels and

predicted exposures were correlated to whole blood Mn levels using robust linear regression. No significant association was found between air exposure and blood Mn when compared across day. A statistically significant association was found between estimated quarterly air Mn exposure and cross-quarter blood Mn. This positive relationship indicated a loose association between increasing exposure and increasing difference in blood Mn levels. However, the overall results of this study indicate that blood Mn does not seem to be an effective short-term biomarker of exposure as analyzed. More data and a different time structure for analysis could lead towards a better understanding of blood Mn's association with air Mn exposure.

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Background

Manganese (Mn) is a trace-element that is essential for the development of proper neurological functioning.[1] However, overexposure to Mn has been linked to a variety of health effects, including pulmonary, reproductive and developmental toxicity.[2] Further, Mn exposure has been associated with irreversible neurological disease, called Manganism, which mimics Parkinson's Disease.[2-3] Current knowledge of low-level exposures to Mn and health effects is inadequate and needs to be more thoroughly researched.

Many occupations expose workers to elevated levels of Mn. These occupations include manganese ore processing,[4] ferroalloy production,[5-7] metalworking, scrap metal recycling,[8] dry cell battery manufacturing,[9] application of certain fungicides[10] and welding.[11-13] Due to the numerous US occupations which pose a Mn exposure risk and the lack of a well understood relationship between exposure and health effects, research in this area is of prime public health concern. Primary to that research is a need to better understand how certain work conditions correlate with exposure and how air exposure correlates with body burden.

Welding is an important trade occupation in the United States with over half a million workers.[14] While the composition of welding fume differs widely by type of welding, Mn can make up to 5% of the welding material by weight.[15] As such, welders are an appropriate population to study to learn about Mn exposure and possible health effects.

Current standards for Mn air exposure come from the American Conference of Governmental Industrial Hygienists (ACGIH) which has established a Threshold Limit Value (TLV) of 0.2 mg/m^3 , however this limit will soon be lowered to 0.02 mg/m^3 for respirable Mn,[16] and from the Occupational Safety and Health Administration (OSHA) which has a

ceiling limit of 5 mg/m³. [17] With these exposure limits in mind, studies have shown welding may lead to overexposure of Mn, reporting exposures from 0.04 to 2.2 mg/m³ for mean personal breathing zone concentrations. [12, 18-26] Further studies have shown that exposure remains even in the presence of local or general exhaust ventilation. [12-13, 23, 27-28] Due to the nature of many of these small studies it is hard to determine an accurate exposure range for Mn in welding workers. For blood, Mn has been analyzed from whole blood, [5, 8-9, 19, 24, 29-32] serum, [22, 24-25, 32] plasma, [6, 33] and the cellular fraction of whole blood. [6] No biological exposure index (BEI) has been set by the ACGIH for Mn in blood. [16]

Previous studies have investigated the possible correlation between air exposure and blood Mn. While some studies have shown only insignificant correlations, [30] others have found blood manganese to correlate with air exposure. [5-6, 19] In these studies, subjects were separated in exposure categories, and blood Mn distributions were determined for each category and then compared. In contrast, this study correlates individual air exposure measurements to blood Mn levels without grouping subjects by exposure categories. Other studies have explored blood Mn concentrations in a longitudinal design. Negligible increases in average blood Mn levels were observed over a four day workweek in welders, regardless of ventilation, by Iarmarcovai, *et al.* [31] Another study examined changes in blood Mn of foundry workers one month after the cessation of work and found a significant decrease. [8] Finally, Roels, *et al.* found no significant change in blood Mn levels in manganese oxide plant workers, sampling over a single workshift. [30] This study marries the longitudinal design to individual air and blood measurements to draw a more conclusive picture of Mn exposure and its expression as blood Mn.

The first aim of this thesis was to create a model to quantify Mn air exposure. Renton Technical College was selected for the study location because subjects' tasks vary in an organized and predictable manner, as opposed to work conditions where monitoring is not always be possible. Further, the ability of this study to take a large number of measurements gives power to the final model and helps validate exposure estimates. To do this analysis we used a mixed-effects regression model, allowing for both fixed and random effects.

The second aim of this study was to determine the association of air exposures and exposure estimates to Mn concentrations in blood. For this aim the selection of Renton Technical College was also advantageous. Students who are learning welding have no prior exposure to welding fume and inhaled Mn and as such a baseline can be established for these subjects. This is important to establish the timing of the exposure in relationship to the biomarker of interest. The relationship between whole blood Mn and air Mn exposure was examined using robust linear regression. Outcome variables included whole blood Mn differences between afternoon and morning samples, Friday and Monday morning samples, Friday afternoon and Monday morning samples, and Friday morning end of the quarter and Monday morning beginning of the quarter samples. Differences were chosen as the outcome variable of interest to account for different baseline Mn levels per time period. The goal was to reduce any possible subject-specific manganese exposure prior to the sampling period, and only look at blood Mn changes resulting from measured or predicted exposures.

Due to the large numbers of workers exposed to welding fume, effects of Mn exposure are of great public health concern. To better understand the association of any effects with exposure, the development of a quantitative and accurate model of exposure is needed. This

study aims to utilize an ideal experimental setting to correlate work environments to air exposures and air exposures to exposure biomarkers over time.

Methods

Design and Recruitment

This study is of a longitudinal cohort type design. We followed a cohort of 34 welding students through three quarters of their training, collecting air and blood data. We created models to predict exposure to Mn on non-sampling days.

Subjects were recruited from Renton Technical College's welding program. The welding program is nominally five quarters long and the first four quarters are intensive in welding containing manganese. On the first day of the each quarter students were recruited to the program. Not all subjects were recruited the first quarter of study, and some subjects only participated in one quarter. Willing participants were enrolled and were actively monitored for the first three quarters and followed for the fourth to observe the hypothesized elimination of manganese in the body. All study protocols were reviewed and approved by the University of Washington Institutional Review Board and subjective provided written consent. Incentives for participation in the study were provided in the form of fifty dollars on each day a pump was worn and blood collected.

Monitoring and Data Collection

Surveys:

Subjects were asked to complete a daily questionnaire at the end of each sampling day. The questionnaire was proctored by trained research personnel. Information gathered on this form included the subject's schedule that day, type of welding, amount of welding (in terms of number of rods used), ventilation use, respirator use, outside welding exposure, smoking, dietary sources of Mn, and welding activity on previous days. Ventilation use was quantified in terms of

the distance between the vent hood and the subject's weld. Answers were given in inches but biased towards easy to estimate distances, e.g. common responses were 12, 18, 24 and 36 in.

Air Sampling:

A personal air pump with sampling train was fitted on each student at the beginning of a sampling day. Pumps could be returned at lunch, but were again donned before afternoon welding. The air pumps were programmed to run at approximately 2 L/min for the entire sampling day. Pumps were attached to MCE filters in closed face cassettes that had been pre-weighed in the laboratory. Personal air samples were also taken on a day or two in the middle of each quarter to increase our sampling number for the predictive model. Air samples were then post-weighed in the lab for total particulate mass and then analyzed for trace metals. Microwave acid digestion prepared samples for instrumental analysis of elements by ICP-MS.[34] Reporting limits ranged from 0.01-0.03 μg Mn per sample depending on background conditions and were based on three times the standard deviation of the blanks, however the final reporting limit was determined by professional opinion of the lab staff. No samples fell below the reporting limits for Mn per sample. For particulate mass, an LOD was established as the mean of absolute values for the blanks plus two times the standard deviation of the blanks. For samples falling below the limit of detection (LOD) for particulate mass, the LOD divided by the square root of two was used for the adjusted weight which was divided by the pump flow rate times welding time to achieve a concentration used in analysis. This calculation resulted in some high concentrations for below LOD samples due to low reported welding time (eg, 35 minutes compared to 3.5 hours). Concentrations were determined by dividing adjusted weight (sample weight minus mean of the blanks in that batch) by welding time times pump flow rate. All hotwork time was included in welding time. We chose to use welding time instead of sampling time based on the

assumption of no exposure during non-hotwork activities (lessons in classroom, lunch, breaks, etc), and that thus using only welding time would give a better understanding of actual exposure concentrations.

Blood sampling:

Blood samples were collected in the mornings and afternoons of sampling days. Six mL of whole blood was collected in 6-mL plastic Vacutainer® evacuated tubes (BD) containing 10.8 mg K₂ EDTA anticoagulant and transported, double sealed, on ice to the lab. In the lab, one mL was transferred to a polytetrafluoroethylene (PFTE) digestion vial and stored at 4 °C until analysis. Microwave acid digestion prepared samples for multi-element analysis of whole blood by ICP-MS.[35] Reporting limits ranged from 0.3-1 ng/mL Mn depending on background conditions and were based on three times the standard deviation of the blanks. No blood samples fell below the reporting limits for Mn.

Statistical Methods

An exposure model was developed based on air monitoring and welding data to predict exposure levels which were then used to estimate individual exposures over the course of a week and quarter. The basic model followed the form:

$$Y_{it} = \beta_0 + \sum_s X_{its} \beta_s + b_i + \varepsilon_{it}$$

where Y_{it} is the log transformed Mn air concentration measured for individual i at time t , X_{its} are individual model predictors (including time welding, type of welding, etc.), b_i is a subject-specific random effect variable that accounts for unmeasured individual behaviors that affect exposure, and ε_{it} is a residual error term. Each of the individual model predictors were analyzed for significance to the model and were removed if found to be statistically insignificant. With the finalized model, individual daily exposures for both sampled and non-sampled days were

predicted based on both the predicted fixed effects and subject-specific random effects. These exposures were in the form of log transformed Mn air concentrations. To achieve a weekly exposure variable, the predicted daily concentrations were exponentiated to achieve non-transformed concentrations of $\mu\text{g}/\text{m}^3$. Each concentration was multiplied by the welding time worked on that day to reach a cumulative exposure for that day. These daily exposures were then summed for the entire week to reach a cumulative weekly exposure of the units $((\mu\text{g}/\text{m}^3) * \text{hr})$. This variable was log-normally distributed; as such, it was transformed before analysis for a final exposure variable having the units $\ln((\mu\text{g}/\text{m}^3)*\text{hr})$ representing the estimated cumulative weekly exposure which was used as the exposure variable in the blood analysis. Unfortunately, sampling numbers of some welding types were low and could not be included in the model. Therefore, welding types with fewer than 10 samples were not included. To avoid losing subjects due to this constraint, subjects performing these welding types had their daily exposures estimated more simply. This was done by taking the geometric mean concentration for that welding type and multiplying it by the hours of welding reported that day. Geometric mean was chosen to remain consistent with the model predictions which are on the log scale. This daily exposure variable was then used according to the above description.

The second focus was on analysis of air exposure and whole blood Mn levels with robust linear regression. To answer the question: does air Mn exposure affect blood Mn levels, and if so, on what time scale? We focused on describing the differences of blood Mn concentrations by subject for each day and week. By comparing each PM data point to the corresponding AM a cross-day outcome was achieved. By comparing Friday AM to Monday AM, and comparing Friday PM to Monday AM two different cross-week outcomes were found. Both of these cross-week outcomes were analyzed to account for the time between exposure and Mn reaching the

blood. These outcomes were correlated to respective air monitoring data from both samples and the predictive models outlined above using robust linear regression. Residual plots were examined to assess the regression model. For the weekly analysis we explored two different possibilities. The difference between Friday PM and Monday AM blood Mn could correlate to an entire workweek's exposure if Mn was quickly taken up by the blood stream. However, if a longer latency existed, we hoped to capture this by investigating the difference between Friday AM and Monday AM correlated to air exposure only from Monday to Thursday.

For analysis across quarter, an exposure variable was estimated along similar lines to the weekly exposure variables. Using a similar model to the one described above, estimates were predicted and then used to generate daily exposures on the log scale. These were then exponentiated, multiplied by the hours of welding that day and summed across quarter. This cumulative exposure variable was then log transformed to reach the final cumulative exposure variable used in analysis. Because welding time is not reported throughout the mid-quarter, days on which subjects attended were assigned the mean welding time of each respective subject. Attendance was ascertained from provided school records. The outcome variable used was the difference between the morning, beginning of week, beginning of quarter sample and the morning, end of week, end of quarter sample.

Data description and modeling was performed using STATA 11.[36]

Results

Air Results

This study encompassed 34 subjects, 30 males and 4 females. On average, 7 air samples were taken per participant over the course of the study (range: 2-15). Not all subjects had data for all three quarters as some were recruited in the second and third quarter of study. Further, some students only were followed for one quarter before leaving the study. Both particulate matter (PM) concentrations and Manganese (Mn) concentrations were examined. It was determined that welding time (including all hotwork such as cutting, grinding, and welding), rather than total sampling time, should be used to calculate the volume of the sample to more accurately calculate exposure concentrations. Due to this decision, 6 of the 239 air samples were removed because the subjects reported no welding time on that sampling day. The removed samples average 2.03 μg per filter compared to 25.57 μg per filter on samples with some recorded welding time. Samples were assigned a welding type based on the subject's reported main welding task that day. This did not prove to be a difficult assumption as multiple welding types were only reported twice. Two Mn data points and one PM data point were removed as outliers, the removed PM result was from the same sample as one of the Mn outliers, a possible explanation could be that a piece of slag got into the filter keeper erroneously and would not normally have been inhaled or a part of subject exposure. The other Mn outlier may be a possible recording error. The outlier values were as follows: 2192.6 $\mu\text{g}/\text{m}^3$ and 902.9 $\mu\text{g}/\text{m}^3$ for Mn and 288.4 mg/m^3 for PM.

