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In vivo neutron activation analysis of bone manganese in workers

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Abstract

Objective: Manganese (Mn) is a neurotoxin. However, the impact of elevated, chronic Mn exposure is not well understood, partially due to the lack of a cumulative exposure biomarker. To address this gap, our group developed a compact *in vivo* neutron activation analysis (IVNAA) system to quantify Mn concentration in bone (MnBn).

Approach: In this study, we used this system and determined MnBn among male Chinese workers and compared results to their blood Mn (MnB), a measure of recent exposure, and the years of employment, a measure of cumulative exposure. A cross-sectional study was conducted with 30 ferroalloy smelters (exposed) and 30 general manufacturing workers (controls). MnBn was assessed using IVNAA, MnB was measured with inductively coupled plasma mass spectrometry, and occupational history and demographics were obtained via questionnaire. Mn-doped phantoms were used to generate a calibration curve; spectra from these phantoms were consistent with *in vivo* spectra.

Main results: The median (interquartile range (IQR)) values for Mn biomarkers were 2.7 μg g^{-1} (7.2) for MnBn and 14.1 μg l^{-1} (4.0) for MnB. In regression models adjusted for age and education, the natural log transformed MnBn (ln(MnBn)) was significantly associated with the exposed/control status ($\beta = 0.44$, $p = 0.047$) and years of employment ($\beta = 0.05$, $p = 0.002$), but not with natural log transformed MnB (ln(MnB)) ($\beta = 0.54$, $p = 0.188$).

Significance: Our results support the use of IVNAA to quantify MnBn and the use of MnBn as a biomarker of cumulative Mn exposure.

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Keywords

manganese (Mn); bone; neutron activation analysis (NAA)

1. Introduction

Manganese (Mn) is an essential micronutrient for human health. It plays a vital role in bone growth, blood sugar regulation, immune cell maintenance and metabolism of lipids, proteins, and carbohydrates (Hurley *et al* 1987). Mn can cause adverse health effects when the intake is either too low or too high. Mn deficiency cases are rare, as dietary Mn typically provides sufficient Mn intake. However, Mn overexposure and its associated detrimental health effects have been documented, which we will briefly review in the following section. The main source of clinically diagnosed Mn intoxication is from occupational exposure. As the fourth most-used industrial metal, inhalation exposure to airborne Mn presents high risks to workers such as miners, ferroalloy smelters, and welders (Couper 1837, Wang *et al* 1989, Lu *et al* 2005, Cowan *et al* 2009, Bouchard *et al* 2011). In addition, there are many environmental sources of Mn that can lead to overexposure in the general population. These sources include pesticides, contaminated water, milk or food (Bouchard *et al* 2011, O'Neal and Zheng 2015), and gasoline additives (Butcher *et al* 1999).

Mn intoxication is primarily associated with neurological disorders (Rodier 1955, Wennberg *et al* 1991, Sassine *et al* 2002, Racette 2014). Generally, the appearance of a constant pattern of behavioral or cognitive abnormalities only becomes obvious after several years of exposure (Iregren 1998). The signs and symptoms include poor hand–eye coordination, bradykinesia, reduced cognitive function, as well as tremor with voluntary movements. In severe cases, a devastating neurological impairment called 'manganism' occurs, a syndrome which closely resembles but is not identical to Parkinson's disease (PD) (Rodier 1955, Schuler *et al* 1957, Whitlock *et al* 1966, Mena *et al* 1967, Emara *et al* 1971, Wennberg *et al* 1991, Ky *et al* 1992, Nelson *et al* 1993, Levy and Nassetta 2003, Crossgrove and Zheng 2004, Williams *et al* 2012). Some studies found that cases of manganism are more likely to happen in patients with a history of prolonged exposure, which suggests that development of the disease is related to cumulative exposure (Rodier 1955, Schuler *et al* 1957).

