# UNIVERSITY OF CINCINNATI

**Date:** May 27th, 2008

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**hereby submit this work as part of the requirements for the degree of:**

Master of Science

**in:**

Environmental Health

**It is entitled:**

Postural instability and chronic low level manganese exposure:
A cross-sectional pilot study of residents in Marietta, Ohio

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**Postural instability and chronic low level manganese exposure:**

**A cross-sectional pilot study**

**of residents in Marietta, Ohio**

A thesis submitted to the

Division of Graduate Studies
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in the Department of Environmental Health
of the College of Medicine

2008

by

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**Objective:** To evaluate postural balance and biological markers of a population chronically exposed to low levels of ambient manganese (Mn). **Methods:** Hair and blood sampling and postural balance testing were performed on participants residing near a ferromanganese refinery. The relationship between blood and hair Mn measurements and postural balance was analyzed with logistic regression. Postural balance of residents was compared with control data by analysis of covariance. **Results:** A significant correlation between hair Mn and postural balance was seen in two test conditions. Blood Mn was not found to be significant. Five of eight postural balance measures were significantly larger when compared with controls. **Conclusion:** Preliminary findings indicate a significant relationship between hair Mn and postural balance. The postural balance outcomes of our study suggest subclinical functional impairment of vestibular and proprioceptive pathways within this study population. These preliminary findings warrant a prospective study with a larger sample size.

**Acknowledgements**

I would like to acknowledge members of my thesis committee: Erin Haynes, Dr. P.H., Dr. Amit Bhattacharya Ph.D., Paul Succop, Ph.D, and Amy Rohs, M.D., M.S. for their support and guidance. I also gratefully appreciate the support of Cyndy Cox, Senior Research Assistant within the University of Cincinnati's Biomechanics and Ergonomic Research Laboratory. We would like to acknowledge Washington State Community College for use of their campus, Dr. Richard Wittberg for assistance with GPS data collection, and Stephanie Wessel for study coordination. A portion of this study was funded by a Career Development Award from the Center for Environmental Genetics at University of Cincinnati. Additional funding was provided by the National Institute of Environmental Health Sciences (NIEHS): 1R21ES013524-02R21, 5T32ES10957, and P30-ES06096.

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## 1. Introduction

Southwestern Ohio is currently home to the only ferromanganese alloy producer in the United States. Located a few miles outside Marietta, Ohio, the refinery has been in operation under different ownership for over fifty years. Local residents have become increasingly concerned about Manganese (Mn) emissions from the refinery. Their concerns have been supported by Environmental Protection Agency (USEPA) Risk-Screening Environmental Indicators (RSEI) using 2002 toxic release inventory (TRI) data. Combining regional population size, emission volume with chemical characteristics, emissions from the refinery were estimated to pose the greatest health risk from air pollution for Ohio residents.[1]

Manganese is a naturally occurring metal as well as an essential trace element. While necessary for metabolism and bone development, chronic inhalation of excessive air manganese concentrations can lead to neurotoxicity. This has been well documented within occupational studies with total Mn dust concentrations approximating 1 $mg/m^3$.[2-4] The health consequences of low level Mn inhalation exposure in a non-occupational environment remain unclear. Additionally, the reliability of biological markers as a measure of Mn exposure, internal dose or as a predictor for neuromotor change has been inconsistent. This has encouraged an increasing number of studies to find utility in neuropsychological testing, upper motor coordination and magnetic resonance imaging (MRI) in the evaluation of Mn associated neurotoxicity.[5-8]

Postural balance testing has proven useful in identifying subtle neuromotor abnormalities secondary to exposures of jet fuel[9], solvents[10], alcohol[11], pesticides[12] and lead[13, 14]. Several occupational studies have utilized postural balance testing in the

context of Mn exposure.[4, 8, 15-18] Only one previous study has evaluated the influence of chronic non-occupational Mn exposure and postural balance.[19]

This pilot study first evaluated the relationship between hair and blood Mn measurements with measures of postural balance and residential distance from the refinery. We then evaluated the postural balance of residents by making two comparisons. First, the postural balance of residents living near the refinery was compared to residents residing farther away. Second, postural balance measurements of residents were compared with measurements from an unexposed control group.

## 2. Materials and methods

### 2.1. Subjects

This cross-sectional study evaluated postural balance and biological samples of Marietta area residents who met specific inclusion and exclusion criteria. This study was approved by the University of Cincinnati's Institutional Review Board (IRB) (06-08-22-02). The study's procedures, risks, and benefits were explained both verbally and in writing to all residents, who then provided written informed consent prior to their participation. Residents were identified from a community profile survey distributed through the local organization Neighbors for Clean Air.

Inclusion criteria required the primary residence of each resident to be within a ten mile radius of the refinery for a minimum of three consecutive years. A distance of 10 miles from the refinery was used in an effort to maximize subject participation by including southwest Marietta. The current residential address of each resident was

confirmed by a global positioning system (GPS). It was also necessary for each resident to complete a self-reported medical history for screening of neurological disorders.

Exclusion criteria were chosen to minimize covariates that would affect postural balance based on the presence of occupational exposure, neurological issues or subject discomfort. Any resident with a known history of occupational Mn exposure was allowed to undergo testing but ultimately excluded from the statistical analysis. Any resident with a history of Meniere's or Parkinson's disease, multiple sclerosis, blindness, stroke, seizure or upper respiratory or inner ear infection within two weeks of testing was excluded from statistical analysis. Those with difficulty standing unaided for at least three minutes or those having pain and discomfort from standing for a short time were not permitted to undergo testing.

Thirty two residents completed balance testing with three residents eventually excluded from all statistical analysis. Two male residents were current or past employees of the refinery. An additional female was excluded as her measures of postural balance under multiple test conditions and hair Mn revealed outlying values. While there was no known diagnosis of diabetic neuropathy in the resident, the potential for this condition to influence postural balance results led to her exclusion from all statistical analysis. The residential distance from the refinery could not be confirmed by GPS for a third female resident leading to her exclusion from analysis involving this variable. Two residents declined to complete bending test conditions due to concern of previous back injury leading to their exclusion from analysis of bending test conditions.

## 2.2 Postural balance testing

Postural balance is a complex function controlled by central and peripheral nervous systems. An upright posture requires an integration of sensory inputs to the brain with motor outputs directing body musculature for proper coordination. Visual, proprioceptive and vestibular pathways provide sensory information relevant for maintaining postural balance. Postural balance testing is a noninvasive and sensitive method to evaluate the integration of these pathways.[20, 21]

Postural balance data was collected using a hall-effect type force platform system, Model ACS-110, Advanced Mechanical Technology Inc. (AMTI), Watertown, MA. As residents stood quietly on the platform, six signals were measured: Fx (force in the horizontal plane perpendicular to the posterior-anterior axis of the resident), Fy (force in the horizontal plane parallel to the posterior-anterior axis of the resident), Fz (force downward, perpendicular to the horizontal plane), Mx (moment about the Fx axis), My (moment around the Fy axis), and Mz (moment around the Fz axis).[10, 22] The three orthogonal forces and three moments created by each resident were captured at a frequency of 50 Hz. The recorded forces and moments were amplified and processed by our custom software, ("Kinelysis" Copyright All Rights Reserved, University of Cincinnati) allowing calculation of x-y coordinates corresponding to the resident's center of pressure (CP). The recorded data was used to calculate the sway length and sway area.

The CP of each resident naturally travels as postural balance is maintained during each test condition. The total sway length (SL) is the distance traveled in cm by the CP during each test condition. The total sway area (SA) includes the area in $cm^2$ enclosed inside the perimeter outlined by the CP movement pattern within the horizontal plane.

All residents underwent a 30 second trial for four different test conditions: (EO): eyes open on the platform, (EC): eyes closed on the platform, (FO): eyes open on a 4 inch thick foam pad placed on the platform and (FC): eyes closed on a 4 inch thick foam pad placed on the platform. These tests were then repeated in reverse order. The repeated measures for each test condition were averaged together for a mean value for SL and SA.

The testing protocol was developed to indirectly challenge or negate three afferent sensory pathways used in maintaining postural balance (Table 1). During the EO condition, the body utilizes all three sensory afferents: visual, proprioceptive and vestibular pathways. With testing in the EC condition, the visual pathway is removed subsequently increasing dependence on proprioceptive and vestibular pathways. The FO condition incorporates standing on foam to challenge proprioceptive input emphasizing use of visual and vestibular inputs. The FC condition impairs both visual and proprioceptive inputs forcing maximum reliance on vestibular function.

Residents denying a history of back injury or recent back discomfort also performed additional semi-dynamic bending testing (Table 2). The first condition (BO) requires standing on the platform with eyes open for 12 seconds and then bending forward at the waist on verbal command. The forward bending position is held for 5 seconds until on verbal command he or she quickly returns to the upright standing position for the remainder of the 30 second test. This test actively challenges all three sensory inputs. The second condition (BC) places greater challenge on vestibular and proprioceptive pathways. The BC test condition uses the same method as the BO test condition with exception that the resident's eyes remain closed throughout the test. Each bending test condition was only completed once.

With exception of three residents who were tested at the University of Cincinnati campus, all residents were tested over a two day period in Marietta, Ohio during October 2006. Daily set up for balance testing followed a well established and standardized protocol to maintain identical test conditions for all residents. A standard 18 kg weight was analyzed on the platform at five specific locations prior to daily testing of residents to confirm calibration of equipment. These measurements were required to locate the CP of the standard 18 kg weight within < 2% error for postural balance testing to proceed.

Each resident was instructed to stand on the center of the platform without socks or shoes. With heels placed together, feet were separated 30º around a triangular wood block placed between the feet. A tracing of the feet was then made onto a thin sheet of white art paper overlying the platform. This tracing was taped to the platform to serve as a guide for consistent foot placement. Between residents, both coordinators confirmed the identification number and reviewed measurements of the previous resident for any possible errors. A standardized calibration using the 18 kg weight periodically tested the platform between residents to identify any unacceptable platform variability.

2.3 Blood and hair sampling

Following postural balance testing, Mn levels were directly measured for each resident in whole blood and hair. A certified phlebotomist collected whole blood samples utilizing single-use syringes for venipuncture and techniques to ensure minimal extraneous lead contamination. A clean alcohol pad was used to wipe the top of all blood tubes. Skin preparation utilized an alcohol pad working outward in a circular fashion from the estimated point of venipuncture. The area was then dried with clean gauze.

These steps were repeated prior to specimen collection. A vacutainer system was used to collect approximately 7 ml of whole blood into two 4 ml purple top tubes containing ethylenediamine tetraacetic acid (EDTA). The second tube was used for metal analysis due to possible manganese contamination from use of a steel needle. The first blood draw was used for DNA extraction, if the resident provided consent. Blood tubes were immediately inverted 7 to 10 times in order to mix whole blood with EDTA. Blood tubes were labeled with resident identification and placed into a refrigerator maintained at 4º Celsius.