Table 1: Air Results by Welding Type

	Mn Conc ($\mu\text{g}/\text{cubic meter}$)					PM Conc ($\text{mg}/\text{cubic meter}$)					
	N	GM	GSD	Min	Max	N	N < LOD	GM	GSD	Min	Max
All	231	34.23	3.22	0.44	345.67	232	52	1.61	2.53	0.33	20.31
Oxy	54	10.95	1.74	1.43	36.62	54	32	0.64	1.72	0.34	3.63
Stick	108	29.50	2.95	0.44	345.67	108	10	2.43	2.36	0.33	19.68
MIG	18	55.27	2.42	4.05	171.17	18	0	1.84	1.82	0.67	5.35
TIG	5	17.05	1.77	8.65	39.05	5	3	0.78	2.45	0.38	3.50
Dual Shield	34	64.72	2.73	3.62	295.29	35	6	1.69	2.24	0.38	20.31
Inner Shield	8	31.63	3.78	1.57	92.30	8	1	2.91	1.82	1.12	9.77
Cutting/Grinding	4	11.29	2.65	2.87	26.24	4	2	1.53	1.12	1.35	1.70

From table 1, we can compare measured exposures by welding type for both Mn and PM. Oxyacetylene Welding (Oxy) has the lowest PM exposures closely followed by TIG welding. This was expected and agrees with our assumptions prior to entering this study. Dual Shield and MIG had the highest Mn concentrations, which is particularly interesting as they did not show the highest PM concentrations. This indicates that welding fume from Dual Shield and MIG welding carries the most potent Mn exposure. Figure 1 displays the histogram of log transformed Mn air concentrations for all welding types. The distribution appears approximately normal after the log transformation, although slightly skewed to the left. Figure 2 shows log transformed PM air concentrations for all welding types in a histogram. The distribution of PM concentrations is less normal than the Mn concentrations; this may be because PM varies more dramatically by welding type than Mn, which results in what appears to be a possibly multi-modal distribution. Also, due to the high number of samples falling below LOD we would expect significant grouping of these samples at the low end of the scale, as observed. Due to the placement of the LOD, it makes sense that the left side of the normal distribution was cut off and replaced with a more compact grouping of samples.

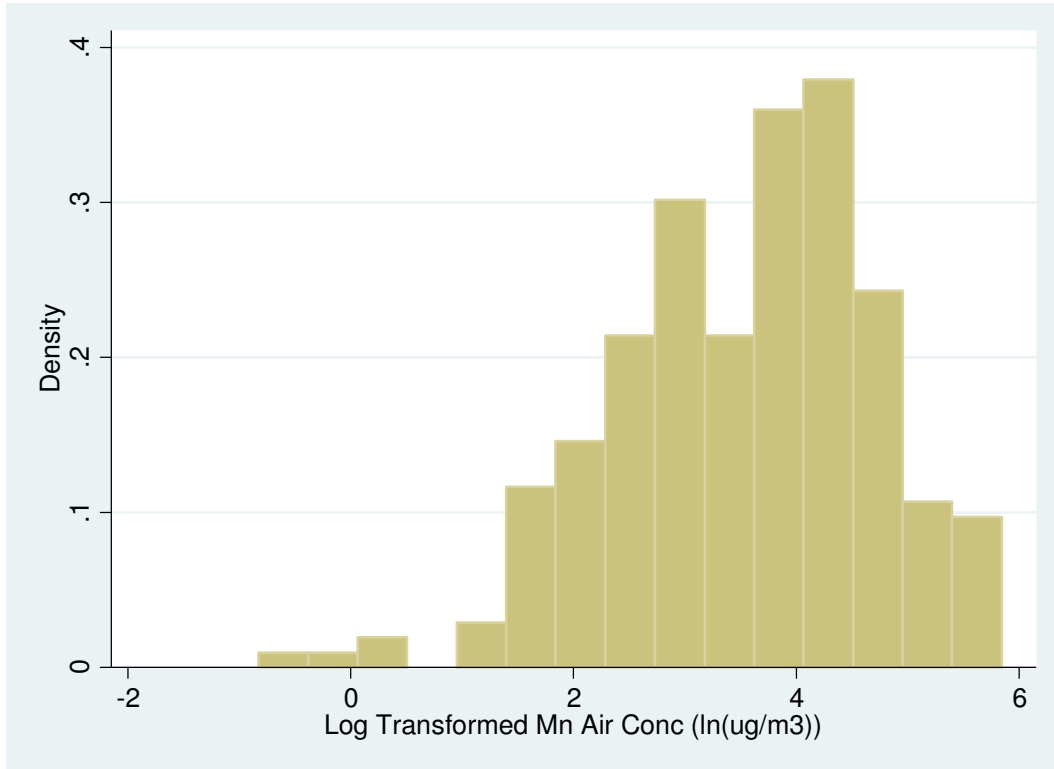


Figure 1: Histogram of Log Transformed Mn Air Concentration ($\ln(\mu\text{g}/\text{m}^3)$). (n=231)

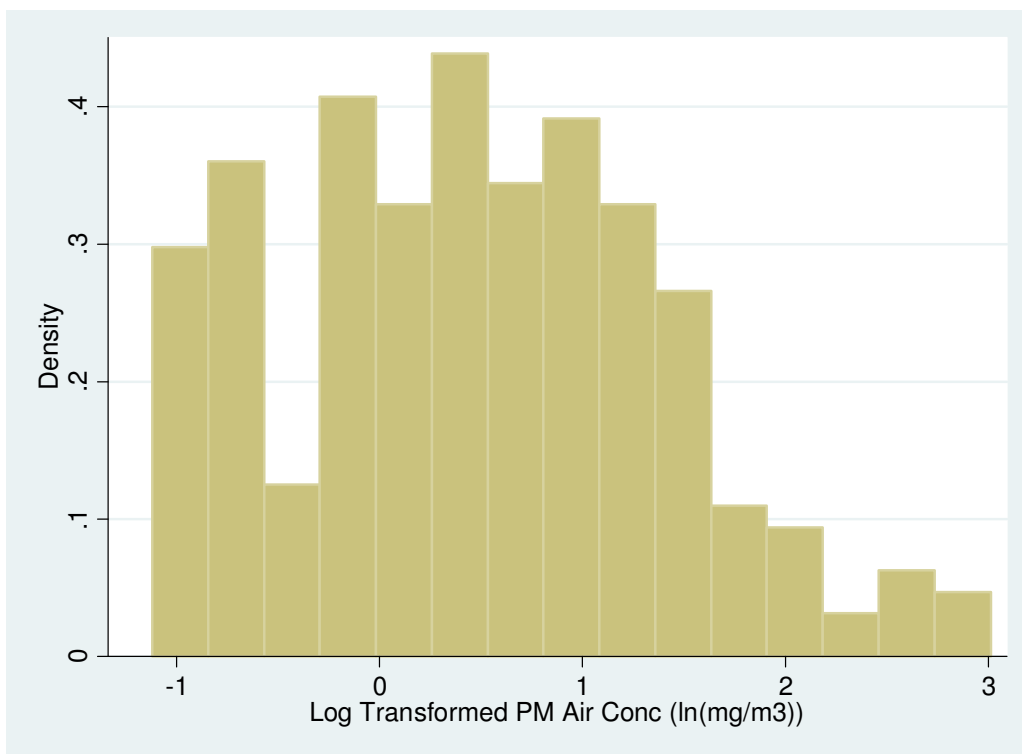


Figure 2: Histogram of Log Transformed PM Air Concentration ($\ln(\text{mg}/\text{m}^3)$) (n=232)

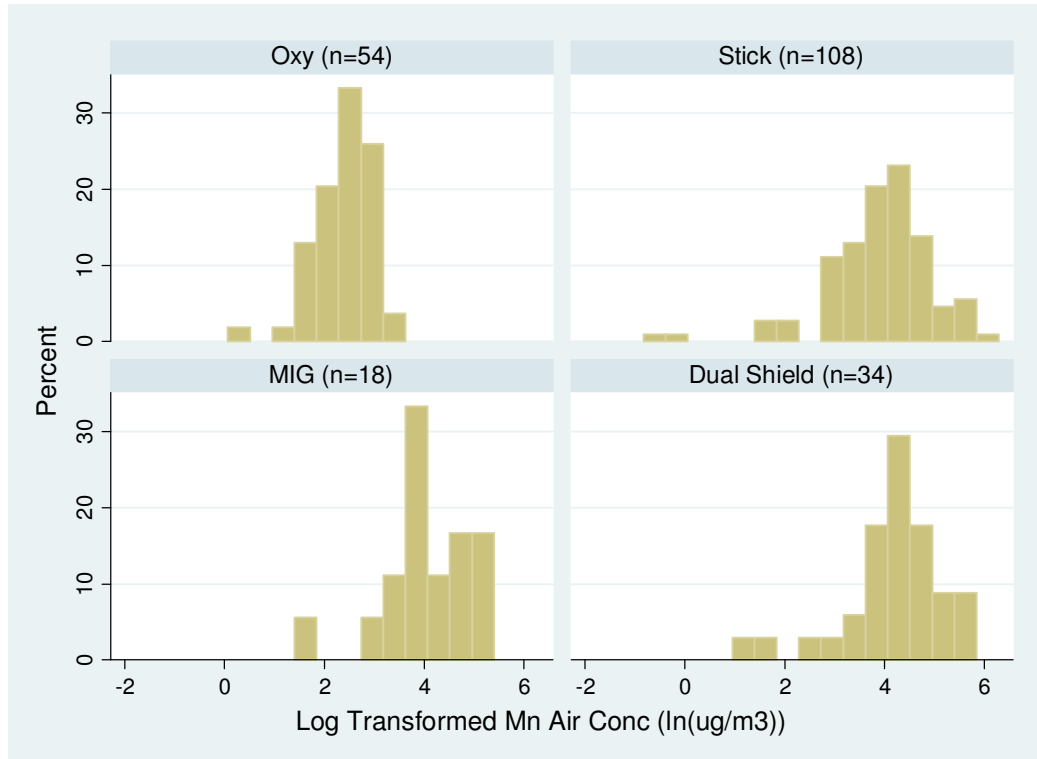


Figure 3: Histogram of log transformed Mn air concentrations by type of welding showing the different distributions of Mn Concentration ($\ln(\mu\text{g}/\text{m}^3)$)

Figure 3 shows the log transformed Mn Concentrations for the four most sampled types of welding. In this way we can compare the distributions of exposures by welding type. All four histograms display a log-normal distribution but with slightly different skews. Again we see the Oxy welding has the lowest average concentration. Figure 4 compares PM concentrations to Mn concentrations. While the correlation is quite significant ($p < 0.001$) it is not tight enough ($r^2 = 0.62$) to accurately predict Mn concentrations from PM levels. This may be due to different correlations by welding type. Figure 5 explores this possibility by correlating Mn to PM concentrations for the four most sampled welding types. While the fit of the line improves for Stick welding, reaching an r^2 value of 0.68, the fit for the other types of welding is reduced from the overall regression line, dipping as low as $r^2 = 0.23$ for Oxy (Table 2). This is in part due to the distribution of samples (greater variability in PM concentration for Stick welding) and the

percentage of leveraging points (much higher for Dual Shield, for example). It should also be noted that Oxy had the highest number and percentage of points below the limit of detection for PM mass, which may have added some instability. More sampling may strengthen this correlation. However, it remains important to measure Mn independent of PM measurements for accurate understanding of Mn air exposure.

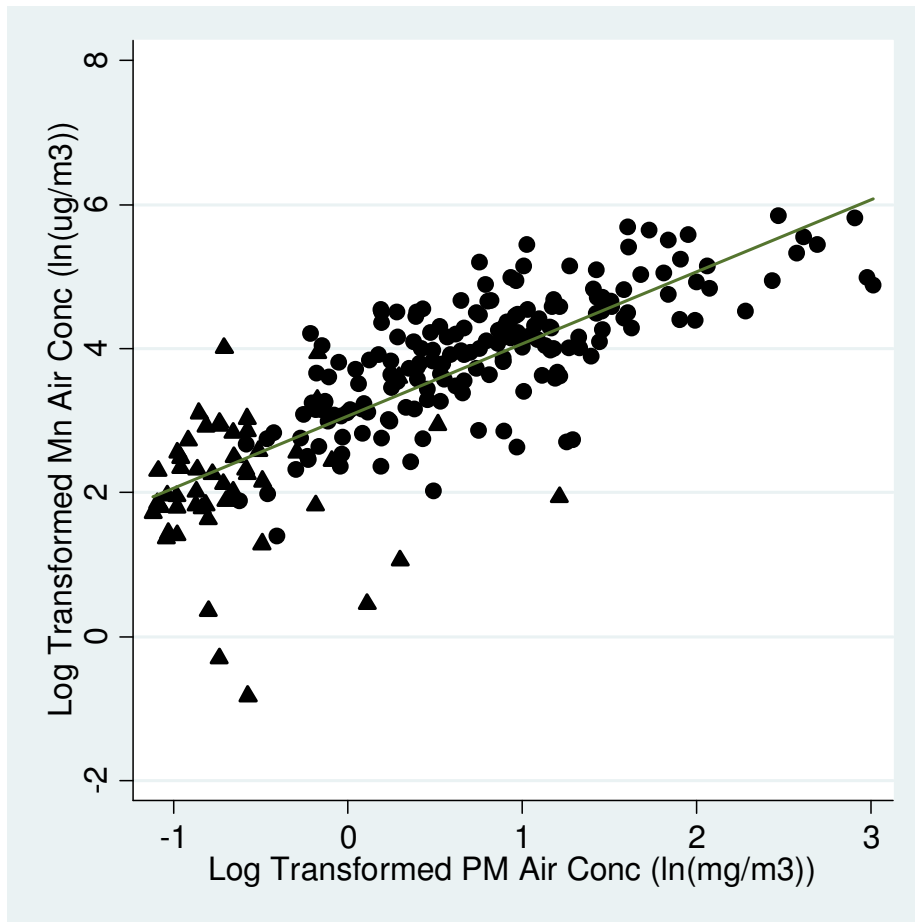


Figure 4: Comparing Mn concentrations to PM concentrations, triangle symbols indicate those points based on PM weights below LOD. (n=231)

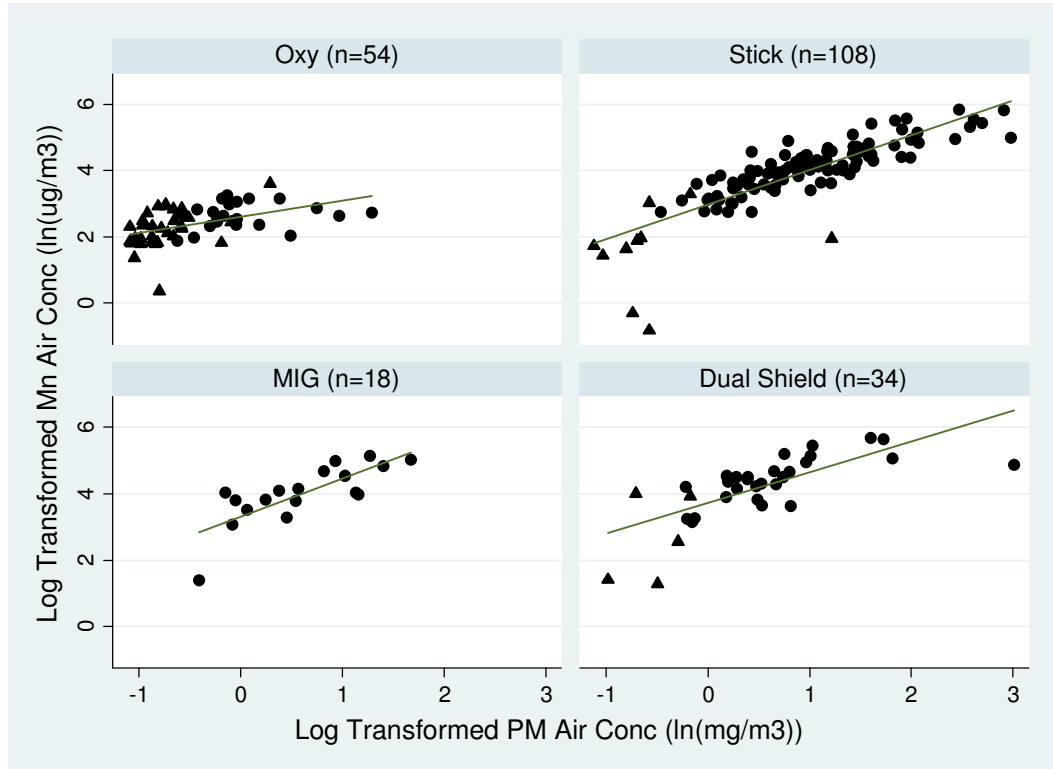


Figure 5: Comparing Mn concentrations to PM concentrations by type of welding, triangle symbols indicate those points based on PM weights below LOD.