Commonly used Mn biomarkers include blood, urine and saliva. Because of the short half-life and large intracellular distribution of Mn, results of Mn concentration in these biometrics are highly variable and are often found to be not significantly associated with Mn-induced neurological effects (Bader *et al* 1999, Zheng *et al* 2000, Crossgrove and Zheng 2004, Wongwit *et al* 2004, Santos *et al* 2014, O'Neal and Zheng 2015). Thus, using Mn levels in blood, urine or saliva as a biomarker of Mn deposition is generally not recommended (O'Neal and Zheng 2015). Hair and nail Mn have also been proposed as Mn biomarkers. However, data show large variations among individuals, although the group-based data appear to be more useful (Bader *et al* 1999, Wongwit *et al* 2004). In addition, hair and nails are potentially subject to external contamination, and hair and nail Mn only reflect the exposure for the past several months. Magnetic resonance imaging (MRI) technology has provided evidence of Mn deposition in the brain even in the absence of clinical symptoms of

Mn toxicity (Jiang *et al* 2006, Dydak *et al* 2011). While brain Mn accumulation can be reflected by an increased MRI signal, one downside of the technology is that Mn may be removed from the brain after a short period of time (i.e. within months) (Arjona *et al* 1997, Kim *et al* 1999). Hence, brain Mn concentrations obtained using MRI only reflect recent Mn exposure. In addition, the MRI signal of Mn in the brain is not specific to Mn, because the signal is not an intrinsic property of the Mn ion alone (Fitsanakis *et al* 2006). Overall, to date, the lack of a reliable body Mn burden biomarker limits the capacity for cumulative Mn exposure assessment.

Bone is a promising potential biomarker for Mn exposure assessment. Around 40% of human body Mn is stored in bone (Liu *et al* 2014). Derived from the value provided by the International Commission on Radiological Protection (ICRP) 23 and ICRP 70 (Snyder 1975, Valentin 2002), there is approximately 1 μg Mn per gram of bone, which is equal to approximately 5 μg Mn per gram of Ca. In a recent study on rats, the data revealed that the half-lives of Mn in various rat bones were between 77 and 690 d, with an average of 143 d in the whole rat skeleton. Taking into account that every 16.7 d of a rat's life is equal to one human year, the half-life of Mn in human bone can be estimated as from 4.6 to 41.3 years, with an average of 8.6 years in the whole human skeleton (Sengupta 2011, O'Neal and Zheng 2015). Because bone is one of the main long-term storage organs for Mn in humans, it is logical to propose that bone manganese (MnBn) is a relevant and valuable biomarker for assessment of cumulative Mn exposure.

Over the past several years, we have developed a method to measure MnBn using neutron activation analysis (NAA), which is a powerful modality for noninvasive *in vivo* elemental measurement (Liu *et al* 2013, 2014, 2017). The general principle of NAA is to convert the stable isotopes of some elements into unstable radioisotopes; the decay information can then be collected for analytical purposes. The stable isotope of Mn, ^{55}Mn, with its natural abundance of 100% and its relatively large thermal neutron capture cross-section, can be readily activated by low energy neutrons. The resulting ^{56}Mn nucleus is radioactive and decays to an excited state of ^{56}Fe by the emission of a β-particle. This is followed by the internal transition of ^{56}Fe from an excited state to a ground state, which emits an 847 keV γ-ray. The branching ratio of the 847 keV γ-ray is 98.8%. By measuring the 847 keV characteristic γ counts, the concentration of ^{55}Mn in the sample can be determined. We have developed and characterized a NAA system with an Mn detection limit of 0.64 μg Mn per gram of bone (ppm) (Liu *et al* 2017). Another *in vivo* NAA (IVNAA) system has also been developed in other labs (Aslam *et al* 2009), which uses a large accelerator. In contrast, the neutron generator utilized in our lab is much more compact, and hence transportable. In this project, the IVNAA system was shipped to China for use in an epidemiology study.

This study was an interdisciplinary collaboration between different research groups including researchers from medical physics, occupational/environmental health, epidemiology, and metal toxicology. In this paper, we report the practical uses of the newly developed IVNAA MnBn measurement technology, the distribution of MnBn concentrations, group differences for MnBn and blood Mn (MnB) concentrations, and the relationship between MnBn and MnB concentrations.

2. Material and methods

2.1. *In vivo* neutron activation analysis system (IVNAA)

Details regarding the NAA system design and setup can be found in our previous publications (Liu *et al* 2014, 2017). In short, the system consists of a customized compact deuterium–deuterium (DD) neutron generator, a customized moderator/reflector/shielding assembly, and an HPGe detection system with a 100% relative high efficiency detector (GMX100P4–95; Advanced Measurement Technology, Oak Ridge, TN).