Previous studies have observed hair analysis as a reliable indicator of exposure with concentrations within different segments of hair strands reflecting the time history of exposure.[23] The proximal aspect of hair is thought to provide a more accurate measure of body burden. Prior to analysis, all hair samples were washed with nitric acid and the collection table was cleaned with a Sani-Cloth® germicidal disposable wipe. For hair analysis, 10 mg from each sample were dissolved in 1 ml 65% nitric acid and kept at 90ºC for 60 min. The lysate was then equilibrated for 24 h at room temperature, diluted with 4 ml of double-distilled water and analyzed by atomic absorption spectroscopy (AAS).[24]

Blades of ceramic scissors were cleaned with an alcohol wipe. Hair collection was taken from the posterior vertex of the head. Gloves were worn throughout the specimen collection protocol. A minimum number of 20 hairs and length of 1 cm was attempted. Using a paper tape measure, hair length measuring ≤ 12 cm was cut as close to the scalp as possible. For hair length measuring ≥ 13 cm, hair was measured 12 cm from the scalp, cut and then discarded. The remaining sample was then cut as closely to the scalp as possible. Hair samples were taped appropriately onto an index card labeled to properly identify the scalp end of the sample and resident identification. Samples

found to be too small to tape were collected in pre-labeled envelopes. Hair samples were maintained at room temperature. A standard hair survey was administered to collect a history of use of hair care products and hair dye.

All blood samples were continually refrigerated at 4º Celsius from collection until laboratory analysis. As with previous studies, blood lead levels were measured using an ESA model 2014 Anodic Stripping Voltammeter with strip chart recording.[25] All sample analysis was performed by the Hematology and Environmental Laboratory at the University of Cincinnati Department of Environmental Health following previously validated methods.[24, 26]

## 2.4 Independent and dependent variables

Independent variables for the study included measurements of blood Mn, hair Mn, blood lead and distance in miles between the resident's home address and the refinery (DISTANCE). Dependent variables were mean values for the measured total sway length (SL) and total sway area (SA) for the four postural balance test conditions. With the two bending test conditions being measured once, the reported resident SL and SA for these conditions represented a single test measurement and not a mean value. Consistent with previous studies, measurements of SL, SA, hair Mn, blood Mn and blood lead were natural log transformed to form a normal distribution for statistical analysis.[12, 19]

## 2.5 Covariates and confounders

Previous studies have identified covariates beyond the study exclusion criteria possibly affecting either sway length or sway area.[9, 10] To control for these and identify

neurologic disorders, each resident completed a detailed questionnaire documenting basic demographics as well as medical, social and occupational history. Covariates included within the analysis were resident age, weight, height, gender, alcohol intake, caffeine intake and smoking. Alcohol consumption was documented as 12 oz. beer equivalents within 48 hours of testing and average number of drinks per week; caffeine consumption as 8 oz. drinks prior to testing; and tobacco usage as average number of cigarettes smoked per day. The height and weight of each resident was measured on the day of balance testing and combined into a height/weight ratio. These variables were evaluated for statistical significance within regression models and an analysis of covariance (ANCOVA).

Prior studies have associated lead exposure with abnormalities in postural balance and decreased iron levels.[13, 14, 27] Studies have also suggested iron deficiency to be associated with increased Mn absorption into the CNS.[28, 29] With excessive lead exposure influencing Mn absorption into the CNS and postural balance, lead exposure was anticipated to be a potential confounder. Lead levels were measured from blood samples and included in analysis and modeling.

2.6 Control data

Using data collected from a previous study[9], the SA and SL of residents were compared to those of a control group for four test conditions. Control data was collected in a previous study evaluating cumulative low-level exposure to jet fuel on aircraft maintenance personnel. Controls were considered unexposed in regard to occupational and environmental neurotoxicants and consisted of 25 volunteers from the military, the

University of Cincinnati and other sources. In this cohort social and occupational histories do not indicate any history of abnormal Mn exposure. To better match the age range of the control data, residents under the age of 20 and over the age of 59 were excluded from this specific analysis. All residents and controls with known consumption of alcohol within 48 hrs of testing or those drinking more than twice a week on average were excluded. These criteria led to the exclusion of three controls and one resident from this analysis. Demographic data for controls used in the statistical analysis are provided in Tables 3 and 4.

Control data collection followed the same testing protocol of the current study with the exception that bending conditions were not tested. Balance measurements for controls were collected on a precursor to our current platform system, Model # SP6-1-400, Advanced Mechanical Technology Inc. (AMTI), Watertown, MA. Control data was converted and processed by our software ("Posture Program" vs. 5.2. Copyright, All Rights Reserved, University of Cincinnati). Measurements for controls were collected and analyzed in the same method as this study enabling a valid comparison of both SA and SL between groups.

2.7 Statistical analysis

Any association between chronic non-occupational exposure to elevated Mn and postural balance abnormalities is currently unclear. Based on previous studies of postural balance testing, a estimated sample size of 31 subjects or residents would be necessary to measure an effect size of 0.25, with an alpha of 0.05 and beta of 0.20 (80% power).[9, 30] Statistical Analysis System (SAS) software was used for statistical analysis. Proc Means

was used in calculation of descriptive statistics. Proc Univariate was utilized to check the normality of dependent and independent variables using the Shapiro-Wilk normality statistic. Dependent variables of SL and SA as well as independent variables of hair Mn, blood Mn and blood lead were transformed to their natural logarithms for a normal distribution. Proc Corr was used to check test-retest reliability with Spearman correlation coefficients. Proc Corr was also used to find Pearson's bivariate correlation coefficients between SL and SA and independent variables as well as among independent variables. Associations reaching a statistical significance of $p \leq 0.10$ were considered a non-zero correlation. Potential confounders were defined as variables with a non-zero correlation with both dependent variables and independent variables. Covariates were designated as variables having a non-zero correlation with dependent variables only.

Proc Reg provided linear multiple regression analysis to determine the relationship between SA and SL and independent variables. Proc Glm was used in an analysis of covariance (ANCOVA). A collective comparison using the least square means (LS means) of SA and SL evaluated between residents subdivided by DISTANCE and between residents and unexposed control data. All calculations of Proc Reg and Proc Glm used a backward elimination method to systematically remove independent variables and potential covariates with the least predictive power until remaining variables all have a p value $\leq 0.10$. Within the final analysis of the linear multiple regression and the ANCOVA, any association with a p value $\leq 0.05$ was considered statistically significant.

## 3. Results

3.1 Descriptive statistics

The ages for all residents included in statistical analysis ranged from 19 to 68 years with a mean just above 50 years (Table 5). Gender distribution was slightly unequal with 18 females involved in comparison to 11 males. The mean distance from refinery to resident residence was 5.8 miles within a range of 0.3 to 9.7 miles.

Blood Mn measurements within our residents range from 4.2 to 21.7 μg/l with a mean of 9.4 μg/l (Table 5). The estimated range of mean daily ambient Mn exposure within this pilot study ranges between 0.10 to 2.0 $\mu g/m^3$.[31] Previous non-occupational studies have reported ambient Mn concentrations ranging from 0.003 to 5.86 $\mu g/m^3$ in Mexico [5] and 0.009 to 0.035 $\mu g/m^3$ in Southwest Quebec [19]. Blood Mn measurements for these studies ranged from 5.0 to 31.0 μg/l (mean of 10.16) and 2.5 to 15.9 μg/l (mean of 7.5) respectively. The estimated ambient exposure to our residents was relatively higher than the Southwest Quebec study and within the lower range seen in the Mexico study. The relationship between blood Mn measurements and ambient Mn estimates in our study is generally consistent with those found in other non-occupational studies.

Hair Mn measurements within our study ranged from 1.2 to 12.4 μg/g (mean of 4.4) (Table 5). There is relatively little published literature of non-occupational studies utilizing hair Mn as a biological marker. An environmental study evaluating the ingestion of water containing very high levels of Mn (mean 610 μg/l) found a mean hair Mn concentration of 6.2 ± 4.7 μg/g.[32] An occupational study evaluating Magnetic Resonance Imaging (MRI) in Mn dioxide exposed workers however did not find a significant association between ambient Mn and hair Mn concentrations.[6] Blood lead measurements of all residents ranged from 0.6 to 6.2 μg/dl (mean 1.8 ± 1.0).

Residents were encouraged not to consume alcohol within 48 hours of balance testing. Questionnaire data found 15 residents periodically consuming alcohol, with one resident consuming 3 drinks within the 48 hours prior to testing (Table 6). Of the residents consuming alcohol, the average number of drinks per week ranged from 0.3 to 1.5. Seven residents smoked cigarettes prior to testing. Eighteen residents consumed caffeine prior to testing. One resident consumed approximately 128 ounces of coffee prior to testing. Despite the impressive amount, the resident states this did not differ from his daily routine. His balance measurements all fall within one standard deviation of the group mean in all tests with exception of SL under the FO test condition (Table 7, Table 8 and Table 9).

Demographic and balance measurements for the three residents excluded from all statistical analysis are provided separately (Table 10, Table 11, and Table 12). In evaluating residents excluded from all statistical analysis, all three lived beyond a 4 mile radius from the refinery. Both workers stated consistent exposure to Mn while working at the refinery. A few months prior to the study, Worker A had retired from the refinery after 23 years of employment. His hair and blood Mn was significantly less than Worker B. Worker B had been employed by the refinery for approximately 30 years. He had not worked in several days prior to testing due to a labor dispute. His balance measurements were larger than Worker A under every test condition.

Resident A was a 42 year old female insulin dependent diabetic with a history of hypertension and high cholesterol. She had not eaten prior to her balance testing in the morning. However, her blood glucose levels were normal prior and following balance testing at 140 and 135 mg/dl respectively. While she did not have a known history or any

complaints of diabetic neuropathy, her balance measurements were larger than the group mean under all test conditions. Her blood lead levels were below the mean for residents included in the analysis (Table 5 and Table 10). In contrast, her hair and blood Mn levels were well above mean resident values at 41.40 μg/g and 15.60 μg/L respectively. With consistent outlying values higher than the rest of residents, Resident A consistently skewed group mean estimates to higher values. Without a clear explanation for her elevated measurements and potential for undiagnosed diabetic neuropathy, Resident A was excluded from all statistical analysis.

3.2 Bivariate correlations within independent and dependent variables

Within dependent variables, correlation coefficients for duplicated test conditions were 0.93 for SL and 0.78 for SA. This is consistent with previous studies and supports acceptable reproducibility for sway variables.[9, 10] Within independent variables, correlation coefficients do not suggest a significant association between DISTANCE and hair Mn or blood Mn (Table 13). The only relationship among biological markers measuring Mn found to be significant was between hair Mn and blood Mn.

3.3 Bivariate correlations between independent and dependent variables

A level of statistical significance was reached between hair Mn concentrations and SL in both EO and EC test conditions (Table 14). Bivariate correlation coefficients between SA and SL and biological markers suggest hair Mn to be positively correlated with all sway variables with exception of SA in the bending test conditions. The only significant association between DISTANCE and postural balance was found in SA under

the FO test condition. While measures of SA and SL were expected to decrease as DISTANCE increased, the association between DISTANCE and SA under the FO test condition was found to be positive. Considering this was the only significant association involving DISTANCE, it appears that there was no consistent relationship between DISTANCE and postural balance within our study population.

Blood lead was significantly correlated with SL in all test conditions with exception of the BO test condition which reached a p value of $\leq 0.07$. Several previous studies have also found a strong association between blood lead and postural balance abnormalities.[13, 14] The strong consistency between blood lead and SL reproduced in this study supports the validity of this pilot study. A recent occupational study using a benchmark approach has estimated a new lower threshold for blood lead levels to be associated with postural sway changes in adults.[33] The 95% confidence limits of their new lower threshold estimate are 12.1 to 17.3 μg/dl (mean 14.4). None of our residents described a significant source of lead exposure and all had blood lead levels well below this new lower threshold estimate. Regardless, blood lead levels were included as a covariate for adjustment when significant within the regression model for the association between hair Mn and postural balance.