Table 2: Linear regression results for comparing Mn concentrations to PM concentrations by welding type.

	N	Const.	Slope	95% CI	p-value	R²
All	231	3.063	1.001	0.904-1.099	<0.001	0.62
Oxy	54	2.609	0.486	0.236-0.736	<0.001	0.23
Stick	108	2.975	1.043	0.854-1.232	<0.001	0.68
MIG	18	3.315	1.141	0.487-1.795	0.002	0.60
Dual Shield	34	3.725	0.927	0.365-1.489	0.002	0.51

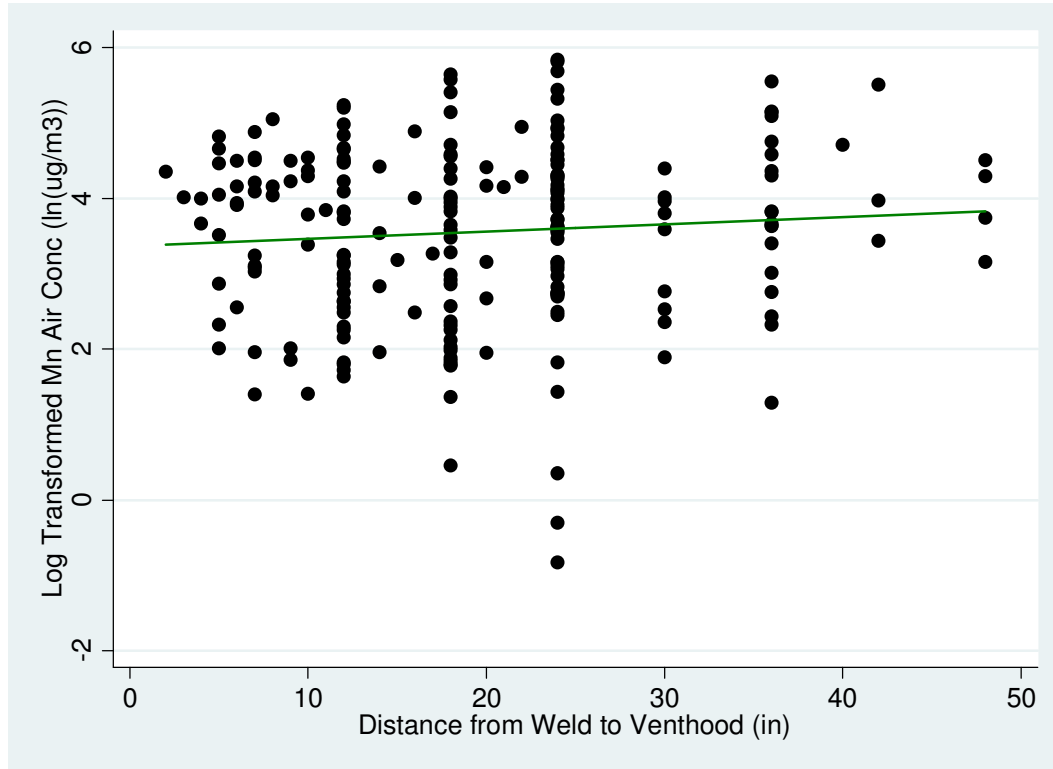


Figure 6: Assessing the effect of venthood distance on Mn air exposure. slope= 0.010, P= 0.16, $r^2= 0.007$. When assessed by welding type, MIG and TIG show significance, however the effect is minimal and the sample size is very small (5 samples for TIG, 17 samples for MIG). (n=215)

To assess possible predictors for Mn exposure that would be part of the model to estimate Mn exposure on non-sampling days, we looked at the correlation between venthood distance and Mn concentration and welding time and Mn concentration. Venthood distance is the measurement in inches from the weld to the local exhaust ventilation hood. In Figure 6, this distance is associated with the log transformed Mn concentrations. The regression line indicates an insignificant slight increase in concentration with increase in venthood distance that is not scientifically meaningful. Thus, the measure of venthood distance from weld is not a key predictor of air exposure in this study. Welding time was also a possible individual predictor of exposure, however Figure 7 shows no association between Mn concentrations and welding time

($p=0.73$). Both venthood distance and welding time were reexamined as predictors when the prediction model was being built.

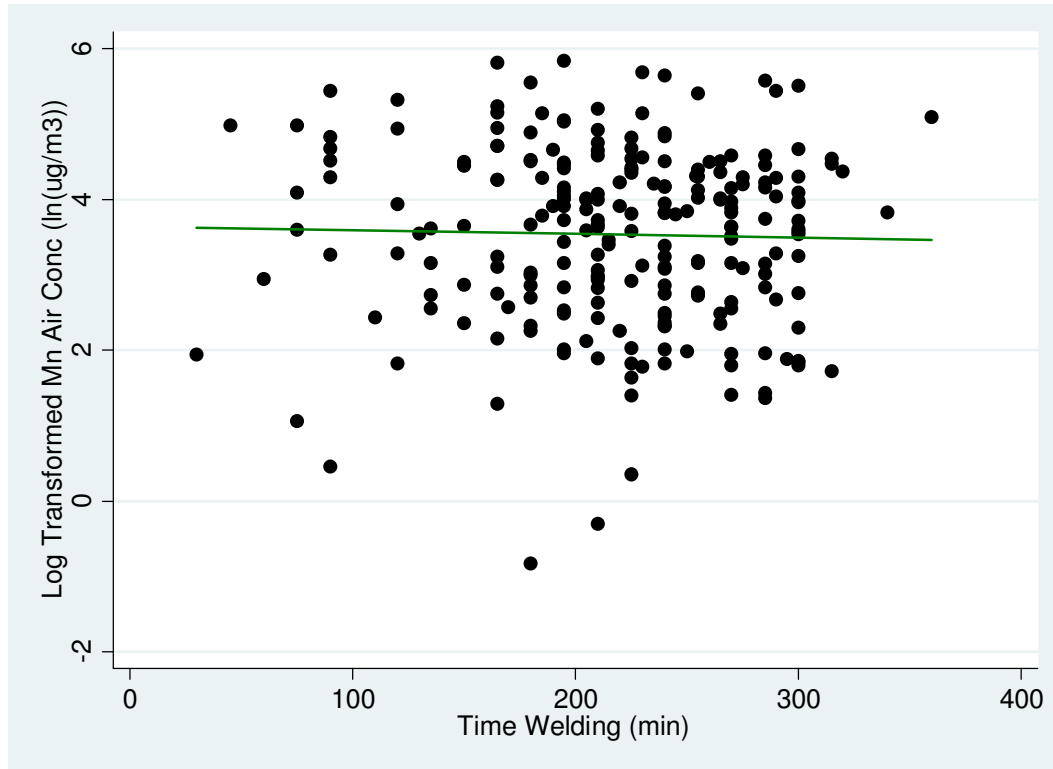


Figure 7: Assessing the effect of welding time on Mn air exposure. $p=0.73$, $r^2 = 0.001$. When separated by type of welding, the result was only significant for Oxy welding, the effect was still minimal. ($n=231$)

A daily exposure variable was reached by multiplying each air Mn concentration by the subject's welding time that day for an intermediary variable which was then log transformed to reach our final exposure variable with the units $\ln((\mu\text{g}/\text{m}^3)\cdot\text{hr})$. For samples with a welding time of zero minutes, an exposure of zero was assigned (6 samples). The distribution for this exposure variable can be seen in Figure 8.

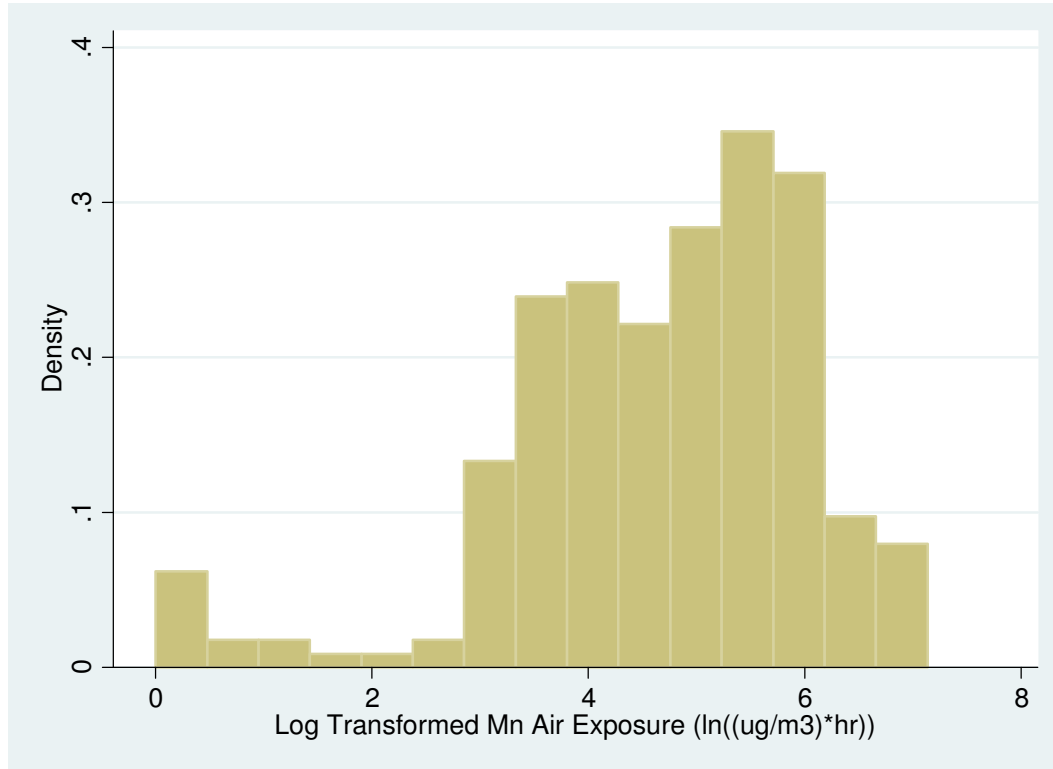


Figure 8: Histogram of log transformed Mn air exposures ($\ln((\mu\text{g}/\text{m}^3) \cdot \text{hr})$) (n=237).

To achieve a weekly exposure variable for use in correlating changes in blood Mn across a sampling week, air exposure must be estimated as we did not sample the air in the mid-week. Due to low sample numbers for some welding types, No Welding, TIG, InnerShield and Cutting/Grinding were eliminated from the model and were not predicted. Subjects performing these tasks had their daily exposures estimated using the geometric mean for each type of welding multiplied by welding time on that day. The results of this model fit on 214 samples are shown in Table 3.

Table 3: Mixed effects model used to predict welding exposures.

Fixed Effects				
	Coefficient	Standard Error	95% Confidence Interval	p value
Constant	2.40	0.14	2.13-2.66	<0.001
Weldtype (reference: Oxy)	-	-	-	-
Stick	1.55	0.16	1.24-1.87	<0.001
MIG	1.69	0.27	1.17-2.21	<0.001
Dual Shield	1.90	0.22	1.47-2.32	<0.001
Random Effects				
	Estimate	Standard Error	95% Confidence Interval	
SD of Subject ID	0.25	0.10	0.12-0.53	
SD of Residuals	0.90	0.05	0.82-1.00	

The prediction model used a mixed effects model to account for the fixed effects of type of welding while also including random effects due to individual subject variations. When running different models venthood distance and welding time were not significant and were thus removed from the model. The final model therefore included only welding type as a fixed effect and subject ID as a random effect. After running this final model, cross validation statistics were used to verify the stability of the model, results are displayed in Table 4. Based on the variability seen in the RMSE and coefficient values, the model is adequately stable and therefore useful for predicting daily air concentrations.

Table 4. Cross validation results for mixed effects model.

	Coefficient or Estimate				
	Model 1	Model 2	Model 3	Model 4	Model 5
Constant	2.39	2.33	2.37	2.44	2.43
Weldtype (reference: Oxy)	-	-	-	-	-
Stick	1.60	1.60	1.49	1.59	1.50
MIG	1.63	2.09	1.51	1.56	1.72
Dual Shield	1.90	2.10	1.79	1.81	1.90
SD of Subject ID	0.32	0.31	0.21	0.19	0.29
SD of Residuals	0.91	0.92	0.95	0.83	0.93
	Goodness of Fit				
	Model 1	Model 2	Model 3	Model 4	Model 5
RMSE	1.25	0.90	0.79	0.85	0.93

Using this model, subject-specific daily air Mn concentrations were predicted. We tested the validity of these predictions with a predicted vs. observed plot (Figure 9). The predicted values are compressed compared to the observed values, but this is expected of any prediction. Further, in this plot the subject-specific random effects add variability to the predicted values that welding type alone would not have predicted. Figure 9 displays two main groups of exposure predictions; nominally, these can be seen as low and high exposure groups. In the low exposure group are exposures from Oxy welding while the high exposure group contains exposures relating to Stick, MIG, and Dual Shield welding.