The DD neutron generator used in this study produces 2.45 MeV neutrons in an approximately isotropic manner. Because the interaction cross-section $\sigma(E)$ (and hence the probability to produce an Mn γ-ray signal) increases with the decrease of the energy of the incident neutron, a moderator and reflector were used to maximize the number of low-energy neutrons present in the irradiation cave. Additional shielding materials were added to minimize the neutron as well as photon dose.

To minimize the radiation dose to the subject for *in vivo* elemental quantification, Mn was measured in the hand bones. The participant's arm was extended away from the body as their hand was placed in an irradiation cave; the rest of the body was shielded from the neutron beam. The hand was irradiated for 10 min and then the participant was transited to the HPGe detector system for a 60 min measurement, with spectra saved at 5 min to obtain the calcium (Ca) counts and 60 min to obtain the Mn counts. The time between the end of the irradiation and the beginning of measurement was fixed at 5 min. Figure 1(a) shows a participant's hand being irradiated. Figure 1(b) shows the HPGe measurement system, with the measurement cave clearly seen. Both systems were covered in customized wooden plates to make them more user-friendly.

2.2. Mn-doped bone-equivalent phantoms and system calibration

We created bone-equivalent phantoms with Mn concentrations of 0, 5, 10, 15 and 20 ppm. The matrix of the phantoms contained the same amount of calcium (Ca) and other elements to mimic real bone, as described previously (Liu *et al* 2013, 2014). The phantoms are used to calibrate the system for *in vivo* MnBn measurement. The net counts from both the Mn and Ca 3084 KeV characteristic γ-rays were calculated by an in-house spectral analysis program. The ratios of Mn/Ca were calculated and plotted against the Mn concentration for system calibration. Because the activated Mn and Ca counts came from the same neutron beam (with thermal neutrons as the major component) and Ca concentration is the same in each phantom, and presumably in the bone in *in vivo* measurements, the fluctuation of activated Ca counts reflects the variation of neutron flux, hand palm attenuation, and counting geometry factors. Therefore, the Mn/Ca ratio was used for system calibration, and normalizing the Mn signal to the Ca signal is expected to correct for the variation of neutron flux, hand palm attenuation, and counting geometry factors.

2.3. Spectrum and statistical analysis

The γ-rays emitted from the irradiated phantoms or human bone were detected by an HPGe detector system. The signals were collected and processed by a DSPEC plus digital pulse

processing system and Maestro γ-ray spectroscopy. The raw data were analyzed by an in-house MATLAB-based program. The Levenberg–Marquardt method was used for calculation of net counts under the Gaussian peak. Multi-peak fitting was applied to differentiate the peaks between 847 keV of Mn and 844 keV of Mg. A reduced Chi-square algorithm was used to determine the goodness of fit for peak fitting: the mean and standard deviation of the reduced chi-square are 1.22 ± 0.22. SYSTAT 13 (Systat, inc., San Jose, CA, USA) was used for statistical analysis to obtain the basic statistics of the data and to perform regression analysis. For all the data analysis in this paper, a significant level is defined as a confidence level >95% (or $p < 0.05$), and a marginally significant level is defined as a confidence level of >90% and <95% (or $0.05 < p < 0.1$).

2.4. Study population

The human study was approved by the Purdue Institutional Review Board (IRB) and Zunyi Medical College Ethical Review Board (ERB). Participants signed an informed consent document prior to participation in the study. Sixty-one male workers were recruited as exposed and control groups from a ferroalloy factory ($n = 31$, exposed) and a manufacturing facility ($n = 30$, control), respectively. Exclusion criteria included individuals who had cognitive symptoms, active neurological or psychiatric disease, and movement impairments that had known causes which were not related to manganese exposure. No participant was excluded based on these exclusion criteria. One worker from the ferroalloy factory did not complete MnBn measurements and was excluded from further analyses, leaving a total of 60 participants. The participants completed the MnBn NAA measurement, had a blood sample collected to test for MnB, and completed a questionnaire. The questionnaire was administered by study staff to obtain data on demographics and work history.

2.5. Bone and blood Mn measurement and analysis

Before a bone Mn measurement, the participant's right hand and lower arm were thoroughly washed with soap. A trained research assistant then cleaned these areas with 50% alcohol to further reduce or eliminate Mn contamination. The participant was guided to sit on a chair in front of the neutron irradiation system and to place his right hand inside the irradiation cave. A bag filled with water was wrapped around the participant's arm to fix the arm and to further reduce the neutron dose to the rest of the body.