3.4 Regression analysis between hair Mn and dependent postural balance variables

Multiple linear regression analysis was used to further evaluate the relationship between hair Mn and SL and SA under all test conditions (Table 15). Initial linear regression models for SA and SL, with b0 to b8 as the regression coefficients, were the following:

Natural logarithm of sway dependent variable = b0 + b1 (Natural logarithm of hair Mn) + b2 (Gender) + b3 (Height/Weight) + b4 (Age) + b5 (Natural logarithm of blood lead) + b6 (Caffeine) + b7 (Tobacco) + b8 (Alcohol).

A backward elimination method systematically removed independent variables and potential covariates with the least predictive power until remaining variables all have a p value $\leq 0.10$. Hair Mn was forced to remain in the model regardless of its p value. Covariates remaining after backward elimination were identified as cofactors of the regression model. As SA and SL were expected to increase with increased exposure, a one-tailed alpha of 0.05 was used for statistical inference.

Within the regression analysis, hair Mn reached statistical significance with SA under EO and EC test conditions. The relationships between hair Mn and SL under EO and EC test conditions were near significance (Table 15). Hair Mn levels also approximated statistical significance with SA under the FO test condition; however the coefficient of determination within this model suggested considerable inherent variability.

3.5 Relationship between postural balance and residential distance from the refinery

A primary objective of the study was to evaluate for any differences in postural balance among residents. Recent modeling predicts residences found within 2 miles of the refinery to have the greatest ambient Mn exposure.[31] The estimates of exposure range from 0.10 to 2.0 $\mu g/m^3$ dependent on residential direction and distance from the refinery.[31] These concentrations were below levels seen in occupational case studies.[2-4] However, a recent study demonstrated an association between exposure to relatively

lower concentrations with abnormal hand position changes and the coordination of two consecutive hand movements.[5]

Assuming shorter distances represent relatively higher levels of exposure, we attempted to find an association between postural balance and DISTANCE. Bivariate correlations did not find a consistent association between the dependent variables of postural balance and DISTANCE (Table 14). An attempt was also made to collectively evaluate the relationship by subdividing the residents into two groups based on residential location. Ideally, residents with the anticipated highest levels of exposure would be compared to those with relatively less exposure. However, only three residents lived within a two mile radius of the refinery. To improve statistical power, a larger radial distance from the refinery was chosen to categorize residents. A distance of six miles was chosen to equally divide the study population into two equal groups of 14 residents (Table 16 and Table 17). As suggested by the bivariate correlations, no consistent difference was found between SA and SL of residents grouped by residential distance under EO, EC, FO and FC test conditions (Figure 1 and Figure 2).

3.6 Comparison between residents and controls

Different postural balance testing systems have developed sets of normative data, but there are no strict guidelines designating "normal" postural balance. Comparisons of postural balance between studies using different protocols should be made cautiously. But studies using the same protocol between various groups have been very effective at identifying subclinical neuromotor deficits that otherwise may not be detected.

All residents within our study were exposed to varying amounts of ambient Mn depending on their residential location and daily migration patterns. To collectively evaluate the residents, unexposed control data from a previous study was used for comparison.[9] A comparison of demographics shows our residents to have larger mean values in age and weight as well as a greater number of females (Table 18). The analysis of SA and SL with each test condition included the covariates of age, height/weight, gender, alcohol as 12 oz. beer equivalents within 48 hours of testing, tobacco usage as average number of cigarettes smoked per day, and caffeine consumption as 8 oz. drinks prior to testing. The initial regression equations with c0 to c6 as the regression coefficients, used in evaluating covariates in residents and controls were the following:

Natural logarithm of sway dependent variable = c0 + c1 (Age) + c2 (Gender) + c3 (Height/Weight) + c4 (Alcohol) + c5 (Tobacco) + c6 (Caffeine).

The natural logarithm of blood lead was not included as a covariate within this analysis. As described earlier, the potential for lead exposure to be a confounding variable was anticipated. Lead analysis was therefore completed on blood samples of all residents as a measure of precaution. In the prior study which provided the unexposed control data, there was no evidence or personal history suggesting abnormal lead exposure. Subsequently, no blood measurements for lead were collected. As discussed earlier, the mean resident blood lead in our study was 1.8 μg/dl with a maximum of 6.2 μg/dl (Table 5). These levels are well below the estimated lower threshold of blood lead anticipated to affect adult postural sway testing.[33]

The covariates were systematically removed with backward elimination until all remaining variables had a p value $\leq 0.10$. Following adjustment for significant

covariates, the LS means for SL and SA were found to be larger for residents than controls under all tested conditions (Figure 3 and Figure 4). Levels of statistical significance were reached for SL under EO and FO test conditions and for SA under EO, FO and FC test conditions.

## 4. Discussion

### 4.1 Resident exposure

Manganese is found naturally within a wide range of foods including green leafy vegetables, nuts, soybeans and oats. Within the soil, Mn does not exist as a pure metal but as a component within minerals. The most common Mn containing minerals include Mn dioxide, Mn carbonate and Mn silicate. Manganese ores are mined and smelted by furnaces to produce ferromanganese, an alloy predominantly used in production of steel. Specific Mn forms involved in ferromanganese production primarily involve manganese oxides. Particulate is generated from several activities within ferroalloy production. Within the production process, furnaces are found to be the largest potential source accounting for 94% of particulate emissions in the form of fumes.[34] Previous studies by the USEPA have characterized emissions from furnaces used in silicomanganese and ferromanganese production to typically range from 0.2 to 0.4 μm and 0.05 to 0.4 μm respectively.[35]

Direct exposure to Mn through food and water likely has limited to no role within this study. Practically all residents within this study limit water usage to the regulated city supply. While there are currently no federal standards for Mn within drinking water, a study sampling nine area residential cisterns did not suggest water to be a significant source of Mn exposure.[36]

There is reasonable evidence available to support ambient Mn exposure to be greater near the refinery than at locations farther away.[31] With limited data available, further characterization of exposure becomes increasingly presumptive. An accurate assessment of ambient exposure at the individual level would require the effort and expense of extended personal sampling. Within this study, differing daily migratory patterns among residents prohibit an accurate estimation of individual ambient exposure. And with over 50 years of refinery production, air monitoring today has limited utility in estimating the true duration, peak and frequency of long-term ambient exposure.

4.2 Manganese pharmacokinetics

Regulatory systems allow healthy adults to generally maintain proper manganese homeostasis without difficulty. The most significant and typical route of Mn exposure is diet intake normally ranging between 1 to 5 mg/day.[37] Almost 98% of ingested Mn is rapidly cleared by the liver and excreted as bile.[38] Passing through feces, bile serves as the major conduit for Mn elimination. A relatively much smaller amount is filtered from blood by kidneys into urine. Ingestion as a source of excessive body burden is typically isolated to cases of hepatobiliary failure or chronic consumption of extremely high Mn levels within drinking water.[32, 39] Almost 90% of study residents had been evaluated by a physician within the previous year and no residents listed a known history of liver disease.

Unlike ingestion, inhalation of Mn particles bypasses hepatobiliary elimination allowing for a relatively greater dose to reach the CNS. Animal models support that penetration of Mn into the CNS is three orders greater with inhaled Mn than by

ingestion.[40] Particularly important considerations influencing the alveolar absorption of ambient Mn include particle size and the specific chemical form of Mn. Particle sizes of 0.5 to 3 μm tend to deposit in lower airways and alveoli, in turn increasing the opportunity for alveolar absorption.[41] Animal studies suggest greater alveolar absorption and subsequent CNS absorption with Mn particles utilizing soluble sulfate groups.[28] Comparatively less absorption is seen with Mn particles having phosphates or tetraoxide.

The potential of direct olfactory transport is also unique to ambient exposure. Animal models have demonstrated direct transport of ambient Mn into the CNS by the olfactory system.[42, 43] However, the true toxicological significance of olfactory transmission remains unclear as evidence of neurological damage from olfactory transport has been inconsistent.[28, 44, 45]

4.3 Manganese neurotoxicity

Rodent and primate studies estimate the half life of Mn within the CNS to be approximately 50 to 75 days.[40] Prolonged periods with Mn intake exceeding elimination can lead to preferential deposition within the striatum and globus pallidus of the CNS. This has been well documented both within animal studies and occupational studies utilizing MRI.[6, 46]

Symptoms of early Mn neurotoxicity are nonspecific and may initially include headaches, malaise, sleep disturbances, and loss of appetite or sexual drive. Changes with behavior may also present with emotional lability, irritability or aggressiveness. Excessive exposure over long periods of time can lead to more advanced parkinsonian symptoms such as postural tremors or difficulties with speech, balance and gait.

4.4 Residential distance from the refinery as measure of ambient Mn exposure

The only parameter available within this study anticipated to potentially correspond with relative ambient Mn exposure was the proximity of resident's home to the refinery (DISTANCE). No consistent association was found between DISTANCE and dependent or other independent variables (Table 13 and Table 14). This was not surprising in light of the variability of resident time in their residence and limited accuracy of air modeling estimates. Daily migratory patterns of residents can vary widely potentially increasing or decreasing their relative frequency and duration of exposure. It may be assumed that a large share of a person's time is spent within their primary residence. However, the variability of daily migration for employment and other daily activities of life are difficult to quantify. This leaves exposure estimation based on residence to be difficult without individual air sampling.

4.5 Biological markers as a measure of ambient Mn exposure

Previous studies have attempted to use biological markers in different settings to quantify exposure and predict clinical progression. There is increasing support that chronic ambient Mn exposure leads to subclinical changes in neuromotor function. It is important that a sensitive measure be identified to quantify subclinical alterations in function and potentially predict a functional deterioration. In the context of ambient Mn exposure, it is unclear which if any surrogate can fill this inadequacy.

Extensive air monitoring can be an expensive and labor intensive endeavor. An inexpensive yet accessible biological marker would provide a better alternative as a

measure of exposure. The correlation between urine Mn and ambient Mn has been inconsistent. There is limited support that urine Mn measurements are capable of differentiating between workers and unexposed controls.[47] The utility of urine as a biological marker is limited to estimates of short-term exposure. And there are several studies suggesting urine as ineffective at reflecting ambient Mn exposure.[18, 24, 48] Considering the inconsistency of urine Mn in both occupational and non-occupational studies, analysis of urine Mn was not included in our study.

Many studies have evaluated for a correlation between blood Mn and ambient Mn exposure. As with urine Mn, blood measurements have also been successful differentiating between workers and unexposed controls.[24, 47] This success is not however widely supported at the individual level. The accuracy of blood Mn likely depends upon characteristics of the exposure and the time from exposure to sampling.[48] And at doses typically lower than the occupational setting, it is likely that individual variability limits the utility of blood Mn as a measure of personal exposure.