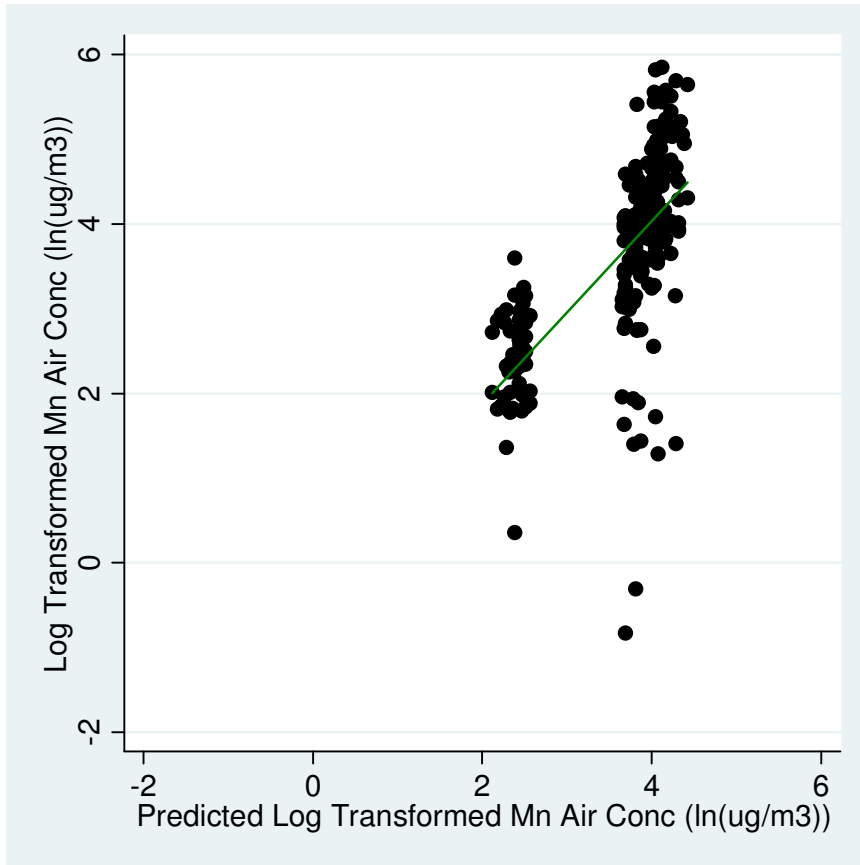


Figure 9: Observed versus predicted plot of log transformed Mn air concentrations. (n=214)

Using the predicted daily exposure concentrations a final variable with the units $\ln((\mu\text{g}/\text{m}^3) * \text{hr})$ was used as a cumulative weekly exposure measurement as described in the methods section. Since this calculation was based on subject reported welding times, a quick assessment of reported mid-week welding times and sampling day welding times (thought to be more accurate) was performed. A t-test showed that midweek welding times were significantly elevated compared to sampling days (mean 245 min compared to 210 min, $p < 0.01$). While this represents bias in our dataset, we do not expect this to introduce error into our results for blood Mn comparisons. Due to the possibility that blood Mn levels likely lag behind air Mn exposure, two different time periods of exposure were used to analyze cross-week blood changes and thus two exposure variables were needed. One needed to account for exposures Monday through

Thursday, and the other Monday through Friday. These variables both displayed roughly normal distributions, the distribution for the variable representing predicted exposures Monday through Thursday can be seen in Figure 10. A total of 88 weeks had complete subject exposure information.

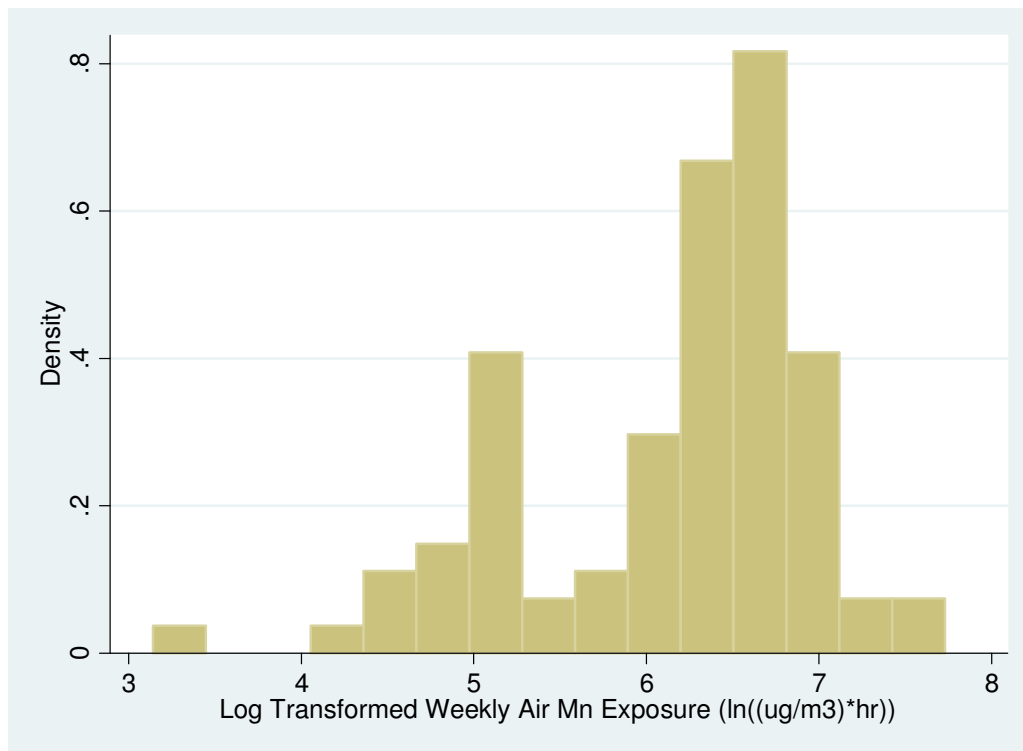


Figure 10. Histogram of estimated weekly air Mn exposures summed from beginning of week through Thursday. (n=88)

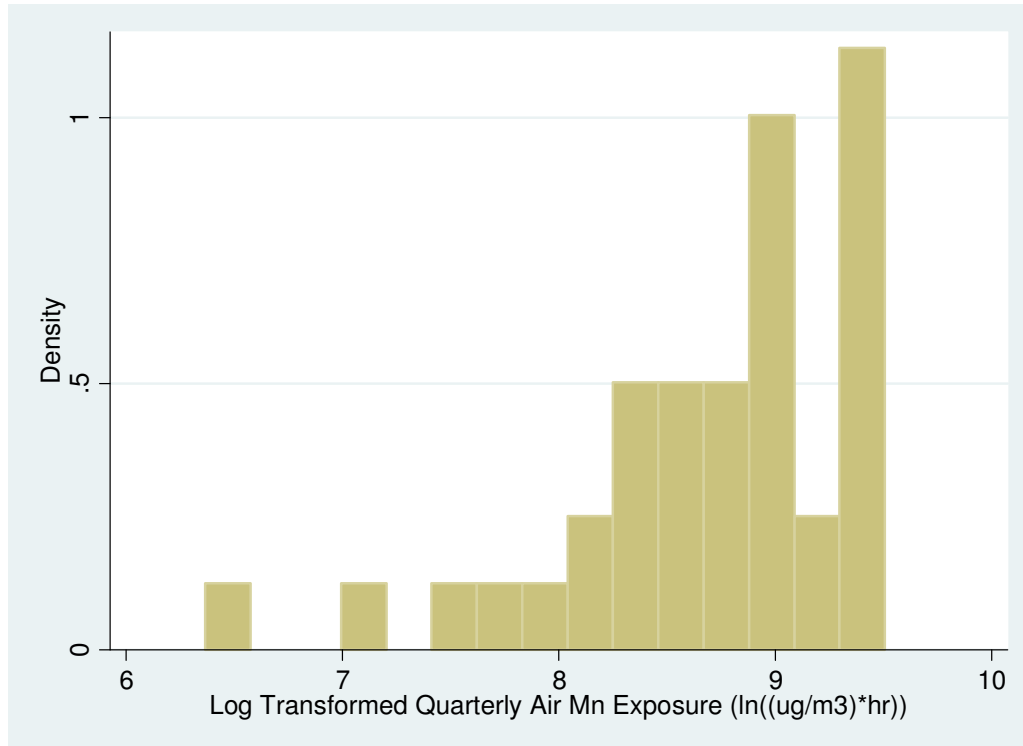


Figure 11: Histogram of estimated cumulative quarterly air Mn exposures summed from the beginning of the quarter through the second to last day. (n=38)

Quarterly air exposures were estimated according to the description in the methods section and here appear to have a slightly irregular distribution, likely due to the relatively small sample size of 38 (Figure 11). In this case the model used to estimate exposures predicted estimates for all the welding types except no welding and grinding/cutting. This was in contrast to the above model which also excluded TIG and Dual Shield. Reasons for this were due to the efficacy of estimating a much higher number of TIG and Dual Shield exposure days from a mixed effects model, rather than using the geometric mean, which was sufficient in the weekly analysis. This change in models did not have any significant effect on the coefficients estimated in the first model. The results of this model appear in Table 5. This model actually displayed slightly greater stability than our previous model, the results of the cross validation are seen in Table 6.

Table 5: Mixed effects model for quarterly exposures.

Fixed Effects				
	Coefficient	Standard Error	95% Confidence Interval	p value
Constant	2.39	0.14	2.13-2.66	<0.001
Weldtype (reference: Oxy)	-	-	-	-
Stick	1.55	0.16	1.24-1.87	<0.001
MIG	1.69	0.27	1.17-2.21	<0.001
TIG	0.53	0.45	-0.35-1.41	0.238
Dual Shield	1.90	0.22	1.47-2.32	<0.001
Inner Shield	1.10	0.36	0.40-1.81	0.002
Random Effects				
	Estimate	Standard Error	95% Confidence Interval	
SD of Subject ID	0.24	0.10	0.10-0.53	
SD of Residuals	0.91	0.05	0.83-1.01	

Table 6: Cross validation results for mixed effects model for quarterly exposures.

Coefficient or Estimate					
	Model 1	Model 2	Model 3	Model 4	Model 5
Constant	2.39	2.44	2.44	2.35	2.33
Weldtype (reference: Oxy)	-	-	-	-	-
Stick	1.62	1.47	1.48	1.53	1.63
MIG	1.68	1.40	1.69	1.77	1.86
TIG	0.54	0.59	0.20	0.60	0.60
Dual Shield	2.00	1.78	1.70	2.03	1.89
Inner Shield	1.12	1.00	0.64	1.21	1.46
SD of Subject ID	0.29	0.14	0.19	0.29	0.31
SD of Residuals	0.92	0.93	0.97	0.90	0.90
Goodness of Fit					
	Model 1	Model 2	Model 3	Model 4	Model 5
RMSE	0.95	1.04	0.88	0.99	0.99

Blood Results

A total of 372 blood samples were collected in the course of our study with a mean Mn concentration of 8.05 ng/mL. One blood record was removed due to having no daily log

associated with it. One Mn whole blood concentration outlier was removed from 371 samples (concentration of 42 ng/mL).

In Table 7, blood data are distributed to the main type of welding the subject was working on that day. Some students did no hot work and are thus classified as being part of “no welding”. However, since Mn in blood is a biomarker, it is expected that activities prior to the sample, such as the welding occurring the day or week before would affect this result. Further, two blood samples are taken per subject per day, and multiple samples are taken on the same subject across the time period of the study, thus samples are divided into groups by time of day sampled and time of week. With these considerations in mind, it is of note that MIG welding seems to have overall higher Mn blood concentrations than the other types. Also of note, Oxy welding has the lowest average concentration, which corresponds well to our hypothesis of Mn in blood being a biomarker of exposure, as the students working on Oxy have been the least exposed and are currently being less exposed than subjects performing other types of welding.

Table 7: Mn Blood Concentration (ng/mL) by type of welding separated by beginning and end of week and morning (AM) and evening (PM) samples.

	Beginning of Week			End of Week		
	N	Mean (ng/mL) (SD)	Range (ng/mL)	N	Mean (ng/mL) (SD)	Range (ng/mL)
Total	186	8.33 (3.35)	1.60-18.0	184	7.76 (3.28)	2.00-18.00
AM						
All	94	8.34 (3.33)	2.4-17.5	93	7.85 (3.25)	2.0-18.0
No Welding	4	10.75 (2.06)	8.0-13.0	1	7.70 (.)	.
Oxy	20	7.00 (2.03)	3.0-11.0	21	6.54 (1.90)	3.0-9.6
Stick	42	7.48 (3.20)	2.4-17.4	45	7.27 (2.87)	2.0-13.0
MIG	6	11.03 (4.50)	6.0-17.5	7	11.29 (4.00)	6.0-17.0
TIG	3	8.53 (2.28)	6.5-11.0	1	11.00 (.)	.
Dual Shield	12	10.61 (3.54)	7.0-17.0	16	9.47 (3.86)	4.0-18.0
Inner Shield	4	9.75 (4.27)	5.0-15.0	2	8.00 (5.66)	4.0-12.0
Cutting/Grinding	3	9.37 (2.28)	8.0-12.0	0	.	.
PM						
All	92	8.32 (3.39)	1.6-18.0	91	7.67 (3.33)	2.2-18.0
No Welding	4	11.63 (3.20)	7.5-15.0	1	6.50 (.)	.
Oxy	19	7.32 (2.13)	3.5-13.0	20	6.86 (2.82)	3.0-15.8
Stick	42	7.51 (3.56)	1.6-18.0	44	7.22 (3.22)	2.2-18.0
MIG	6	11.30 (3.93)	7.8-18.0	7	10.50 (3.49)	6.5-16.0
TIG	2	8.25 (1.77)	7.0-9.5	2	8.00 (1.41)	7.0-9.0
Dual Shield	12	9.69 (3.02)	5.0-15.0	15	8.86 (3.82)	4.0-16.0
Inner Shield	4	9.50 (3.70)	6.0-14.0	2	7.00 (4.24)	4.0-10.0
Cutting/Grinding	3	8.63 (2.91)	6.9-12.0	0	.	.

The distribution of all Mn blood levels appears normal (Figure 12). The distribution of log transformed Mn blood values appeared even more skewed than the non-transformed, and therefore it was decided that these values should not be log transformed for analysis.