The hand was irradiated for 10 min to activate the ^{55}Mn atoms in the hand bone to ^{56}Mn. The hand-equivalent dose was measured as less than 50 mSv and the whole body effective dose was estimated to be about 17 μSv (Liu *et al* 2014). The participant was moved to another laboratory with a high-purity germanium (HPGe) detector to collect the γ-ray signals released from the hand bone. A spectrum from a 5 min measurement was collected to obtain signals from short-lived radionuclides and a spectrum from a 60 min measurement was collected to obtain Mn and Ca signals. The spectra were analyzed and the MnBn concentrations were calculated using the calibration line obtained from the Mn-doped bone-equivalent phantoms.

A trained phlebotomist collected a whole blood sample from the participant using standard protocols and a trace-metal-free vacutainer. The participant's skin was cleaned with an

alcohol swab before sampling. All the samples were frozen and kept at −80 °C immediately after the collection. Blood samples were shipped to the Chinese Centers for Disease Control and Prevention in Beijing for assessment of blood MnB using inductively coupled plasma mass spectrometry (ICP-MS) (Ding *et al* 2012). Briefly, the collected blood samples were digested in 0.5% ultrapure nitric acid. The samples were then diluted and analyzed using XSERIES 2 ICP-MS (Thermo Fisher, USA) (Zhang *et al* 2015).

2.6. Statistical analysis

Stata (College Station, TX) was used to analyze MnBn and MnB data.A p-value <0.05 was considered statistically significant. Our data analysis showed that both MnB and MnBn follow lognormal distributions. Therefore, these variables are summarized using medians and interquartile range (IQR) and natural log transformations were used in regression analyses. A constant of 5.99 was added to all MnBn concentrations to ensure that all values were positive prior to log transformation (Atkinson 1985). Spearman rank-order correlations were used to assess associations between exposed/control status, years of employment, MnB, and MnBn. Unadjusted and adjusted linear regression models were created to assess how exposure/control status, years of employment, and MnB predicted MnBn. Covariates included in the adjusted models were age and education.

3. Results

3.1. Phantom spectrum versus *in vivo* spectrum

To verify that the phantom was a good representative of hand bone, the spectra of phantom measurement and *in vivo* measurement were compared. Figure 2 shows the 60 min measurement of a 20-ppm Mn phantom and the 60 min *in vivo* measurement of human hand after 10 min of irradiation and 10 min of decay. The Mn peak is also shown as an enlarged section in the plot. Most of the large peaks are from natural radioisotopes. The background region at the Mn peak for the phantom is similar to that for the *in vivo* measurement. In addition, Ca peaks are comparable to each other. However, the peaks for Na and Cl are significantly different, which indicates that the Na and Cl added to the phantom may not be equivalent to concentrations in real bone.

3.2. Calibration line

The calibration line for the system was established from a set of Mn-doped bone-equivalent phantoms. Each of the phantoms was placed in the irradiation cave and irradiated for 10 min. During the neutron irradiation, Mn along with other elements such as sodium (Na), chlorine (Cl), magnesium (Mg) and calcium (Ca) were activated and characteristic γ-rays were emitted. The activated ^{56}Mn had a half-life of 2.58 h, which gave us sufficient time to transfer the phantom from the irradiation site to the measurement site. A 5 min spectrum was collected using the 100% high-efficiency HPGe detector after a 5 min decay to collect the spectrum for short-lived radioisotopes, such as ^{28}Al, which will not be discussed in this paper. After the 5 min spectrum collection, another 60 min spectrum was collected to obtain Mn and Ca signals. The activation product for Ca was ^{49}Ca, which decays with a half-life of 8.8 min. Although 60 min is not an ideal time period to collect a Ca signal, using the data collected from the same time period will make the Mn/Ca ratio more consistent and does not

impact calculation of Ca because of the low background at the energy range of the Ca peak. Table 1 shows the Mn counts under 847 keV and Ca counts under 3084 keV for 60 min measurements. The uncertainties for the counts were calculated from a peak fitting routine, and the uncertainties for the Mn/Ca ratios were calculated from the uncertainties of the Mn and Ca γ-ray counts.

The calibration line of Mn/Ca ratio versus Mn concentration is illustrated in figure 3. This was used to calculate the MnBn concentrations from the human study.