Hair as a biological marker is relatively more convenient and less invasive to collect than blood or urine. The reliability of hair analysis as a measure of ambient Mn exposure is unclear. A previous survey cites excessive variance, which may bring the accuracy of hair mineral testing into question.[49] Other factors thought to potentially influence the metal composition within hair include nutritional status, age, gender, hair color and hygiene.[24]

There are few studies specifically evaluating hair Mn as a measure of ambient exposure. The relationship between scalp hair and Mn dose resulting from ambient exposure has not been well studied. There is support that axillary hair Mn measurements

are useful in comparing groups of exposed and unexposed workers.[24] This correlation has been inconsistent and not supported at the individual level.[6, 24] There is preliminary evidence supporting an association between Mn concentrations in scalp hair and Mn exposure from water ingestion.[32, 50] The utility of scalp hair in the setting of ambient Mn exposure remains largely unstudied. A previous group based comparison did find greater scalp hair Mn concentrations in exposed workers in comparison to unexposed workers.[51]

Within our study there is no association suggesting a significant correlation between hair or blood Mn and ambient Mn exposure. Mean values for hair and blood Mn were slightly higher for residents living within 6 miles of the refinery when compared to those living farther away (Table 16 and Table 17). This did not however translate into a significant association between DISTANCE, our surrogate for relative ambient Mn exposure, and biological markers of hair and blood Mn. Ambient Mn concentrations within this study are elevated, but far below levels typical of occupational studies. It is possible that measures of blood and hair Mn do not strongly correlate with ambient concentrations at relatively lower exposure levels. However, this can not accurately be inferred from our study due to uncontrolled resident variability. As mentioned earlier, the daily migratory patterns of residents can vary widely potentially increasing or decreasing their relative frequency and duration of exposure. Blood Mn and hair Mn did show a statistically significant association with each other. This may lend support for hair Mn serving as a measure of internal dose.

4.6 Relationship between biological markers and neuromotor abnormalities

It is recognized that chronic inhalation of excessive Mn leads to absorption within the CNS subsequently causing neuromotor abnormalities clinically resembling Parkinson's disease.[52, 53] It remains unclear if any biological marker can consistently serve as a sensitive indicator of early neuromotor change. Concentrations of blood lead for instance have been strongly correlated with postural balance abnormalities.[13, 14]

Several occupational studies have considered the relationship between biological markers representing internal dose and postural balance abnormalities.[4, 15, 16, 18] A study of Japanese refinery workers found no significant correlation between blood and urine Mn concentrations and postural balance.[16] Another study evaluating mill factory workers also did not find a significant correlation between urine Mn and abnormal findings of postural balance.[4] More recently, a study selected and balance tested ten welders based on work history, symptoms and specific Unified Parkinson' Disease Rating Scale (UPDRS) criteria.[18] Significance was found between blood Mn and "mean sway" and "sway area" under conditions mirroring the EC, FO and FC within our protocol.

Only one prior study has evaluated the interaction between postural balance and blood Mn within a non-occupational population chronically exposed to low levels of ambient Mn.[19] The study involved a predominantly ambient Mn exposure of 306 people within Southwest Quebec. Exposure was primarily attributed to pollution from a Mn-alloy plant several years prior to the study and potentially from methylcyclopentadienyl manganese tricarbonyl (MMT) in automobile emissions. Air sampling from four areas estimated a mean total particulate Mn concentration of 0.022 $\mu g/m^3$ within a range of 0.009 to 0.035 $\mu g/m^3$. The mean blood Mn level was 7.5 ± 2.3 µg/l within a range of 2.5 to 15.9 µg/l. This study did not find a correlation between

blood Mn and postural stability in the overall or female population. There were however a few significant correlations between log transformed blood Mn measurements and postural balance in male subjects. During testing under eyes open and eyes closed conditions, the velocity of body sway in males decreased as blood Mn levels increased. It was also noted that as blood Mn levels increased, postural sway changed from an anterior-posterior direction to a more lateral direction.

The findings of the previous study were inconsistent with our hypothesis that increased Mn exposure would lead to greater measurements of postural balance. In contrast to the previous study, our study did not find a significant positive correlation between SL or SA and blood Mn under any test condition. Interestingly, the previous study also did not find a correlation when all residents were included in the analysis. The decrease in body sway velocity was found only when the population was stratified into male residents. Unfortunately, our small sample size lacked the power for an analysis stratified by gender.

No previous study has compared the association between postural balance and internal dose as measured by hair Mn. Of the 29 residents in our study, hair Mn analysis was completed on 23 residents. Laboratory analysis was not completed on 6 residents due to insufficient sample. And as mentioned previously, a single female resident was excluded from statistical analysis involving hair Mn based on her outlying value of 366 μg/g and recent use of a hair dying product.

Of the six postural balance tests performed, hair Mn was positively related to both SA and SL under four test conditions, with significant findings under two test conditions. Following adjustment for covariates, SA was significantly associated with hair Mn in EO

and EC test conditions (Table 15). As described previously, a few studies have found axillary hair to be inadequate as a biological marker of ambient Mn exposure.[6, 24] In contrast, a study looking at scalp hair did find a significant difference between measurements of workers in comparison to unexposed controls.[51] There are intrinsic differences between scalp hair and axillary hair that may influence their metal concentration such as differences in growth rate, thickness and activity of sweat and sebum glands.[54-56] The relationship between scalp hair and internal Mn dose from ambient exposure is unclear and inference from our pilot study is limited. But considering significant associations were found between hair Mn with blood Mn and several measures of postural balance, the need for further research is supported.

As discussed previously, an upright posture requires the integration of sensory inputs to the brain with motor outputs directing body musculature for proper coordination. Visual, proprioceptive and vestibular pathways provide the sensory information relevant for maintaining proper postural balance. As testing progresses through EO, EC, FO and FC test conditions, visual and proprioceptive inputs are selectively stressed placing greater dependence on other pathways (Table 1 and 2). This specific order of test conditions progressively challenges higher centers within the CNS. This increased difficulty is evident as SA and SL both progressively increase with modification of visual pathways (EO to EC & FO to FC) and proprioceptive pathways (EO to FO & EC to FC) (Figure 3 and Figure 4). The anticipated increases in SA and SL with the increasing challenge of progressive test conditions were comparable between residents and controls.

4.7 Comparison between residents and unexposed controls

Specific changes in either SA or SL can also provide insight into dysfunction of particular pathways. A study by Yasuda et al. has demonstrated that patients with vestibular dysfunction secondary to bilateral canal paresis had much greater SA than SL.[57] In contrast, patients with proprioceptive deficits due to severe decreased sensory vibration sensitivity were found to have much higher SL than SA. Within our study following covariate adjustment, the LS mean for SA were significantly greater for residents in comparison to controls under EO (100%), FO (50%), and FC (30%) test conditions (Figure 4). The LS mean for SL was also significantly larger for residents in comparison to controls under EO (3%) and FO (5%) test conditions (Figure 3). Under the test condition in which all sensory afferents were available for postural balance (EO), the exposed population had significantly poorer balance under both SA and SL. With the progression of test conditions, postural balance increasingly depends on higher centers within the CNS. The postural balance outcomes of exposed residents remained significantly greater for SA under the FO and FC test condition and SL under the FO test condition. And while three test conditions did not reach a level of statistical significance, the postural balance of exposed residents was greater than unexposed control data. Based on Yasuda et al, our study results suggest that prolonged exposure to ambient Mn may have an association with vestibular and/or proprioceptive functional impairment. The primary sites of ambient Mn deposition are the globus pallidus and putamen. And an important function of the basal ganglia is the integration of sensory feedback from the visual, proprioceptive and vestibular systems.[58] It is plausible that Mn

may interfere with the integration of sensory afferents within the CNS and/or the efferent motor response necessary to maintain proper balance.

## 4.8 Limitations

It is theoretically possible for iron deficiency to serve as a confounder between postural balance and ambient Mn exposure. Several studies have suggested iron status to play a role in the absorption of Mn.[27] And it may be assumed that severe iron deficiency and subsequent anemia could lead to abnormalities in postural balance. In reviewing the medical histories provided by each resident, no resident documented a recent history of iron deficiency anemia.

To be included in the study, residents must have lived within a ten mile radius of the refinery over the three previous consecutive years. This effectively focuses our evaluation to residents with a reasonable amount of chronic exposure. Chronic exposure does not however necessarily represent cumulative exposure. The majority of residents have lived in close proximity to the refinery their entire lives, albeit at different locations. But with limited historical data, the relationships between percentage of life at specific distances, exposure and actual absorbed dose will be difficult to determine. Without more accurate estimates of ambient exposure over an extended period of time, a valid estimation of cumulative exposure would be difficult.

Any study with voluntary non-random participant selection has potential for selection bias. All interested residents meeting inclusion and exclusion criteria were given an opportunity to take part in our study within the allotted testing period. With residents identified through a community survey, it is likely residents interested in

personal or community health would also be interested in participating in our study. It is noteworthy that the only information regarding balance testing given to residents prior or during the study was for the purposes of consent and the instructions necessary for completion of the protocol. No specific information regarding how postural balance was being measured or different test conditions was discussed with residents.

## 5. Conclusion

This study population provided the unique opportunity to evaluate the influence of low level chronic ambient Mn exposure on the postural balance of a residential population. This was the first study to evaluate the relationship between hair Mn and postural balance outcomes of SA and SL. Within the regression analysis, hair Mn reached statistical significance with SA under EO and EC test conditions. An association between blood Mn concentrations and postural balance outcomes was not demonstrated.

This was only the second study to evaluate the postural balance of a population chronically exposed to low levels of ambient Mn in a non-occupational setting. There were significant differences between residents when compared with unexposed control data. Following covariate adjustment, SA was significantly greater for residents in comparison to controls under EO, FO, and FC test conditions. Sway length was also significantly larger for residents in comparison to controls under EO and FO test conditions. These identified postural balance outcomes suggest subclinical impairment of vestibular and proprioceptive pathways. These preliminary findings warrant a prospective study with a larger sample size for a greater characterization of neuromotor abnormalities within this population.

**Table 1**

| Postural Balance Test Conditions | | |
|---|---|---|
| **Test 1 (EO)** | **Eyes open; Bare platform** | Evaluates collective effect of all 3 sensory systems controlling postural sway<br>***Tests visual, proprioceptive and vestibular systems.*** |
| **Test 2 (EC)** | **Eyes closed; Bare platform** | Visual system is removed.<br>***Tests the proprioceptive & vestibular systems***. |
| **Test 3 (FO)** | **Eyes open; 4-inch foam placed over the platform** | This test modifies the proprioceptive system.<br>***Tests the visual and vestibular systems.*** |
| **Test 4 (FC)** | **Eyes closed; 4-inch foam placed over the platform** | This test removes the visual system and modifies the proprioceptive system.<br>***Vestibular system becomes the primary control of postural sway.*** |
| **Tests 1 – 4 are then repeated once in reverse order** | | |

**Table 2**

| Postural Bending Test Conditions | | |
|---|---|---|
| **(BO) Bending Eyes Open Bare Platform** | 1) Stand for 12 seconds<br>2) Bend torso at the waist on verbal command<br>3) Stay in that position for 5 seconds<br>4) Return to upright position on verbal command<br>5) Stand for the remainder of the 30 second test | Dynamic challenge to visual, proprioceptive and vestibular systems |
| **(BC) Bending Eyes Closed Bare Platform** | Test Repeated with Eyes Closed | Dynamic challenge to the proprioceptive and vestibular systems |

**Table 3**

| Demographic data for controls included in statistical analysis | | | | | |
|---|---|---|---|---|---|
| Variable | Controls | Mean | Standard Deviation | Minimum | Maximum |
| Age (years) | 22 | 35.0 | 8.1 | 24.0 | 57.0 |
| Height (inches) | 22 | 69.8 | 13.8 | 52.4 | 106.6 |
| Weight (pounds) | 22 | 168.9 | 8.8 | 152.0 | 185.4 |
| Gender | 22 | Female = 10; Male=12 | | | |
| Race | 22 | Asian = 7, Black = 1, White = 14 | | | |