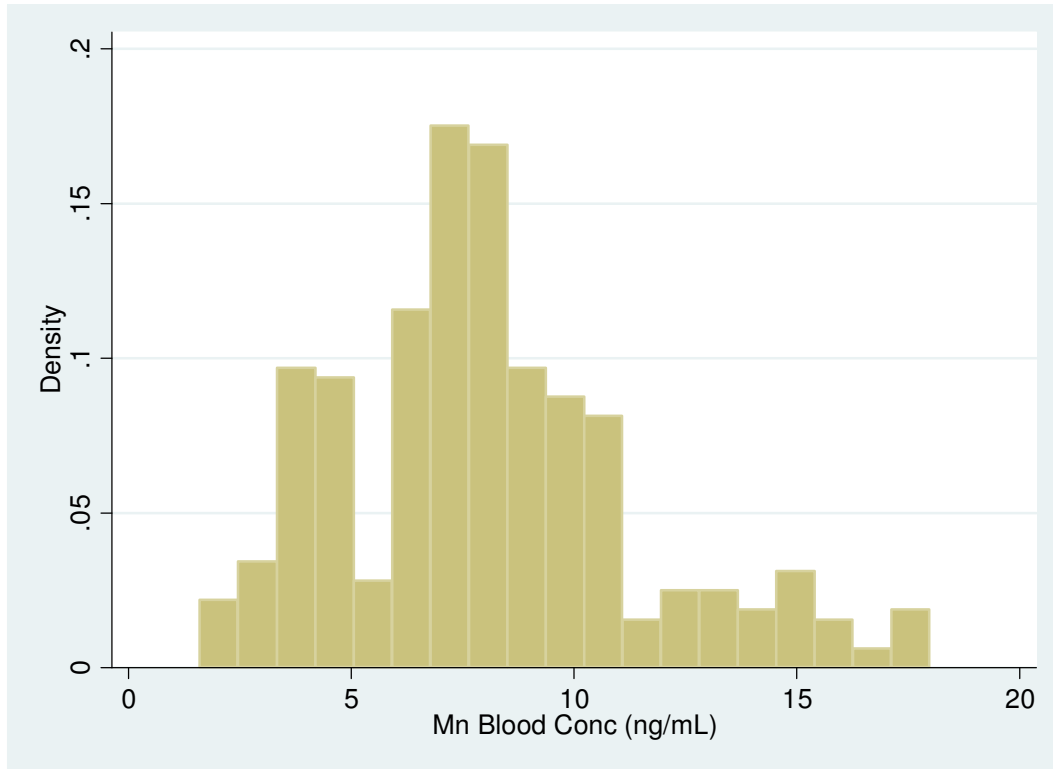


Figure 12: Histogram of all Mn Blood Concentrations (ng/mL) (n=371)

Since we hope to use blood Mn as a biomarker of exposure one question of interest is how does blood Mn change over time? To begin to understand this we can look more closely at the different time periods described in Table 7. Morning (AM) samples compared to afternoon samples (PM) seem to be roughly equivalent (means: 8.34 and 8.32 respectively at the beginning of the week, 7.85 and 7.67 respectively at the end of the week). This likely indicates that no change in blood Mn is seen across a 7 hour time span of exposure and that rather a lag might be occurring between exposure and a rise in blood Mn. Further, this table includes repeated measures on the same individuals, so it will be important to account for this bias in later analysis. Interestingly, blood levels appear to decrease across the week (means: 8.34 and 7.85 for AM samples, 8.32 and 7.67).

First, daily exposures were correlated with daily blood Mn changes. The chosen outcome variable was the difference between a subject's afternoon and morning sample. This difference was correlated to the subject's air exposure on the day the blood samples were taken as described in the air results section. The resulting regression line showed the correlation was not significant, nor scientifically meaningful (Figure 13a, slope = -0.14, $p = 0.19$). Results are reported in Table 8. One leverage point, with a blood difference of almost -10 ng/mL, may be driving this result. When analyzed with this point removed, the slope of the regression remains negative and the results are still statistically insignificant. Since no scientific reason was present for removing this data point, it was included in the analysis. A graph of the residuals from this regression model was viewed. Residuals appeared to be well distributed with no trends apparent. Further analysis included clustering the regression on subject ID. While this increased the robust standard error slightly compared to that in Table 8, the values were not impacted in a way that changed the scientific meaning of our results. The analysis was also performed on a native scale with a non-log transformed exposure variable. The results were not scientifically different (negative slope, insignificant p value, low r^2) than the displayed results and were not included in this paper. Because respirator use should prevent air Mn from entering the body and exposing the subject, subjects were divided into those using respirators at all and those who did not (Figure 13b). Seven days of observation did not have respirator use data, and were removed from this analysis. Noticeably the sign of the regression line changed between the two groups with those using respirators experiencing a slight increase in their blood Mn difference as their exposure increased while those not using respirators had a slight negative trend.

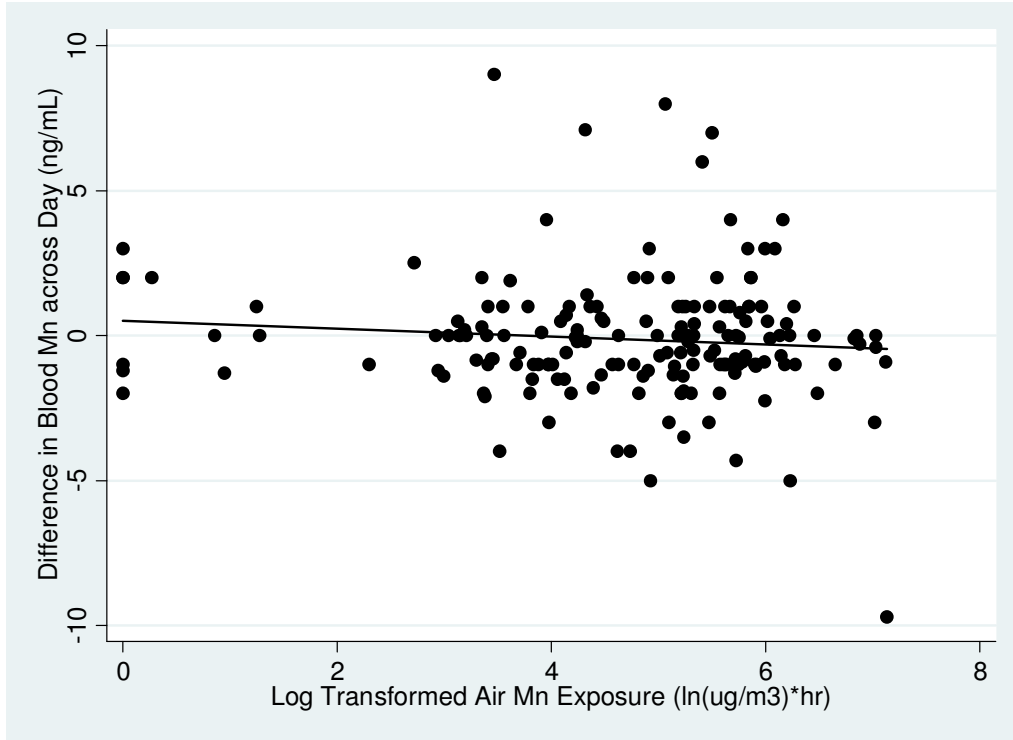


Figure 13a: Comparing Mn air exposure to the change in Mn blood levels over the course of that day. (n=175)

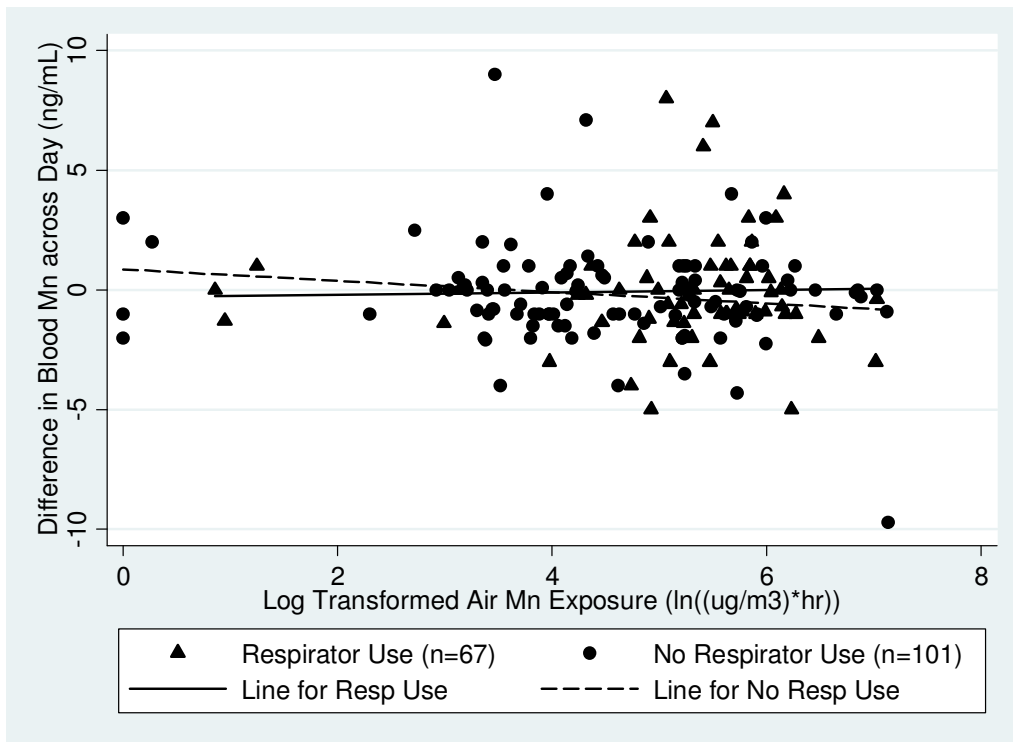


Figure 13b: Relationship between daily air Mn exposure and blood Mn differences across day, grouped by respirator use.

Second, weekly predicted air exposures were correlated with weekly changes in blood Mn. Due to a possible lag in time between exposure and biomarker response two different cross-week analyses were performed. First, the change in blood levels across the week was determined by finding the difference between the Friday AM blood sample and the Monday (or beginning of week) AM blood sample. Since the Friday AM blood sample could not be influenced by welding done on Friday (occurring after the blood draw) the exposure variable representing Monday through Thursday exposures was used as the predictor. The correlation between these two variables was found not to be statistically significant nor scientifically meaningful (Figure 14a, slope = -0.15, $p=0.34$). Results remained not statistically significant when grouped by respirator use (Figure 14b).

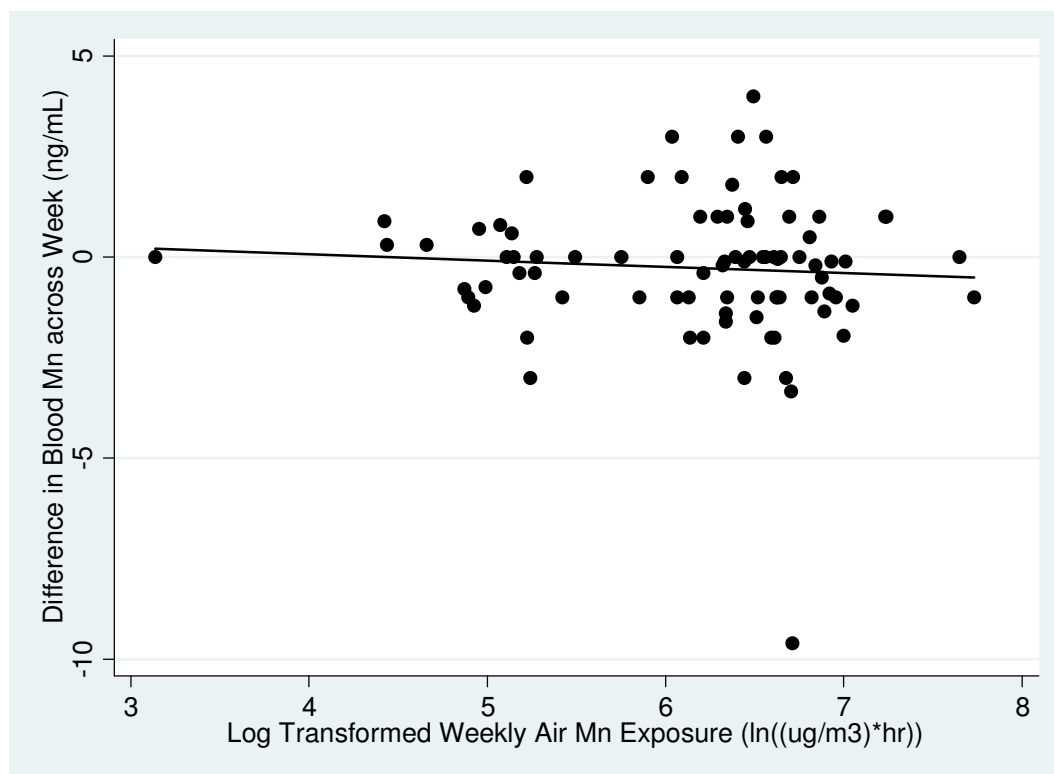


Figure 14a: Monday AM to Friday AM Mn Blood levels correlated to Predicted Weekly Mn Air Exposures from Monday through Thursday. (n=85, $p=0.34$, $r^2=0.01$)

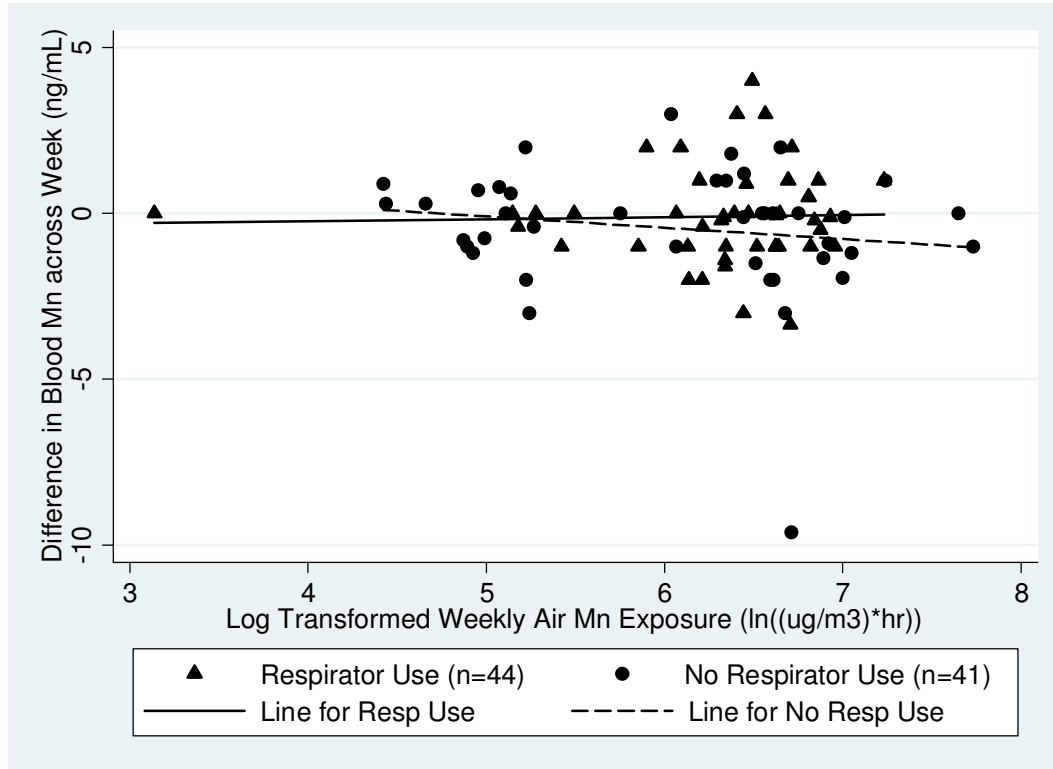


Figure 14b: Relationship between weekly (Monday through Thursday) air Mn exposure and blood Mn differences across week, grouped by respirator use.