3.3. Participant demographics and Mn biomarkers

Data in table 2 summarize the study population's age, education, years of employment at their current job, as well as MnBn and MnB concentrations. Among all 60 participants, age was 47.4 ± 7.9 years (mean ± standard deviation), education was 10.0 ± 3.9 years, and years of employment in their current job was 9.0 ± 6.8 years. The mean of all three variables was slightly lower in exposed versus control groups, but these differences were not statistically significant.

Among all participants, the median ± interquartile range of Mn biomarkers were 14.1 ± 3.9 μg l^{-1} for MnB and 2.6 ± 7.2 μg g^{-1} for MnBn. Both median MnBn and MnB were higher in the exposed group versus the control group, although this was of borderline significance for MnBn (MnBn $p = 0.056$; MnB $p = 0.033$). Both mean MnBn and MnB were significantly higher in the exposed group versus the control group (MnBn $p = 0.037$; MnB $p = 0.029$). The variability in MnBn concentrations is also substantial: the IQR is 16.3 μg g^{-1} in the exposed group and 4.6 μg g^{-1} in the control group.

3.4. Correlation between Mn biomarkers and years of employment

Spearman correlation coefficients between exposed/control status, years of employment, MnB, and MnBn are shown in table 3. There is a significant correlation between exposed/control status and MnB ($p = 0.032$) and a marginally significant correlation between exposed/control status and MnBn ($p = 0.055$). There is also a significant correlation of MnBn with years of employment ($p = 0.001$), but the correlation of MnB with years of employment is only marginally significant ($p = 0.068$). MnB and MnBn were not significantly correlated with each other ($p = 0.226$).

Results from unadjusted regression models and regression models adjusted for age and education are presented in table 4. Regression model results do not vary substantially after adjustment, and results are similar to those in table 3. Employment at the ferroalloy factory (versus the manufacturing facility) and years of employment in the current job, but not ln(MnB) are statistically significant predictors of ln(MnBn). Employment in the ferroalloy factory is related to higher ln(MnB), but is of borderline statistical significance following adjustment for age and education (unadjusted $p = 0.017$; adjusted $p = 0.058$). Years of employment in the current job is not a significant predictor of ln(MnB) (unadjusted $p = 0.413$; adjusted $p = 0.372$). To further clarify the result, ln(BnMn) was plotted against year of employment, as shown in figure 4, with ferroalloy and manufacturing factories presented separately.

4. Discussion

A DD-neutron-generator-based IVNAA system was transported to China for the purpose of measuring MnBn in the human body and determining the relationship between MnBn with MnB as well as years of employment. This is the first time a transportable IVNAA system was used to quantify Mn in bone in an occupationally exposed population, and the second time it was used in a human population. In an initial pilot study using the system among US adults, we found a mean MnBn of 0.66 μg g^{-1} (Wells *et al* 2017). *In vivo* MnBn levels have also been reported by Pejovic-Milic *et al* with the MnBn obtained by a larger accelerator-based NAA system (Pejovic-Milic *et al* 2009). They found mean MnBn levels of 2.9 ± 0.4 and 0.1 ± 0.7 μg Mn g^{-1} Ca among the exposed and control groups, which correspond to 0.73 ± 0.10 and 0.03 ± 0.18 μg Mn g^{-1} dry bone respectively (ICRP 70, p 44). Reported means from both studies are substantially lower than the mean (7.1 μg g^{-1}) within this population, although relatively similar to our median concentration (2.7 μg g^{-1}).

Based on the current blood analysis, the median MnB was 15.9 μg l^{-1} in the exposed group and 13.2 μg l^{-1} in the control group. This is notably higher than MnB reported among several community-based studies: geometric mean MnB was reported to be 9.3 μg l^{-1} within a study of Ohio adults (Kim *et al* 2015), the arithmetic mean MnBn from United States adult males was estimated at 9.2 μg l^{-1} (Oulhote *et al* 2014), and median MnB among Chinese males living in a Beijing suburb was 9.6 μg l^{-1} (Zhang *et al* 2015). However, it is within the range of MnB values reported in studies of occupationally exposed populations, many of which fall between 5 and 20 μg l^{-1} (Baker *et al* 2014).