**Table 4**

| Descriptive statistics for controls | | | | | |
|---|---|---|---|---|---|
| Variable | Controls | Mean | Standard Deviation | Minimum | Maximum |
| *Tobacco** cigarettes smoked on day of testing | 2 | 0.7 | 1.8 | 1 | 6 |
| *Alcohol* ** alcoholic drinks consumed within 48 hrs of testing | 0 | 0 | 0 | 0 | 0 |
| *Alcohol* Average number alcoholic drinks consumed per week | 19 | 0.67 | 0.59 | 0.05 | 2 |
| *Caffeine*** caffeinated ounces consumed on day of testing | 11 | 18.8 | 15.6 | 1 | 48 |

* Data missing for 1 Control

**Data missing for 5 Controls

**Table 5**

| Descriptive statistics for all residents included in statistical analysis | | | | | |
|---|---|---|---|---|---|
| Variable | Residents | Mean | Standard Deviation | Minimum | Maximum |
| Age (years) | 29 | 50.7 | 10.9 | 19.0 | 68.0 |
| Height (inches) | 29 | 65.7 | 3.9 | 58.8 | 73.5 |
| Weight (pounds) | 29 | 189.7 | 59.5 | 89.5 | 360.0 |
| Distance (miles) | 28 | 5.8 | 2.7 | 0.3 | 9.7 |
| Hair Mn (µg/g) | 22 | 4.4 | 3.3 | 1.2 | 12.4 |
| Ln Hair Mn | 22 | 1.23 | 0.70 | 0.17 | 2.52 |
| Blood Mn (µg/L) | 28 | 9.4 | 3.7 | 4.2 | 21.7 |
| Ln Blood Mn | 28 | 2.17 | 0.36 | 1.44 | 3.08 |
| Blood Lead (µg/dl) | 28 | 1.8 | 1.0 | 0.6 | 6.2 |
| Ln Blood Lead | 28 | 0.44 | 0.46 | -0.51 | 1.83 |
| Gender | 29 | Female = 18; Male=11 | | | |
| Race | 29 | White = 29 | | | |

**Table 6**

| Covariates for all residents included in statistical analysis | | | | | |
|---|---|---|---|---|---|
| Variable | Residents | Mean | Standard Deviation | Minimum | Maximum |
| *Tobacco* cigarettes smoked on day of testing | 7 | 7.9 | 3.4 | 1 | 10 |
| *Alcohol* alcoholic drinks consumed within 48 hrs of testing | 1 | 3 | 0 | 3 | 3 |
| *Alcohol* Average number alcoholic drinks consumed per week | 15 | 0.9 | 0.5 | 0.3 | 1.5 |
| *Caffeine* caffeinated ounces consumed on day of testing | 18 | 14.7 | 25.8 | 4.0 | 128.0 |

**Table 7**

| Balance Measurements for all residents included in statistical analysis (SA in $cm^2$, SL in cm) | | | | | |
|---|---|---|---|---|---|
| Test | Dependent Variable | Mean | Standard Deviation | Minimum | Maximum |
| EO | SA | 3.58 | 1.88 | 0.90 | 9.38 |
| | SL | 50.71 | 13.70 | 35.83 | 93.88 |
| EC | SA | 5.31 | 4.11 | 0.94 | 18.81 |
| | SL | 68.69 | 32.45 | 39.74 | 183.83 |
| FO | SA | 5.11 | 1.49 | 2.40 | 8.05 |
| | SL | 66.82 | 17.03 | 42.12 | 108.52 |
| FC | SA | 10.18 | 6.12 | 3.28 | 29.50 |
| | SL | 106.40 | 52.48 | 44.56 | 310.60 |
| BO | SA | 13.11 | 4.98 | 6.91 | 25.2 |
| | SL | 85.65 | 20.59 | 60.15 | 134.11 |
| BC | SA | 17.57 | 8.56 | 4.80 | 44.24 |
| | SL | 111.30 | 46.67 | 63.34 | 288.39 |

**Table 8**

| Balance Measurements for all residents included in statistical analysis (natural log transformed) | | | | | |
|---|---|---|---|---|---|
| Test | Dependent Variable | Mean | Standard Deviation | Minimum | Maximum |
| EO | SA | 1.14 | 0.54 | -0.10 | 2.23 |
| | SL | 3.90 | 0.23 | 3.58 | 4.54 |
| EC | SA | 1.45 | 0.66 | -0.06 | 2.93 |
| | SL | 4.15 | 0.36 | 3.68 | 5.21 |
| FO | SA | 1.59 | 0.31 | 0.88 | 2.09 |
| | SL | 4.17 | 0.25 | 3.74 | 4.69 |
| FC | SA | 2.18 | 0.52 | 1.19 | 3.38 |
| | SL | 4.58 | 0.41 | 3.80 | 5.74 |
| BO | SA | 2.51 | 0.36 | 1.93 | 3.23 |
| | SL | 4.43 | 0.22 | 4.10 | 4.90 |
| BC | SA | 2.77 | 0.46 | 1.57 | 3.79 |
| | SL | 4.65 | 0.32 | 4.15 | 5.67 |

**Table 9**

| | Mean balance measurements of resident consuming 128 ounces of coffee prior to balance testing | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **EO-SA** | **EO-SL** | **EC-SA** | **EC-SL** | **FO-SA** | **FO-SL** | **FC-SA** | **FC-SL** |
| SA ($cm^2$)<br>SL (cm) | 1.49 | 56.51 | 2.27 | 58.99 | 6.01 | 97.18 | 9.43 | 129.56 |
| Natural log transformed | 0.40 | 4.03 | 0.82 | 4.08 | 1.80 | 4.58 | 2.24 | 4.86 |

**Table 10**

| Descriptive statistics for residents excluded from all statistical analysis | | | | | | | |
|---|---|---|---|---|---|---|---|
| Resident | **Age (years)** | **Height (inches)** | **Weight (pounds)** | **Hair Mn (µg/g)** | **Blood Mn (µg/L)** | **Blood Lead (µg/dl)** | **Distance (miles)** |
| Worker A | 55 | 71 | 221 | 0.8 | 3.0 | 1.1 | 7.4 |
| Worker B | 51 | 71 | 254 | 97.6 | 10.2 | 1.3 | 4.3 |
| Resident A | 42 | 66 | 244 | 41.1 | 15.6 | 1.2 | 4.9 |

**Table 11**

| Mean balance measurements of residents excluded from all analysis (SA in $cm^2$ and SL in cm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **EO-SA** | **EO-SL** | **EC-SA** | **EC-SL** | **FO-SA** | **FO-SL** | **FC-SA** | **FC-SL** |
| Worker A | 2.50 | 45.78 | 3.53 | 54.73 | 3.81 | 52.69 | 5.96 | 77.35 |
| Worker B | 4.69 | 54.66 | 7.87 | 98.21 | 8.77 | 77.49 | 16.10 | 134.02 |
| Resident A | 4.17 | 78.61 | 12.78 | 150.29 | 6.18 | 89.25 | 20.23 | 184.08 |

**Table 12**

| Mean balance measurements of residents excluded from all analysis (natural log transformed) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **EO-SA** | **EO-SL** | **EC-SA** | **EC-SL** | **FO-SA** | **FO-SL** | **FC-SA** | **FC-SL** |
| Worker A | 0.92 | 3.82 | 1.25 | 4.00 | 1.33 | 3.96 | 1.78 | 4.35 |
| Worker B | 1.54 | 4.00 | 2.06 | 4.58 | 2.17 | 4.35 | 2.77 | 4.89 |
| Resident A | 1.43 | 4.36 | 2.53 | 5.00 | 1.80 | 4.49 | 2.97 | 5.21 |

**Table 13**

| Bivariate Correlations between Independent Variables | | | | | |
|---|---|---|---|---|---|
| | | **Ln Hair Mn** | **Ln Blood Mn** | **Ln Blood Lead** | **Distance** |
| **Ln Hair Mn** | r | ….. | 0.59 | 0.32 | 0.06 |
| | (p) | | 0.004* | 0.14 | 0.78 |
| **Ln Blood Mn** | r | 0.59 | ….. | 0.06 | 0.19 |
| | (p) | 0.004* | | 0.75 | 0.34 |
| **Ln Blood Lead** | r | 0.32 | 0.06 | ….. | -0.034 |
| | (p) | 0.14 | 0.75 | | 0.86 |
| **Distance** | r | 0.06 | 0.19 | -0.03 | ….. |
| | (p) | 0.78 | 0.34 | 0.86 | |

* Denotes statistical significance reaching $p \leq 0.05$
Ln denotes natural logarithm transformed

**Table 14**

| Bivariate Correlations between Dependent and Independent Variables | | | | | |
|---|---|---|---|---|---|
| | | Independent Variables | | | |
| Test Condition | Dependent Variables | **Ln Hair Mn** | **Ln Blood Mn** | **Ln Blood Lead** | **Distance** |
| EO | SA r | 0.24 | 0.16 | 0.09 | 0.29 |
| | (p) | 0.28 | 0.42 | 0.64 | 0.13 |
| | SL r | 0.42 | 0.11 | 0.46 | -0.019 |
| | (p) | 0.05* | 0.57 | 0.01* | 0.93 |
| EC | SA r | 0.29 | 0.11 | 0.19 | 0.24 |
| | (p) | 0.18 | 0.58 | 0.33 | 0.23 |
| | SL r | 0.45 | 0.05 | 0.42 | 0.06 |
| | (p) | 0.03* | 0.79 | 0.02* | 0.77 |
| FO | SA r | 0.32 | 0.11 | 0.24 | 0.44 |
| | (p) | 0.15 | 0.59 | 0.21 | 0.02* |
| | SL r | 0.21 | -0.02 | 0.48 | 0.12 |
| | (p) | 0.35 | 0.93 | 0.01* | 0.54 |
| FC | SA r | 0.24 | -0.06 | 0.33 | 0.12 |
| | (p) | 0.28 | 0.76 | 0.08 | 0.53 |
| | SL r | 0.22 | -0.06 | 0.37 | -0.01 |
| | (p) | 0.33 | 0.76 | 0.05* | 0.97 |
| BO | SA r | -0.09 | -0.38 | 0.33 | -0.15 |
| | (p) | 0.71 | 0.05* | 0.10 | 0.46 |
| | SL r | 0.14 | -0.17 | 0.44 | -0.11 |
| | (p) | 0.56 | 0.40 | 0.02* | 0.58 |
| BC | SA r | -0.02 | -0.18 | 0.38 | -0.01 |
| | (p) | 0.92 | 0.40 | 0.06 | 0.95 |
| | SL r | 0.14 | 0.02 | 0.37 | -0.19 |
| | (p) | 0.56 | 0.91 | 0.07 | 0.36 |

* Denotes statistical significance reaching $p \leq 0.05$

All dependent and independent variables with exception of DISTANCE were natural logarithm transformed