Second, to look at cross-week blood Mn changes in a different way, we used the difference between Friday PM blood Mn and Monday AM blood Mn as the outcome variable. This was correlated to predicted air exposures summed from the beginning of the week through Friday (Figure 15a). This result was statistically significant ($p=0.03$) but shows a negative association between increasing predicted exposure and blood change across week. The association becomes less significant when subjects are grouped by respirator use (Figure 15b).

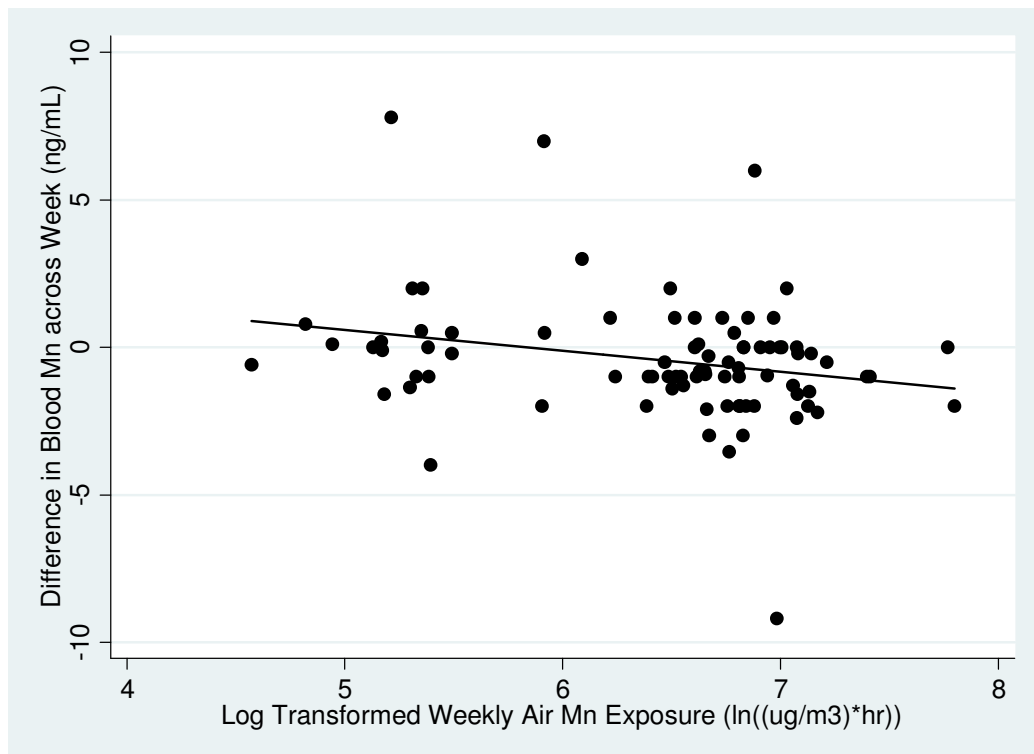


Figure 15a: Monday AM to Friday PM Mn Blood levels correlated to Predicted Weekly Mn Air Exposures from Monday through Friday. (n=84, p=0.03, $r^2=0.06$)

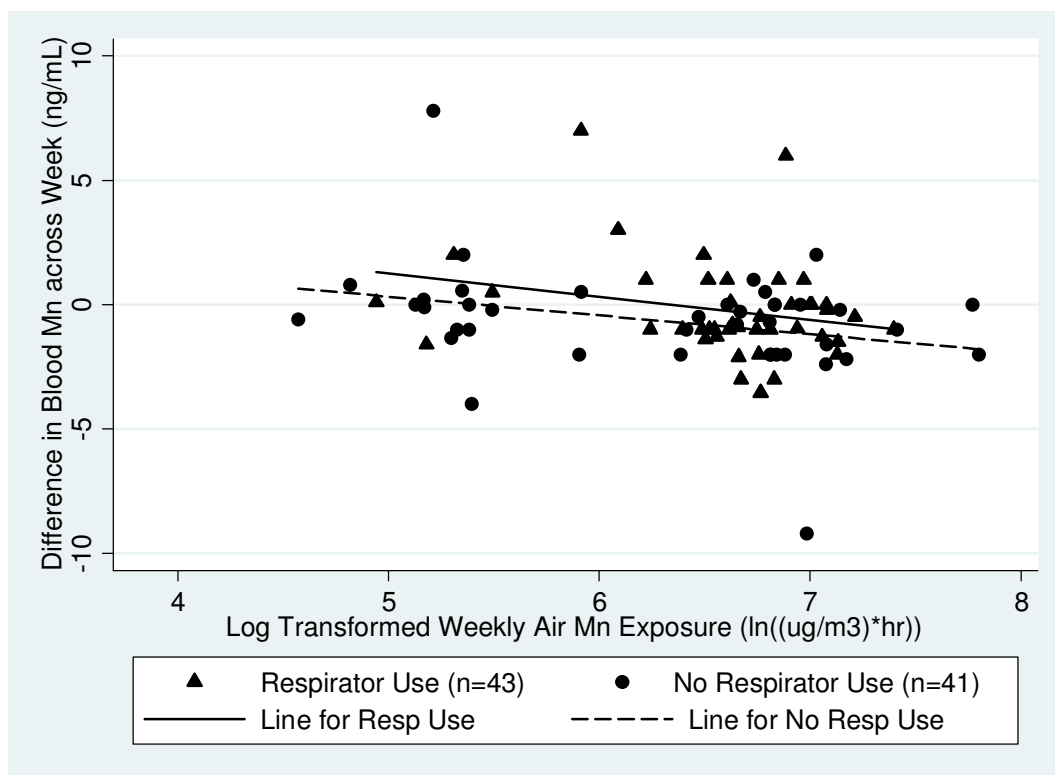


Figure 15b: Relationship between weekly (Monday through Friday) air Mn exposure and blood Mn differences across week, grouped by respirator use.

Similar diagnostics were used to assess the validity of the cross-week regression results as were employed for the cross-day results. One leverage point, with a blood difference of almost -10 ng/mL, seems to be influencing these results as well. When analyzed with this point removed, the slopes of the regression lines remain consistent and the results do not meaningfully change. Since no scientific reason was present for removing this data point, it was included in each analysis. A graph of the residuals from each regression model was viewed. Residuals appeared to be well distributed with no trends apparent. Further analysis included clustering the regression on subject ID. This again did not impact our results in a meaningful way. The above analyses were also performed on a native scale with non-log transformed exposure variables. The results were not scientifically different than the displayed results and were not included in this paper.

To better understand how Mn levels change over time we examined the relationship between cumulative air Mn exposure and blood Mn levels across quarter. Figure 16 shows a striking increase in blood Mn difference as estimated exposure increases. This association was significant when examined with robust linear regression ($p=0.036$, $r^2=0.13$), see Table 8. A residual plot showed no trends with relatively good distribution of data. Clustering on subject ID also did not influence results significantly. However, when the exposure variable was analyzed using the native scale the result became even more significant with a much tighter association (Figure 17, $p=0.003$, $r^2=0.24$).

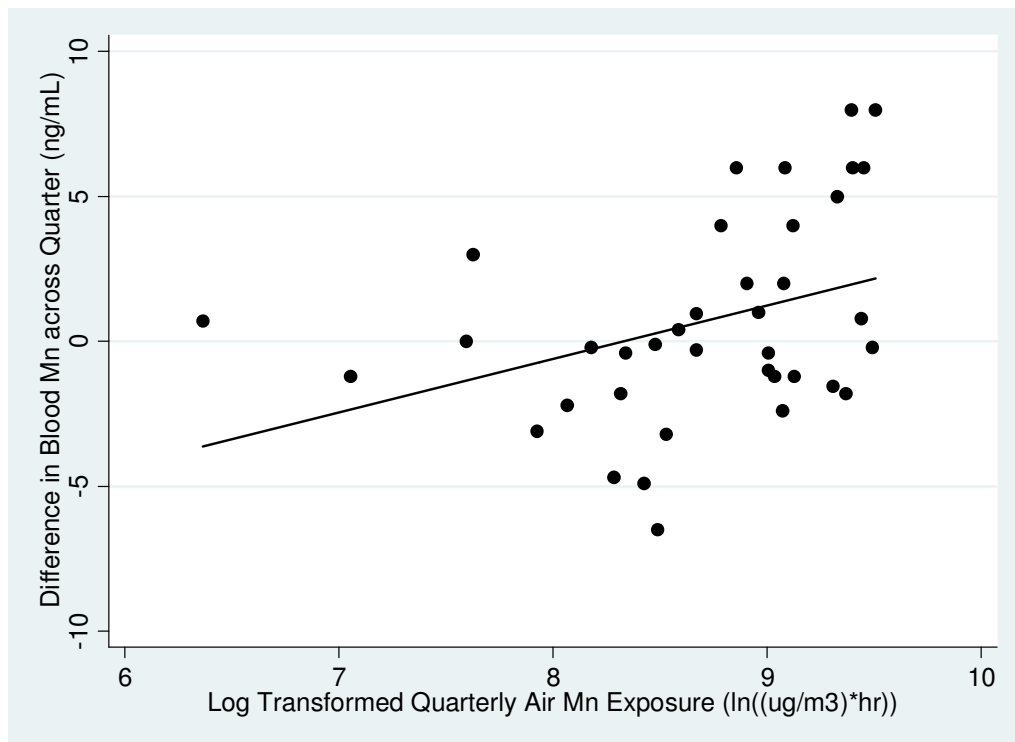


Figure 16: Cross-quarter association between blood Mn levels and quarterly air Mn exposure. (n=38)

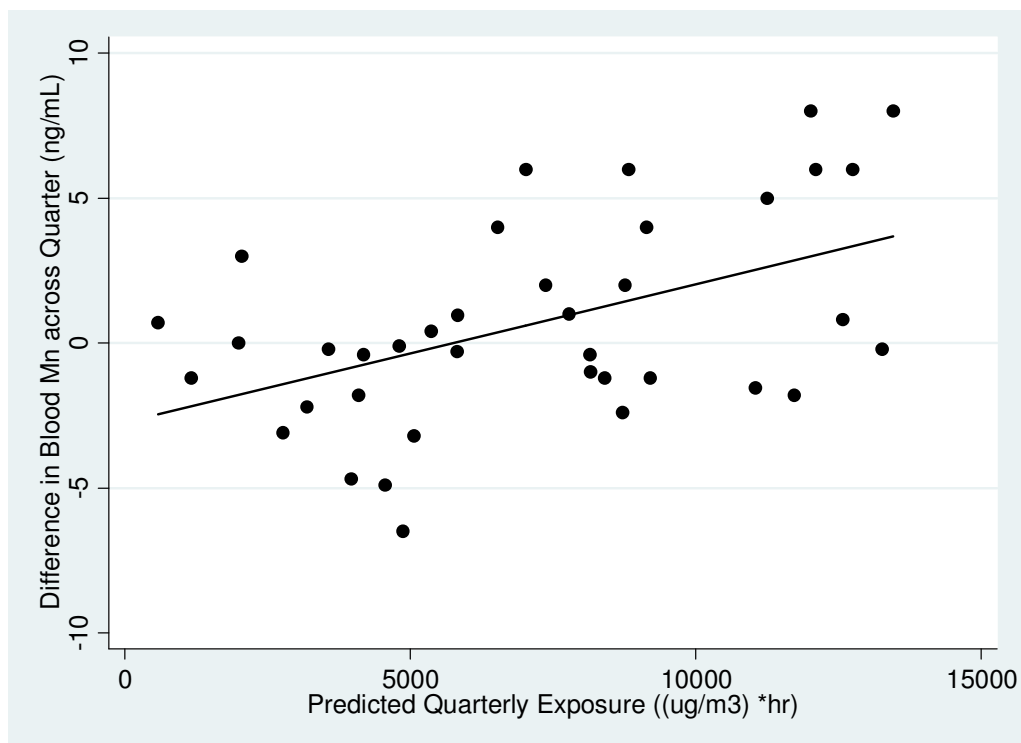


Figure 17: Cross-quarter association between blood Mn levels and quarterly air Mn exposure on native scale. (n=38)

Table 8: Linear regression results for Blood Mn and Air Mn associations. Seven samples were missing respirator use data.

Cross Day Analysis							
	N	Constant	Slope	Robust SE	95% CI	p value	r ²
All	175	0.51	-0.14	0.10	-0.34 - 0.07	0.19	0.01
Resp Use	67	-0.32	0.05	0.15	-0.24 - 0.34	0.73	0.00
No Resp Use	101	0.86	-0.24	0.17	-0.57 - 0.10	0.16	0.03
Cross Week Analysis, Monday AM to Friday AM							
	N	Constant	Slope	Robust SE	95% CI	p value	r ²
All	85	0.69	-0.15	0.16	-0.47 - 0.17	0.34	0.01
Resp Use	44	-0.49	0.06	0.16	-0.26 - 0.39	0.69	0.00
No Resp Use	41	1.62	-0.34	0.26	-0.86 - 0.18	0.19	0.03
Cross Week Analysis, Monday AM to Friday PM							
	N	Constant	Slope	Robust SE	95% CI	p value	r ²
All	84	4.16	-0.71	0.32	-1.36 - -0.07	0.03	0.06
Resp Use	43	5.95	-0.94	0.59	-2.14 - 0.26	0.12	0.06
No Resp Use	41	4.10	-0.76	0.41	-1.58 - 0.07	0.07	0.08
Cross Quarter Analysis, Beg Monday AM to End Friday AM							
	N	Constant	Slope	Robust SE	95% CI	p value	r ²
All (log scale)	38	-15.40	1.85	0.85	0.13 - 3.57	0.04	0.13
All (native scale)	38	-2.75	0.0005	0.0001	0.0002- 0.0008	0.003	0.24

Overall our data showed a negligible trend of higher exposures associating with decreases in blood Mn difference over short time periods (Table 8). Further analysis showed that when individual factors were controlled for, regression coefficients were unchanged for daily and weekly analysis. The individual factors examined were: sex, age, race, smoking status, and alcohol consumption. Likelihood ratio tests showed no significance for any of these variables when included in the above regression models for daily and weekly analysis. The main finding here is that estimated daily exposures summed across quarter associate significantly with changes in blood Mn levels across quarter (p=0.04 and p=0.003).