On average, both MnB and MnBn were higher among the ferroalloy workers (exposed) than the manufacturing workers (controls). The unadjusted association of MnB with exposed/control was statistically significant, but for MnBn it was of borderline statistical significance. After adjustment for age and education, we did observe a statistically significant association of MnBn with exposed/control groups. We originally hypothesized that there would be a clearer distinction of MnBn between these groups; however, a closer look at job tasks and occupational history could explain these results. Current jobs the workers were reported performing at both factories could reasonably result in either high or low Mn exposure. For example, current roles among ferroalloy workers include Mn powder processing, ore exaction, ore grinding, Mn electrolysis, and Mn filtration, all of which are likely to have high Mn exposure; however, some reported working in sewage treatment and factory management, which may not result in substantial occupational Mn exposure. Similarly, some participants from the manufacturing facility reported job tasks that would likely have little occupational Mn exposure, such as managers, drivers, and marketing; however, several also reported at least some welding-related tasks, which could result in substantial Mn exposure.

It is important to remember that while we are comparing two different biomarkers of Mn exposure, these measures are expected to represent different time periods of exposure. MnBn, with a half-life of >8 years, is more of a cumulative Mn exposure biomarker, while MnB reflects exposures over the last day or few days before, and is noted to be highly variable (Zheng *et al* 2011, O'Neal *et al* 2014). Thus, it is not surprising that MnB and

MnBn would not be significantly correlated with each other in our analysis, as our participants' occupational Mn exposure may have changed over time. Further support from this comes from reports from our participants that several of the workers from the manufacturing facility had previously been employed at the ferroalloy facility or a similar facility involved with Mn ore processing, which would likely influence the MnBn measurements. This is consistent with our observations that MnBn, but not MnB, was associated with years of employment in the current position.

Some limitations of this study were identified. First, some measurements may be affected by external Mn contamination. Some of these workers' responsibilities require hands-on work with solutions containing Mn, and many of them did not use gloves or other personal protective equipment. This was identified during the data collection period, after noting that the fingernails of some of the participants were discolored. All participants had their fingernails cut and thoroughly washed their hands and lower arms using soap before the MnBn measurement. Alcohol was also used to clean the skin surface of the hand and the lower arm. For several exposed participants, the research assistant further brushed their hand and fingernails using a soft brush to reduce the contamination. While these protocols reduced the potential for external contamination, it is possible that this still may have influenced our results.

A second limitation is that the IVNAA system did not perform at the expected efficiency. This was due to the impaired performance of the HPGe detector induced by the change of the utility frequency from 60 Hz in the US to that of 50 Hz in China. The frequency change downgraded the detector efficiency and elevated background counts. The sensitivity of the system may have been compromised because of the degradation of the HPGe detector. Both issues need to be addressed in our future studies.

Despite these limitations, the study does demonstrate that it is feasible to apply the IVNAA technology to quantify Mn in bone and to use MnBn as a biomarker for cumulative Mn exposure assessment. The study also shows a higher MnBn in ferroalloy workers versus manufacturing workers; and this was strengthened in a posthoc analysis comparing participants based on job title rather than factory. We also observe a significant correlation of MnBn versus current years of employment, which supports that MnBn is a useful biomarker for cumulative Mn exposure.

In conclusion, our data supports use of the IVNAA technology described in this paper to noninvasively quantify Mn concentration in human bone, and MnBn is a promising biomarker for cumulative Mn exposure assessment in larger epidemiologic studies.

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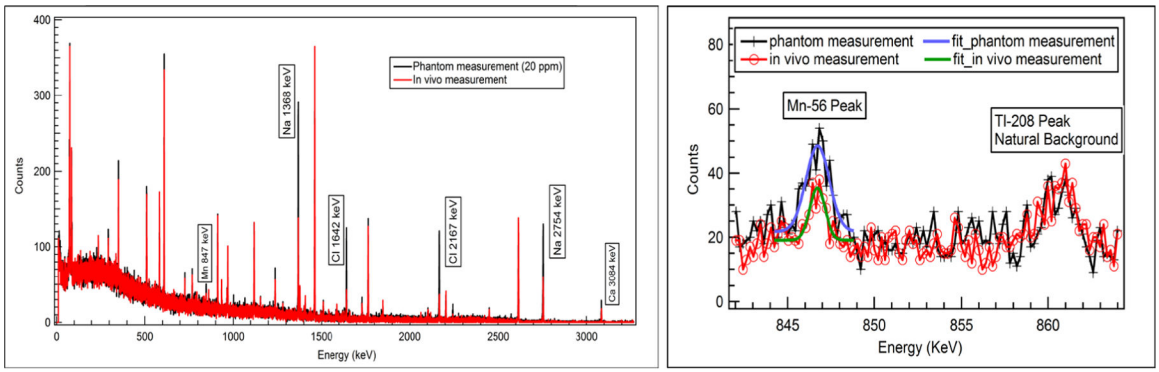
(a) (b)

Figure 1.
The neutron irradiation system (a) and the HPGe γ-ray measurement system (b).