**Table 15**

| Linear regression models relating total sway area and total sway length to Ln hair Mn | | | | | | |
|---|---|---|---|---|---|---|
| Test | Dependent Variable | Independent Variable | Parameter Estimate | Standard Error | P Value (one tailed) | Model $R^2$ |
| EO | SA | Intercept | 0.88 | 0.16 | | 0.51 |
| | | Ln Hair Mn | 0.20 | 0.12 | 0.05 | |
| | | Alcohol | -0.32 | 0.13 | 0.01 | |
| | | Gender | 0.15 | 0.18 | 0.20 | |
| | | Tobacco | -0.024 | 0.012 | 0.02 | |
| | SL | Intercept | 3.69 | 0.076 | | 0.36 |
| | | Ln Hair Mn | 0.08 | 0.056 | 0.09 | |
| | | Ln Blood Lead | 0.19 | 0.079 | 0.01 | |
| EC | SA | Intercept | 1.92 | 0.42 | | 0.31 |
| | | Ln Hair Mn | 0.30 | 0.15 | 0.03 | |
| | | HTWT | -2.47 | 0.99 | 0.01 | |
| | SL | Intercept | 3.79 | 0.11 | | 0.42 |
| | | Ln Hair Mn | 0.12 | 0.078 | 0.06 | |
| | | Gender | 0.17 | 0.12 | 0.08 | |
| | | Ln Blood Lead | 0.20 | 0.11 | 0.04 | |
| FO | SA | Intercept | 1.40 | 0.13 | | 0.10 |
| | | Ln Hair Mn | 0.14 | 0.10 | 0.08 | |
| | SL | Intercept | 4.03 | 0.10 | | 0.28 |
| | | Ln Hair Mn | 0.015 | 0.07 | 0.4 | |
| | | Ln Blood Lead | 0.26 | 0.10 | 0.01 | |
| FC | SA | Intercept | 1.85 | 0.22 | | 0.13 |
| | | Ln Hair Mn | 0.11 | 0.16 | 0.3 | |
| | | Ln Blood Lead | 0.28 | 0.23 | 0.1 | |
| | SL | Intercept | 4.34 | 0.16 | | 0.18 |
| | | Ln Hair Mn | 0.049 | 0.12 | 0.3 | |
| | | Ln Blood Lead | 0.28 | 0.16 | 0.05 | |
| BO | SA | Intercept | 2.45 | 0.14 | | 0.09 |
| | | Ln Hair Mn | -0.065 | 0.10 | 0.7 | |
| | | Gender | 0.20 | 0.16 | 0.1 | |
| | SL | Intercept | 4.31 | 0.076 | | 0.34 |
| | | Ln Hair Mn | 0.0057 | 0.057 | 0.5 | |
| | | Gender | 0.26 | 0.089 | 0.005 | |
| BC | SA | Intercept | 2.72 | 0.14 | | 0.59 |
| | | Ln Hair Mn | -0.074 | 0.10 | 0.8 | |
| | | Gender | 0.45 | 0.16 | 0.006 | |
| | | Alcohol | -0.36 | 0.10 | 0.002 | |
| | SL | Intercept | 4.51 | 0.099 | | 0.44 |
| | | Ln Hair Mn | 0.0012 | 0.074 | 0.50 | |
| | | Gender | 0.41 | 0.12 | 0.001 | |

Ln of sway dependent variable = b0 + b1 (Ln Hair Mn) + b2 (Gender) + b3 (HTWT) + b4 (Age) + b5 (Ln blood lead) + b6 (Caffeine) + b7 (Tobacco) + b8 (Alcohol)

Ln denotes natural logarithm transformed
Gender: 0 = male, 1 = female
HTWT: height to weight ratio
Age: age in years
Caffeine: ounces of caffeine in 12 hours prior to testing
Tobacco: mean number of cigarettes per day
Alcohol: number of alcoholic drinks in 48 hours prior to testing

**Table 16**

| Demographic data for residents living ≤ 6 miles from refinery | | | | | |
|---|---|---|---|---|---|
| Variable | Number | Mean | Standard Deviation | Minimum | Maximum |
| Age (years) | 14 | 51.6 | 7.07 | 33.0 | 60.0 |
| Height (inches) | 14 | 66.1 | 4.46 | 58.8 | 73.5 |
| Weight (pounds) | 14 | 196.8 | 73.54 | 89.5 | 360.0 |
| Distance (miles) | 14 | 3.8 | 2.0 | 0.3 | 6.0 |
| Hair Mn (µg/g) | 11 | 5.1 | 3.7 | 1.2 | 12.4 |
| Ln Hair Mn | 11 | 1.35 | 0.80 | 0.17 | 2.52 |
| Blood Mn (µg/L) | 14 | 9.9 | 4.7 | 4.2 | 21.7 |
| Ln Blood Mn | 14 | 2.20 | 0.42 | 1.44 | 3.08 |
| Blood Lead (µg/dl) | 14 | 2.0 | 1.4 | 0.9 | 6.2 |
| Ln Blood Lead | 14 | 0.55 | 0.50 | -0.11 | 1.83 |
| Gender | 14 | Female = 8; Male = 6 | | | |

Ln denotes natural logarithm transformed

**Table 17**

| Demographic data for residents living > 6 miles from refinery | | | | | |
|---|---|---|---|---|---|
| Variable | Number | Mean | Standard Deviation | Minimum | Maximum |
| Age (years) | 14 | 48.7 | 13.53 | 19.0 | 68.0 |
| Height (inches) | 14 | 65.4 | 3.59 | 59.5 | 70.5 |
| Weight (pounds) | 14 | 185.3 | 45.15 | 102.5 | 265.0 |
| Distance (miles) | 14 | 7.9 | 1.4 | 6.5 | 9.7 |
| Hair Mn (µg/g) | 11 | 3.7 | 2.7 | 1.5 | 10.2 |
| Ln Hair Mn | 11 | 1.11 | 0.61 | 0.40 | 2.32 |
| Blood Mn (µg/L) | 14 | 8.8 | 2.4 | 4.3 | 14.1 |
| Ln Blood Mn | 14 | 2.14 | 0.29 | 1.46 | 2.65 |
| Blood Lead (µg/dl) | 14 | 1.5 | 0.5 | 0.6 | 2.2 |
| Ln Blood Lead | 14 | 0.34 | 0.42 | -0.51 | 0.79 |
| Gender | 14 | Female = 9; Male = 5 | | | |

Ln denotes natural logarithm transformed

**Table 18**

| Descriptive statistics between residents and controls | | | | | |
|---|---|---|---|---|---|
| Residents | | | | | |
| Variable | Number | Mean | Standard Deviation | Minimum | Maximum |
| Age (years) | 22 | 47.6 | 10.3 | 19.0 | 59.0 |
| Height (inches) | 22 | 65.7 | 4.2 | 58.8 | 73.5 |
| Weight (pounds) | 22 | 193.6 | 65.3 | 89.5 | 360.0 |
| Hair Mn (µg/g) | 16 | 5.01 | 3.57 | 1.24 | 12.40 |
| Blood Lead (µg/dl) | 22 | 1.81 | 1.15 | 0.60 | 6.20 |
| Gender | 22 | Female = 13; Male=9 | | | |
| Race | 22 | White = 22 | | | |
| Controls | | | | | |
| Age (years) | 22 | 35.0 | 8.1 | 24.0 | 57.0 |
| Height (inches) | 22 | 69.8 | 13.8 | 52.4 | 106.6 |
| Weight (pounds) | 22 | 168.9 | 8.8 | 152.0 | 185.4 |
| Gender | 22 | Female = 10; Male=12 | | | |

**Table 19**

| Sway Length of Residents (n=22) and Controls (n=22) (natural log transformed) | | | | | |
|---|---|---|---|---|---|
| Test Condition | Dependent Variables | **Mean** | **Standard Deviation** | **Minimum** | **Maximum** |
| EO | Residents | 3.92 | 0.26 | 3.58 | 4.54 |
| | Controls | 3.81 | 0.12 | 3.57 | 4.02 |
| EC | Residents | 4.18 | 0.40 | 3.68 | 5.21 |
| | Controls | 4.07 | 0.19 | 3.79 | 4.47 |
| FO | Residents | 4.16 | 0.28 | 3.74 | 4.69 |
| | Controls | 3.96 | 0.15 | 3.55 | 4.15 |
| FC | Residents | 4.58 | 0.46 | 3.80 | 5.74 |
| | Controls | 4.33 | 0.24 | 4.04 | 4.96 |

**Table 20**

| Sway Area of Residents (n=22) and Controls (n=22) (natural log transformed) | | | | | |
|---|---|---|---|---|---|
| Test Condition | Dependent Variables | **Mean** | **Standard Deviation** | **Minimum** | **Maximum** |
| EO | Residents | 1.24 | 0.51 | 0.37 | 2.24 |
| | Controls | 0.62 | 0.38 | -0.096 | 1.31 |
| EC | Residents | 1.53 | 0.65 | 0.70 | 2.94 |
| | Controls | 1.14 | 0.39 | 0.24 | 1.90 |
| FO | Residents | 1.59 | 0.31 | 0.88 | 2.09 |
| | Controls | 1.06 | 0.40 | 0.022 | 1.728 |
| FC | Residents | 2.19 | 0.57 | 1.189 | 3.384 |
| | Controls | 1.68 | 0.39 | 0.973 | 2.422 |

**Figure 1**

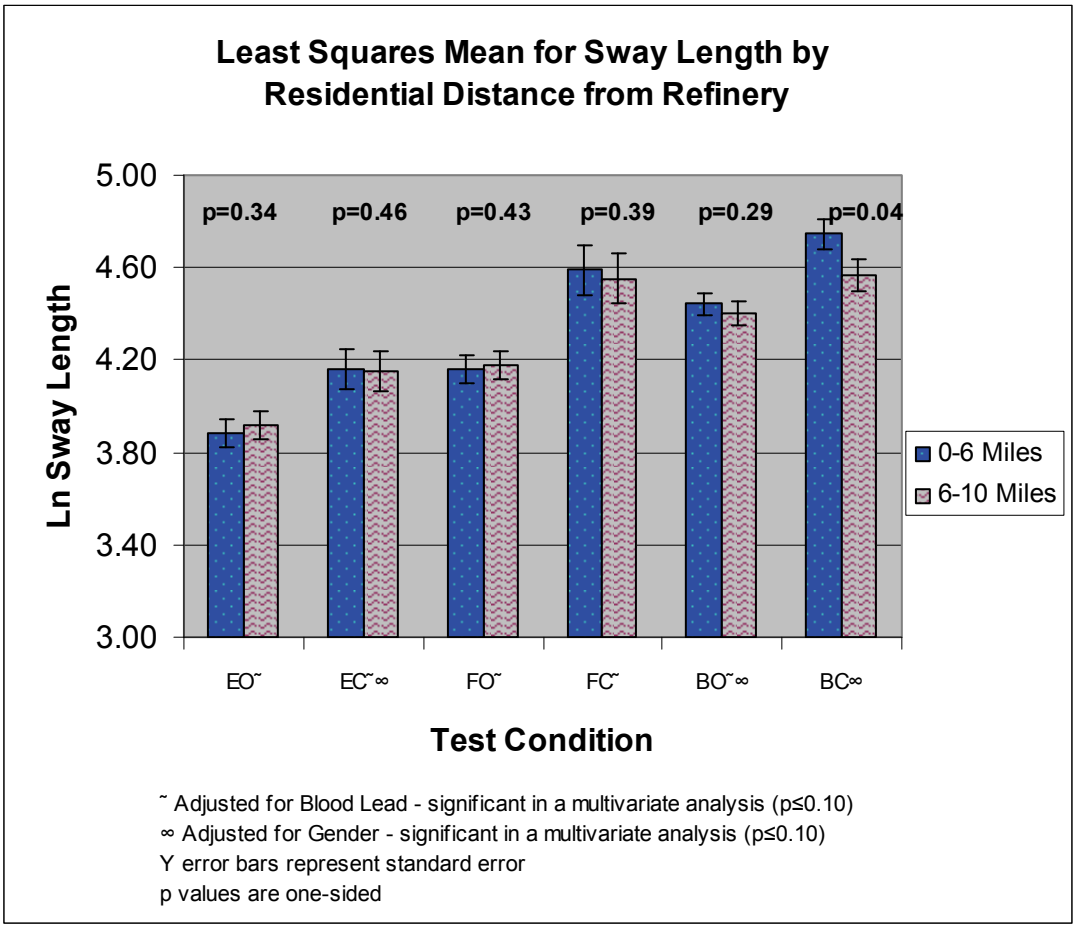

Least Squares Mean for Sway Length by Residential Distance from Refinery
5.00
4.60
4.20
3.80
3.40
3.00
Ln Sway Length
p=0.34
p=0.46
p=0.43
p=0.39
p=0.29
p=0.04
EO˜
EC˜∞
FO˜
FC˜
BO˜∞
BC∞
Test Condition
0-6 Miles
6-10 Miles
˜ Adjusted for Blood Lead - significant in a multivariate analysis (p≤0.10)
∞ Adjusted for Gender - significant in a multivariate analysis (p≤0.10)
Y error bars represent standard error
p values are one-sided