Discussion

The first aim of this thesis, to create a predictive model of air exposures, was completed and then used to fulfill the second aim, correlating air predictions to blood results. While the aims of this thesis were accomplished, the results were not highly significant and not all of them trended in our hypothesized direction: that blood Mn could act as a short-term biomarker of exposure. However, there were some results that had scientific meaning and confirmed parts of our hypothesis. First, in the air data, PM concentrations were strongly associated with Mn concentrations, Mn concentrations differed significantly between welding types, and Mn concentrations reflected our *a priori* assumptions of which types of welding would pose a more significant exposure risk. Our air results fell on the low end of reporting welding exposure (geometric mean of 0.034 mg/m³ compared to the range 0.04 to 2.2 mg/m³ for mean personal breathing zone concentrations previously reported[12, 18-26]), but were above the new TLV of 0.02 mg/m³ respirable.[16] Further, descriptive blood Mn results indicated that types of welding associated with higher air Mn exposure also had higher blood Mn levels, indicating some level of biomarker potential.

Perhaps most scientifically interesting is the positive association between predicted quarterly air exposure and difference in blood Mn across quarter. This confirmed our hypothesized result that increases in exposure would associate with increases in blood Mn as a biomarker of exposure. However, the result is not on the time scale we expected, as blood Mn is thought to be a short-term biomarker of exposure. Due to the time constraints of this project we were unable to examine this relationship more fully. Also, in terms of using blood Mn as a biomarkers of exposure, the association appears to be relatively weak ($r^2 = 0.24$). It should be

noted that these results correspond well to the results of previous studies and that blood Mn should be re-examined as possibly a long-term biomarker.

Therefore, from this study and the time periods used in analysis, blood Mn does not seem to be an effective short-term biomarker of exposure, in that it is poorly associated with air Mn exposure as analyzed here over the time periods one would expect for a short-term biomarker. Further, when an association was significant, the slope is of the opposite sign compared to expected results, failing the biological plausibility test (in the cross-weekly analysis). This indicates that blood Mn does not directly correspond to inhaled Mn as studied here on a short-term basis. This may be due to the study's limitations or other variables such as dietary Mn.

As a long-term biomarker, blood Mn seems to have some prospects. However, it is interesting to note that in the cross-quarter analysis, blood levels are still decreasing across quarter for lower exposures. Possibly, these students are coming from a higher exposure welding type and are thus seeing a decrease in exposure not captured by our time scale. Or, Mn might be experiencing competition with iron in the blood, thus as exposure to welding fume increases (welding fume contains iron), the increased amount of iron out competes Mn until Mn exposure reaches a threshold at which it begins to increase, despite competition with iron. Other explanations may also present themselves as the study continues.

This study included many features that may have limited our ability to gain good insight into the question of whether airborne Mn exposure is detectable in blood and the timescale for this relationship. Reporting bias on questionnaires likely impacted on our results. Subjects likely exaggerated welding time during the mid-week, but it was unfeasible for our research team to validate the numbers subjects reported. Subjects would sometimes forget to pick up their pumps

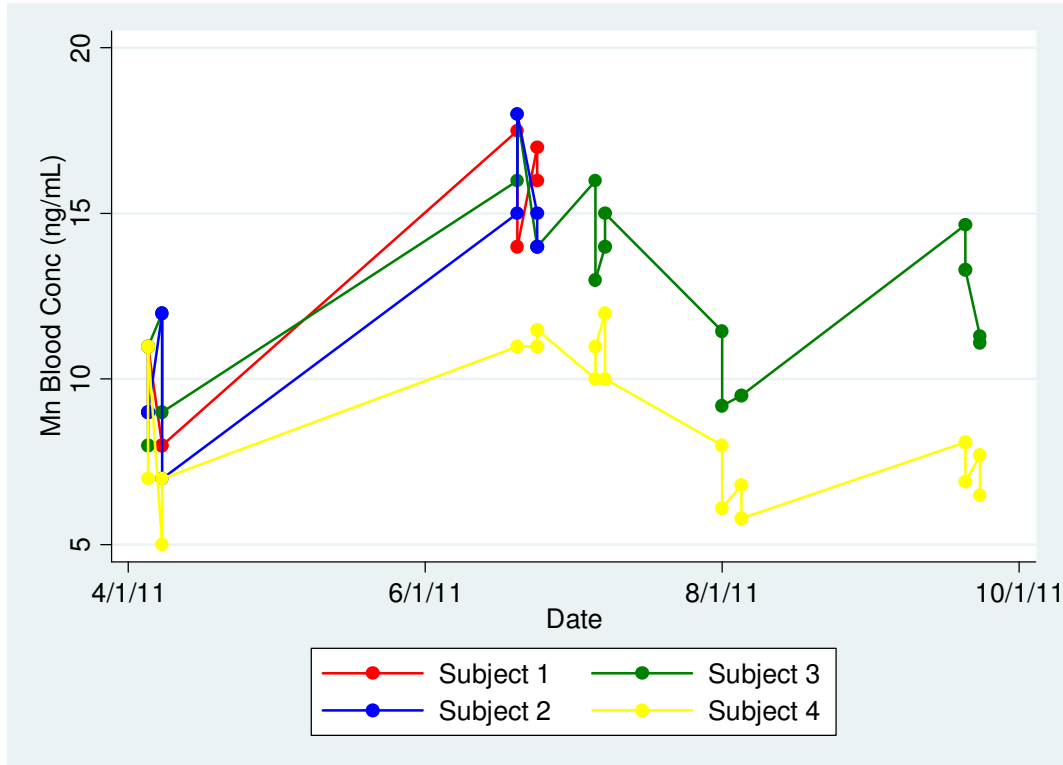
after lunch and begin welding again without their pumps, this exposure was then lost. Due to scheduling difficulties such as holidays and school finals, some weeks were not able to be scheduled Monday through Friday but were rather Tuesday through Friday, or even on one occasion, Wednesday through Friday. Another limitation of this study was significant missing data regarding respirator use. This lost data may have clarified some of the results that were grouped by respirator use. Small sample size was also a limitation for certain welding types, this limited the prediction model in that we could not estimate exposures using the model for four different welding categories. Further, some subjects had very limited exposure data, more samples per subject could have added stability to our model and affected the results of the later analysis.

Throughout the regression analysis, different diagnostic tools were employed to assure valid results. Overall, we have confidence that our results are scientifically meaningful and that possible issues such as high leverage points, missing data, and correlated data have not impacted our results in a significant fashion as reported in the results section.

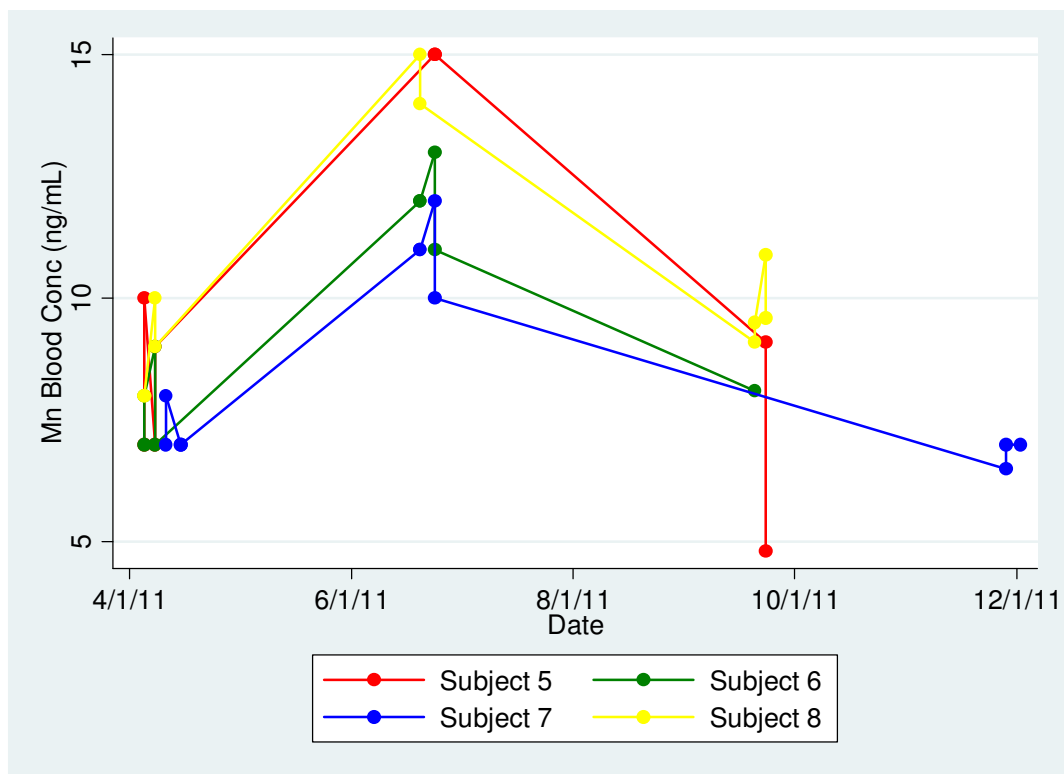
More analysis needs to be done looking at cross-quarter blood Mn changes as those may be promising based on the analysis shown above and longitudinal graphs included in Appendix A. Particular attention should be paid to the type of welding being performed during each quarter, and which quarter a student is in, during analysis. It would also be interesting to analyze blood and air results based on different time periods of lag between the air exposure and the blood sample draw. Next steps should include investigation of other Mn biomarkers such as hair and toe nails, which may be more stable and thus easier to associate with air exposure.

Appendix A

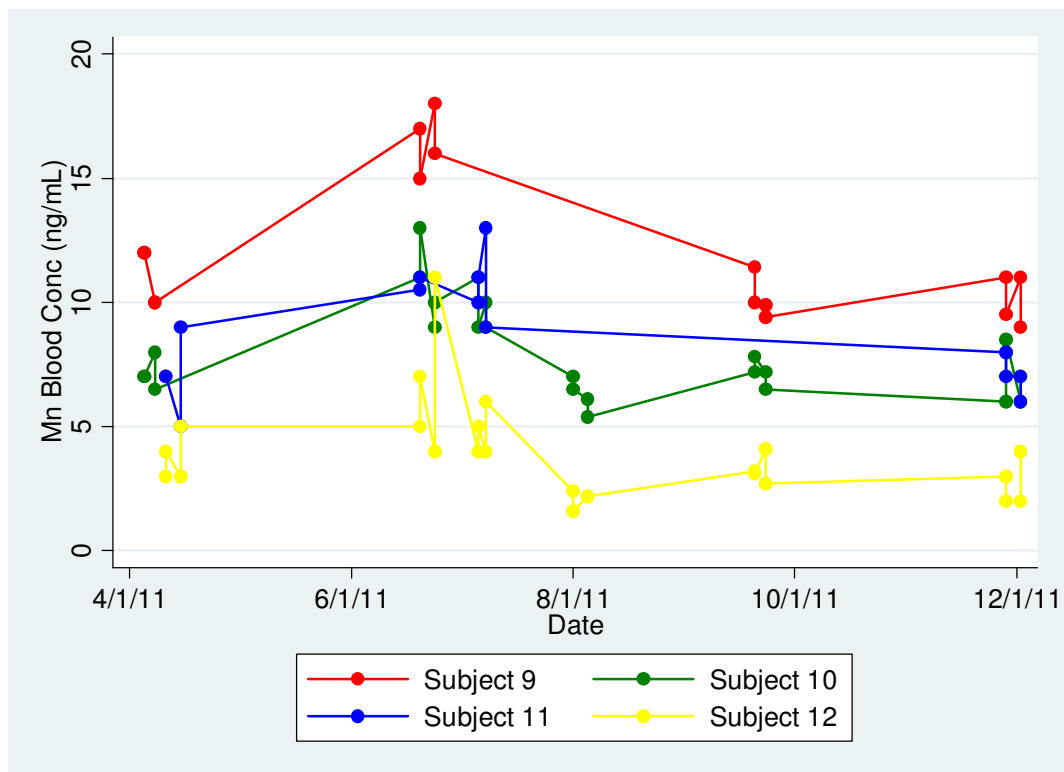
Below are graphs of longitudinal blood Mn data by subject with four subjects per graph to increase readability. It appears that there might be a general trend of increasing blood Mn during the first quarter a subject is in our study. However, since subjects entered into our study at different points in their program this may not correlate directly with an increase in blood Mn during their first quarter of study. To read these graphs: every group of ~4 data points represents a sampling week, sampling weeks occur at the beginning and end of each quarter. One can roughly assume that there is a quarter of exposure between the first and second group of dots, between the third and fourth group of dots, etc. It should be noted that summer quarter is significantly shorter than the other quarters and that there is a large summer break between summer quarter and fall quarter.