(a) (b)

Figure 2.

(a) Spectra collected by the HPGe detector for a 60 min measurement of a 20 ppm Mn phantom and a 60 min *in vivo* measurement of the human hand with MnBn concentration of 20.6 ppm. (b) The enlarged spectra to demonstrate the Mn-56 peaks and their Gaussian fit.

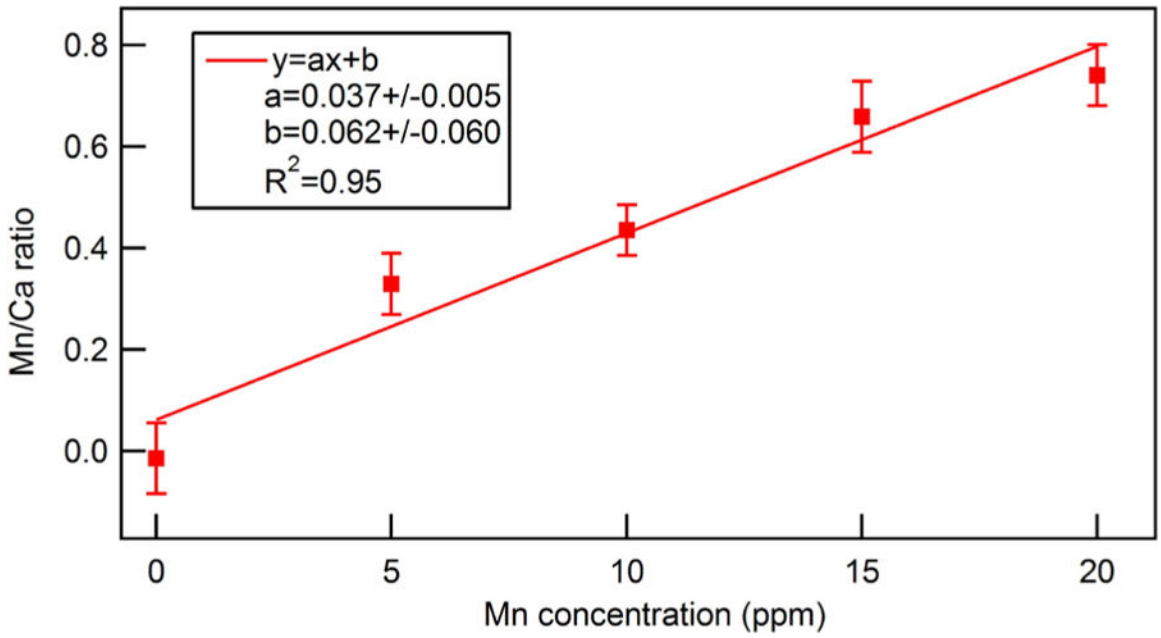


Figure 3.
The calibration line of Mn/Ca ratio per Mn concentration (ppm).