**Figure 2**

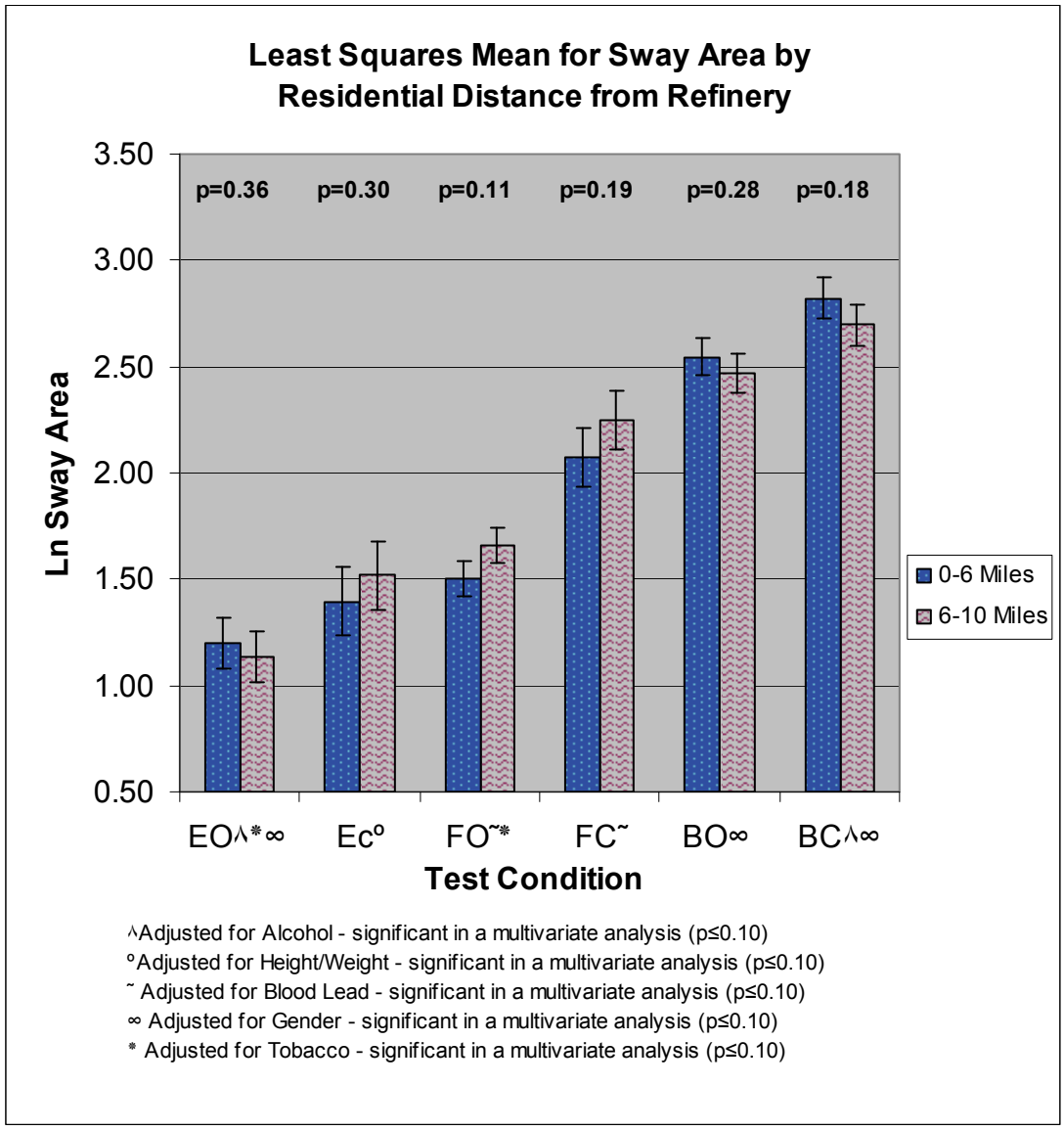

Least Squares Mean for Sway Area by Residential Distance from Refinery
3.50
3.00
2.50
2.00
1.50
1.00
0.50
Ln Sway Area
p=0.36
p=0.30
p=0.11
p=0.19
p=0.28
p=0.18
EO^*∞
Ec°
FO~*
FC~
BO∞
BC^∞
Test Condition
0-6 Miles
6-10 Miles
^Adjusted for Alcohol - significant in a multivariate analysis (p≤0.10)
°Adjusted for Height/Weight - significant in a multivariate analysis (p≤0.10)
~ Adjusted for Blood Lead - significant in a multivariate analysis (p≤0.10)
∞ Adjusted for Gender - significant in a multivariate analysis (p≤0.10)
* Adjusted for Tobacco - significant in a multivariate analysis (p≤0.10)

**Figure 3**

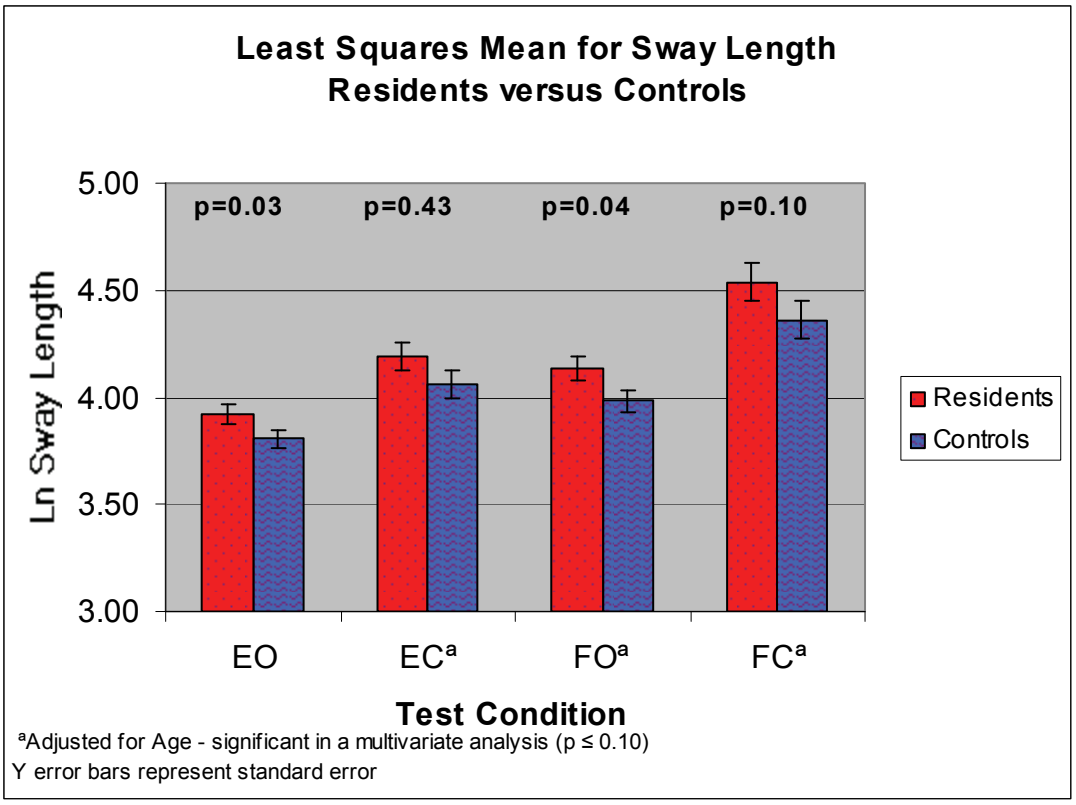
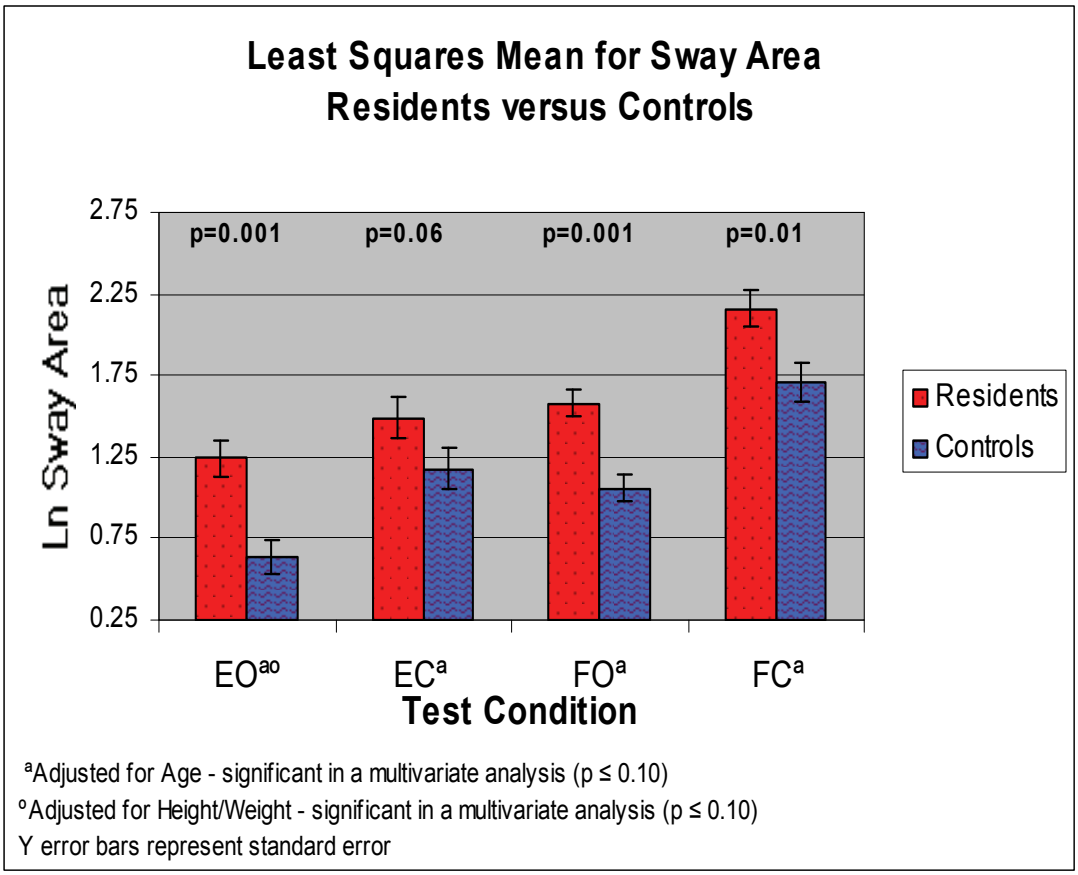

Least Squares Mean for Sway Length
Residents versus Controls
5.00
4.50
4.00
3.50
3.00
Ln Sway Length
p=0.03
p=0.43
p=0.04
p=0.10
EO
EC[a]
FO[a]
FC[a]
Test Condition
Residents
Controls
[a]Adjusted for Age - significant in a multivariate analysis (p ≤ 0.10)
Y error bars represent standard error

**Figure 4**




[a]Adjusted for Age - significant in a multivariate analysis ($p \leq 0.10$)

[o]Adjusted for Height/Weight - significant in a multivariate analysis ($p \leq 0.10$)

Y error bars represent standard error

**Bibliography**

1. Political Economy Research Institute. *Ohio's 'Toxic Twelve' Identifies Top Air Polluters in State.* Available at: http://www.peri.umass.edu/Ohio-Toxics.266.0.html. Accessed April 27, 2008.