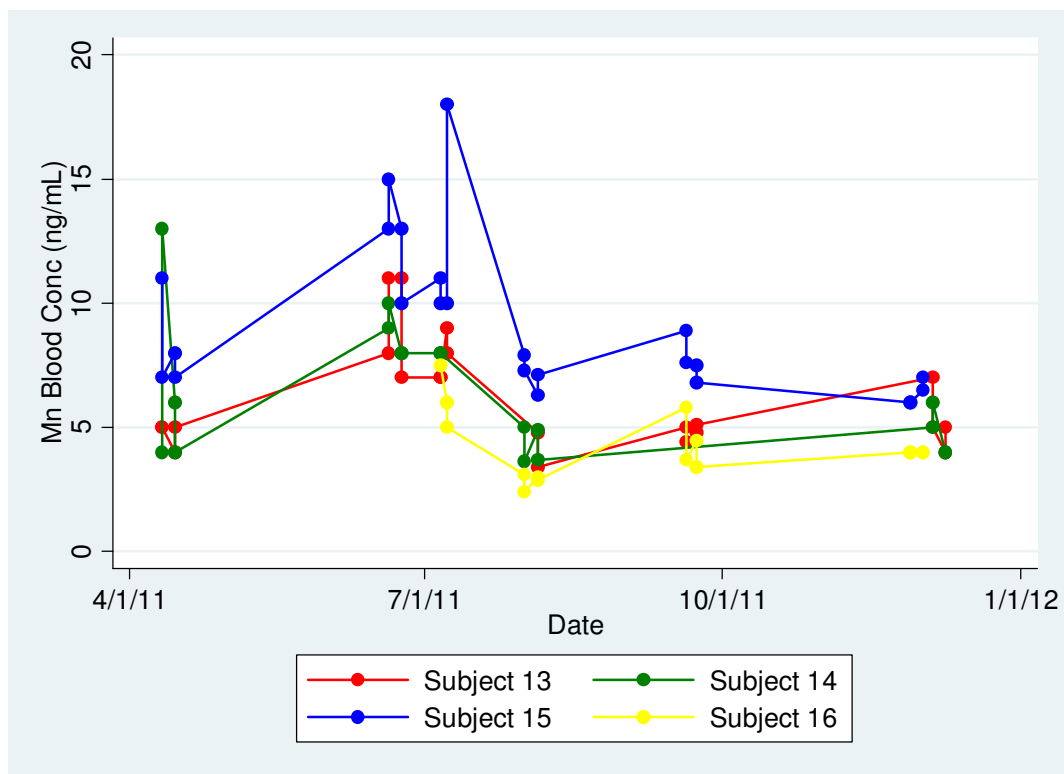
Longitudinal graphs of Mn blood concentrations over time by subject.
Subjects 1-4



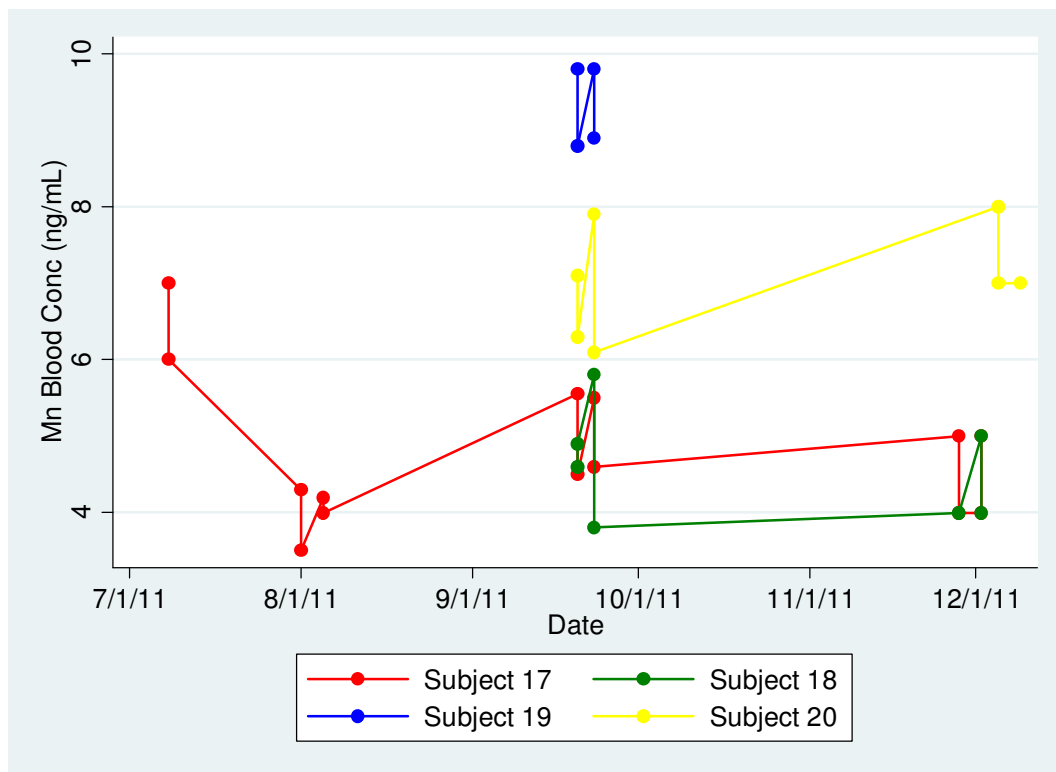
Subjects 5-8



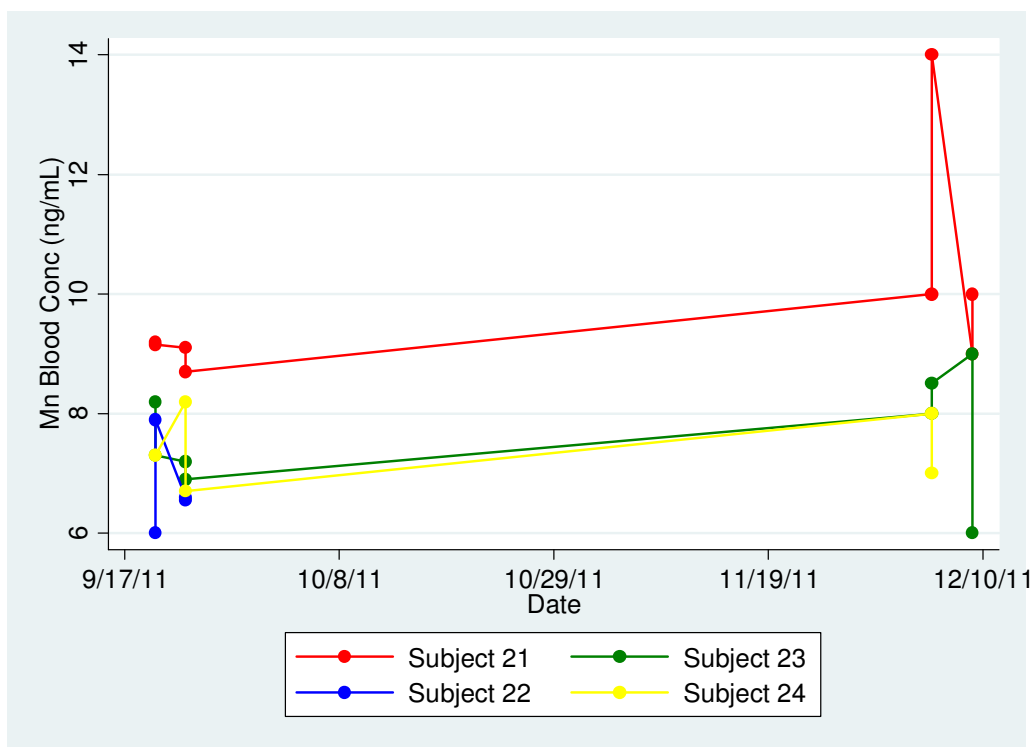
Subjects 9-12



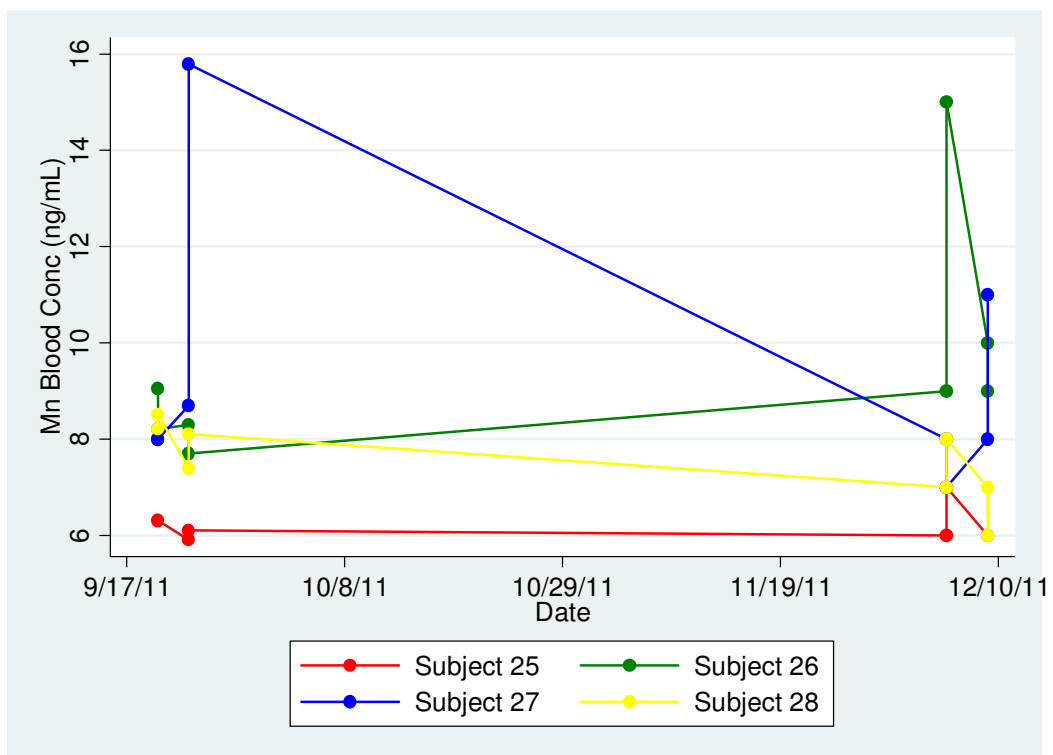
Subjects 13-16



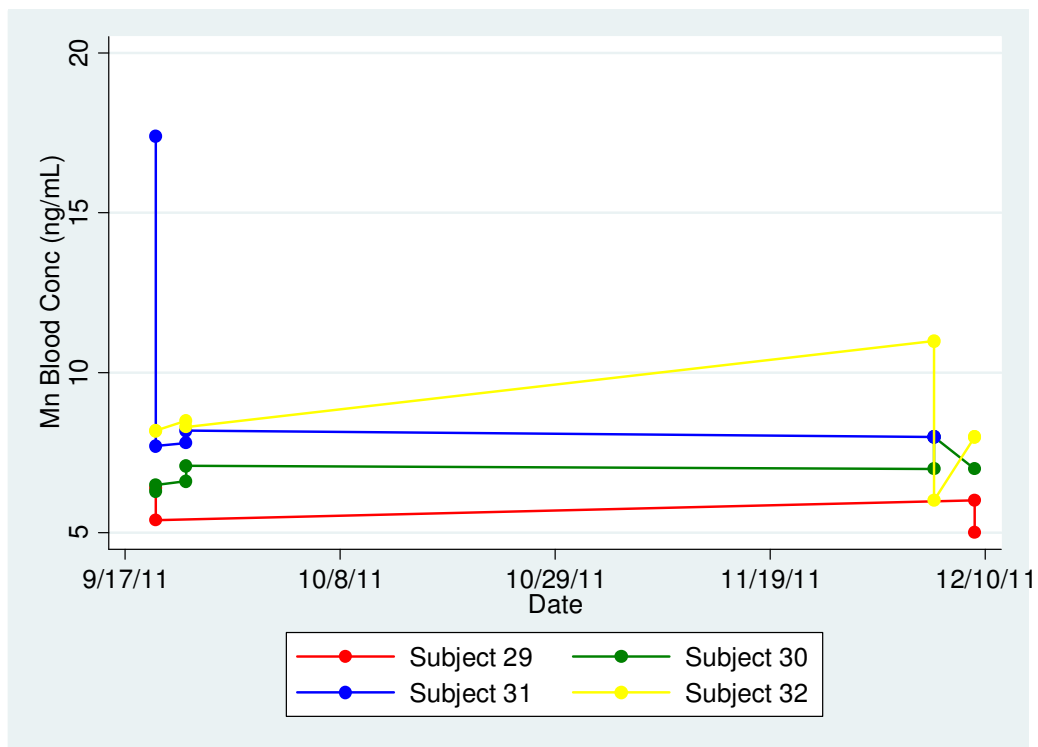
Subjects 17-20



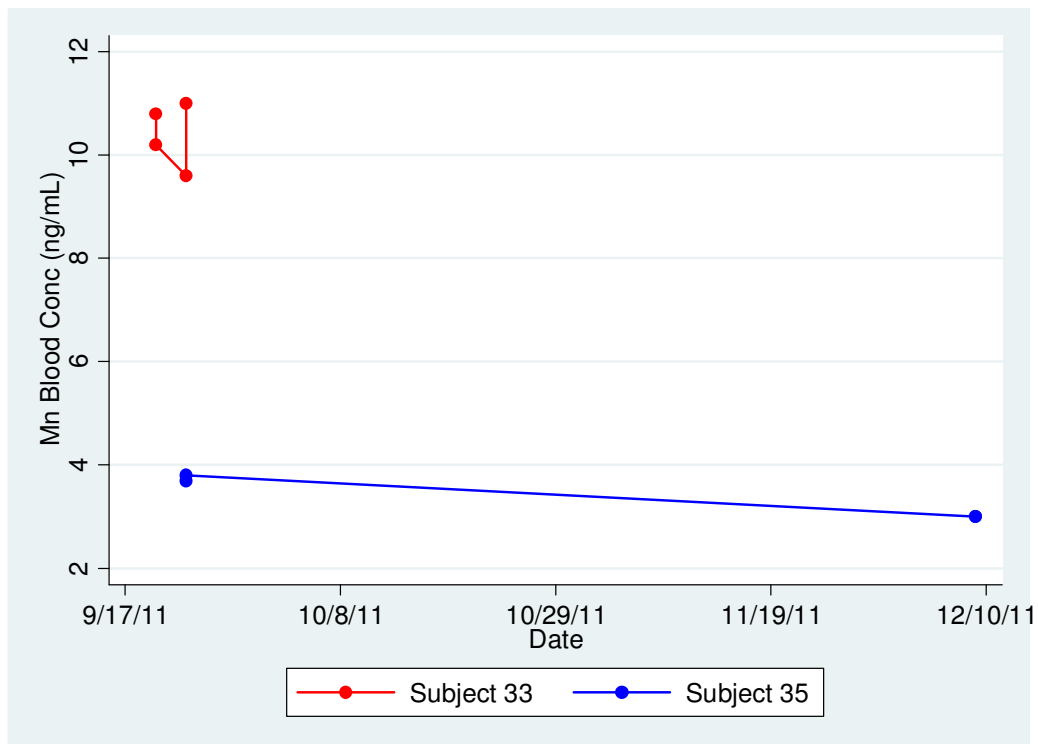
Subjects 21-24



Subjects 25-28



Subjects 29-32



Subjects 33 and 35 (34 was omitted from the study before accumulating any data)

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