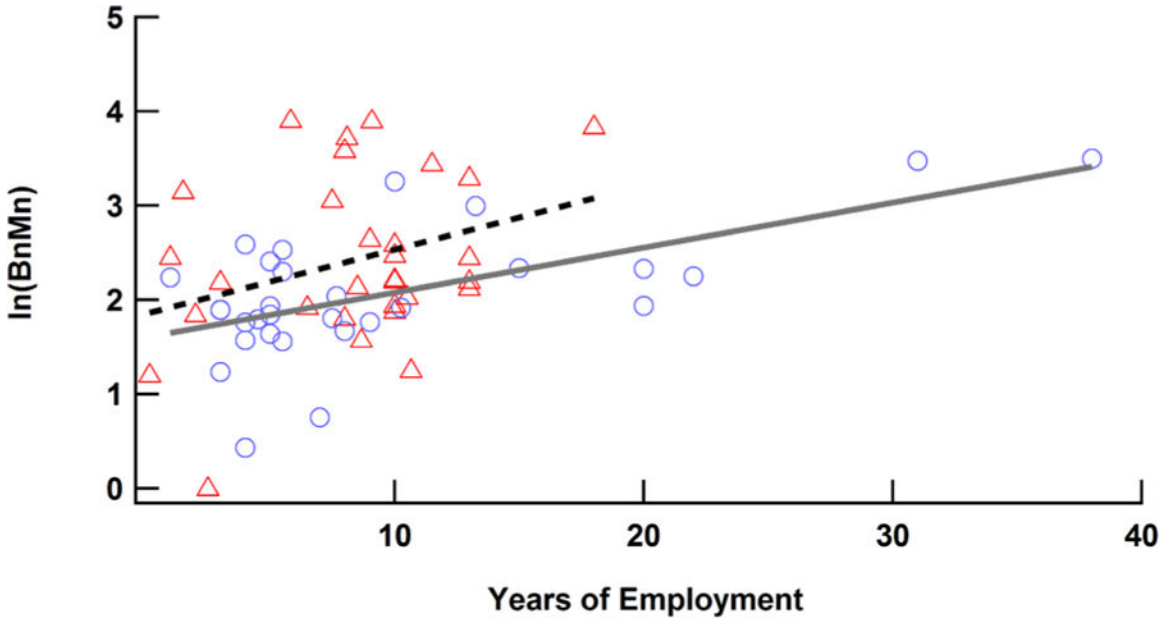


Figure 4.

Natural log transformed bone manganese (ln(BnMn)) by year of employment at current job. Circles represent manufacturing factory (control) participants; triangles represent ferroalloy factory (exposed) participants. The solid and dashed lines represent the unadjusted linear association of ln(BnMn) by years of employment among those from the manufacturing factory (control) and ferroalloy factory (exposed), respectively.

Table 1.

The Mn, Ca counts and Mn/Ca ratio of the Mn-doped bone phantoms from 60 min measurements.

Phantom	^{56}Mn	^{49}Ca	Mn/Ca ratio
0 ppm	−6 ± 18	385 ± 7	−0.01 ± 0.07
5 ppm	87 ± 19	295 ± 6	0.29 ± 0.06
10 ppm	137 ± 21	387 ± 6	0.35 ± 0.05
15 ppm	193 ± 23	326 ± 6	0.59 ± 0.07
20 ppm	240 ± 21	324 ± 6	0.74 ± 0.06

1 ppm = 1 μg g^{-1} phantom. Values are counts or ratio ± uncertainty.

Table 2.

Population characteristics.

	N	Minimum	Maximum	Median	IQR	Mean	SD
Ferroalloy factory (exposed)							
Age (years)	30	33	58	46	8	46.7	6.1
Education (years)	30	1	15	9	6	9.1	3.6
Years of employment	30	0.2	18.0	9.0	4.0	8.4	4.1
Blood Mn (μg l^{-1})	30	9.6	39.7	15.2	5.9	15.9	5.6
Bone Mn (μg g^{-1} dry bone)	30	−5.0	43.0	3.1	16.3	10.3	14.5
Manufacturing factory (control)							
Age	30	29	62	51	17	48.2	9.4
Education (years)	30	2.5	17	11	5	10.9	4.0
Years of employment	30	1.0	38.0	5.5	6.3	9.5	8.7
Blood Mn (μg l^{-1})	30	8.4	22.4	13.5	3.1	13.2	3.3
Bone Mn (μg g^{-1} dry bone)	30	−4.4	27.0	0.9	4.6	3.9	7.9

SD = Standard deviation; IQR = Interquartile range. Years of employment refers to current position.

Table 3.

Correlations between factory of employment, years of employment, MnB, and MnBn.

	Factory of employment	Years of employment	MnB	MnBn
Factory of employment	—			
Years of employment	0.118 (0.371)	—		
MnB	0.277 (0.032)	0.268 (0.068)	—	
MnBn	0.249 (0.055)	0.409 (0.001)	0.159 (0.226)	—

$N = 60$. Values are Spearman's ρ (p-value).

Table 4.

Results from regression models predicting ln(MnBn).

	Unadjusted model			Adjusted model		
Independent variable	***β***	**95% CI**	***p***	***β***	**95% CI**	***P***
Factory of employment	0.37	−0.05, 0.78	0.080	0.44	0.01, 0.87	0.047
Years of employment	0.05	0.02, 0.08	0.001	0.05	0.02, 0.08	0.002
ln(MnB)	0.46	−0.30,1.22	0.233	0.54	−0.27, 1.36	0.188

CI = Confidence interval. $N = 60$. Adjusted models include age and education as covariates.