2. Roels H, Lauwerys R, Buchet JP, et al. Epidemiological survey among workers exposed to manganese: Effects on lung, central nervous system, and some biological indices. *Am J Ind Med*. 1987;11:307-327.

3. Roels HA, Ghyselen P, Buchet JP, Ceulemans E, Lauwerys RR. Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. *Br J Ind Med*. 1992;49:25-34.

4. Chia SE, Goh J, Lee G, et al. Use of a computerized postural sway measurement system for assessing workers exposed to manganese. *Clin Exp Pharmacol Physiol*. 1993;20:549-553.

5. Rodriguez-Agudelo Y, Riojas-Rodriguez H, Rios C, et al. Motor alterations associated with exposure to manganese in the environment in mexico. *Sci Total Environ*. 2006;368:542-556.

6. Dietz MC, Ihrig A, Wrazidlo W, Bader M, Jansen O, Triebig G. Results of magnetic resonance imaging in long-term manganese dioxide-exposed workers. *Environ Res*. 2001;85:37-40.

7. Bowler RM, Koller W, Schulz PE. Parkinsonism due to manganism in a welder: Neurological and neuropsychological sequelae. *Neurotoxicology*. 2006;27:327-332.

8. Kim EA, Cheong HK, Choi DS, et al. Effect of occupational manganese exposure on the central nervous system of welders: 1H magnetic resonance spectroscopy and MRI findings. *Neurotoxicology*. 2007;28:276-283.

9. Smith LB, Bhattacharya A, Lemasters G, et al. Effect of chronic low-level exposure to jet fuel on postural balance of US air force personnel. *J Occup Environ Med*. 1997;39:623-632.

10. Kuo W, Bhattacharya A, Succop P, Linz D. Postural stability assessment in sewer workers. *J Occup Environ Med*. 1996;38:27-34.

11. Bhattacharya A. Quantitative posturagraphy as an alternative noninvasive tool for alcohol/drug/chemical testing--preliminary thoughts. *Drug Chem Toxicol*. 1999;22:201-212.

12. Sack D, Linz D, Shukla R, Rice C, Bhattacharya A, Suskind R. Health status of pesticide applicators: Postural stability assessments. *J Occup Med*. 1993;35:1196-1202.

13. Bhattacharya A, Shukla R, Bornschein RL, Dietrich KN, Keith R. Lead effects on postural balance of children. *Environ Health Perspect*. 1990;89:35-42.

14. Bhattacharya A, Shukla R, Dietrich KN, Bornschein RL. Effect of early lead exposure on the maturation of children's postural balance: A longitudinal study. *Neurotoxicol Teratol*. 2006;28:376-385.

15. Chia SE, Gan SL, Chua LH, Foo SC, Jeyaratnam J. Postural stability among manganese exposed workers. *Neurotoxicology*. 1995;16:519-526.

16. Kaji H, Ohsaki Y, Rokujo C, Higashi T, Fujino A, Kamada T. Determination of blood and urine manganese (mn) concentrations and the application of static sensography as the indices of mn-exposure among mn-refinery workers. *J UOEH*. 1993;15:287-296.

17. Young T, Myers JE, Thompson ML. The nervous system effects of occupational exposure to manganese--measured as respirable dust--in a south african manganese smelter. *Neurotoxicology*. 2005;26:993-1000.

18. Bowler RM, Nakagawa S, Drezgic M, et al. Sequelae of fume exposure in confined space welding: A neurological and neuropsychological case series. *Neurotoxicology*. 2007;28:298-311.

19. Hudnell HK. Effects from environmental mn exposures: A review of the evidence from non-occupational exposure studies. *Neurotoxicology*. 1999;20:379-397.

20. Bhattacharya A, Shukla R, Bornschein R, Dietrich K, Kopke JE. Postural disequilibrium quantification in children with chronic lead exposure: A pilot study. *Neurotoxicology*. 1988;9:327-340.

21. Bhattacharya A, Shukla R, Dietrich K, Bornschein R, Berger O. Effect of early lead exposure on children's postural balance. *Dev Med Child Neurol*. 1995;37:861-878.

22. Bhattacharya A, Morgan R, Shukla R, Ramakrishanan HK, Wang L. Non-invasive estimation of afferent inputs for postural stability under low levels of alcohol. *Ann Biomed Eng*. 1987;15:533-550.

23. Foo SC, Khoo NY, Heng A, et al. Metals in hair as biological indices for exposure. *Int Arch Occup Environ Health*. 1993;65:S83-6.

24. 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;72:521-527.

25. Dietrich KN, Krafft KM, Bornschein RL, et al. Low-level fetal lead exposure effect on neurobehavioral development in early infancy. *Pediatrics*. 1987;80:721-730.

26. Vitayavirasuk B, Junhom S, Tantisaeranee P. Exposure to lead, cadmium and chromium among spray painters in automobile body repair shops. *J Occup Health*. 2005;47:518-522.

27. Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. *J Pediatr*. 2003;142:9-14.

28. Dorman DC, Struve MF, Clewell HJ,3rd, Andersen ME. Application of pharmacokinetic data to the risk assessment of inhaled manganese. *Neurotoxicology*. 2006;27:752-764.

29. Erikson KM, Syversen T, Steinnes E, Aschner M. Globus pallidus: A target brain region for divalent metal accumulation associated with dietary iron deficiency. *J Nutr Biochem*. 2004;15:335-341.

30. Dick RB, Bhattacharya A, Shukla R. Use of a computerized postural sway measurement system for neurobehavioral toxicology. *Neurotoxicol Teratol*. 1990;12:1-6.

31. Agency for Toxic Substances and Disease Registry. *Health Consultation: Washington County Air Quality Marietta Ohio*. Atlanta, GA: US Department of Health and Human Services; June 18, 2007. Available at: http://www.atsdr.cdc.gov/HAC/pha/marietta3/ATSDRHealthConsultation2007.pdf. Accessed September 10, 2007.

32. 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;115:122-127.

33. Iwata T, Yano E, Karita K, Dakeishi M, Murata K. Critical dose of lead affecting postural balance in workers. *Am J Ind Med*. 2005;48:319-325.

34. Pacific Environmental Services, Inc., US Environmental Protection Agency. *Background Report AP-42 Section 12.4 Ferroalloy Production*. Research Triangle Park, NC. Available at: http://www.epa.gov/ttn/chief/ap42/ch12/bgdocs/b12s04.pdf. Accessed September 4, 2007.

35. US Environmental Protection Agency. *Locating and estimating air emissions from sources of manganese.* Research Triangle Park, NC: Office of Air and Radiation, &

Office of Air Quality Planning and Standards; September 2005. Available at: http://www.epa.gov/ttnchie1/le/manganes.pdf. Accessed September 10, 2007.

36. Agency for Toxic Substances and Disease Registry. *Health Consultation: Warren Township Cistern Sampling Investigation*. Atlanta, GA: US Department of Health and Human Services; June 18, 2005. Available at: http://www.atsdr.cdc.gov/hac/PHA/WarrenTwnshipCistern/WarrennTownshipCisternHC.pdf. Accessed September 14, 2006.

37. Agency for Toxic Substances and Disease Registry. *Toxicological profile for manganese*: September 2000. Atlanta, GA: US Department of Health and Human Services; 2000.

38. Levy BS, Nassetta WJ. Neurologic effects of manganese in humans: A review. *Int J Occup Environ Health*. 2003;9:153-163.

39. Aggarwal A, Vaidya S, Shah S, Singh J, Desai S, Bhatt M. Reversible parkinsonism and T1W pallidal hyperintensities in acute liver failure. *Mov Disord*. 2006;21:1986-1990.

40. Andersen ME, Gearhart JM, Clewell HJ,3rd. Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. *Neurotoxicology*. 1999;20:161-171.

41. Jr. Sullivan J, Krieger GR. *Clinical Environmental Health and Toxic Exposures.* Second ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2001.

42. Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC, Wong BA. Olfactory transport: A direct route of delivery of inhaled manganese phosphate to the rat brain. *J Toxicol Environ Health A*. 2002;65:1493-1511.

43. Elder A, Gelein R, Silva V, et al. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environ Health Perspect*. 2006;114:1172-1178.

44. Henriksson J, Tjalve H. Manganese taken up into the CNS via the olfactory pathway in rats affects astrocytes. *Toxicol Sci*. 2000;55:392-398.

45. Dorman DC, McManus BE, Parkinson CU, Manuel CA, McElveen AM, Everitt JI. Nasal toxicity of manganese sulfate and manganese phosphate in young male rats following subchronic (13-week) inhalation exposure. *Inhal Toxicol*. 2004;16:481-488.

46. Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA, Wong BA. Tissue manganese concentrations in young male rhesus monkeys following subchronic manganese sulfate inhalation. *Toxicol Sci*. 2006;92:201-210.

47. Apostoli P, Lucchini R, Alessio L. Are current biomarkers suitable for the assessment of manganese exposure in individual workers? *Am J Ind Med*. 2000;37:283-290.

48. Smith D, Gwiazda R, Bowler R, et al. Biomarkers of mn exposure in humans. *Am J Ind Med*. 2007.

49. Shamberger RJ. Validity of hair mineral testing. *Biol Trace Elem Res*. 2002;87:1-28.

50. Boojar MM, Goodarzi F, Basedaghat MA. Long-term follow-up of workplace and well water manganese effects on iron status indexes in manganese miners. *Arch Environ Health*. 2002;57:519-528.

51. Sukumar A, Subramanian R. Elements in the hair of workers at a workshop, foundry, and match factory. *Biol Trace Elem Res*. 2000;77:139-147.

52. Huang CC, Chu NS, Lu CS, Chen RS, Schulzer M, Calne DB. The natural history of neurological manganism over 18 years. *Parkinsonism Relat Disord*. 2007;13:143-145.

53. Perl DP, Olanow CW. The neuropathology of manganese-induced parkinsonism. *J Neuropathol Exp Neurol*. 2007;66:675-682.

54. Porter AM. Why do we have apocrine and sebaceous glands? *J R Soc Med*. 2001;94:236-237.

55. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. normal sweat gland function. *J Am Acad Dermatol*. 1989;20:537-563.

56. Barth JH, Ridden J, Philpott MP, Greenall MJ, Kealey T. Lipogenesis by isolated human apocrine sweat glands: Testosterone has no effect during long-term organ maintenance. *J Invest Dermatol*. 1989;92:333-336.

57. Yasuda T, Nakagawa T, Inoue H, Iwamoto M, Inokuchi A. The role of the labyrinth, proprioception and plantar mechanosensors in the maintenance of an upright posture. *Eur Arch Otorhinolaryngol*. 1999;256 Suppl 1:S27-32.

58. Visser JE, Bloem BR. Role of the basal ganglia in balance control. *Neural Plast*. 2005;12:161-74; discussion 263-72.