

# Toenail, Blood, and Urine as Biomarkers of Manganese Exposure

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**Objective:** This study examined the correlation between manganese exposure and manganese concentrations in different biomarkers. **Methods:** Air measurement data and work histories were used to determine manganese exposure over a work shift and cumulative exposure. Toenail samples ( $n = 49$ ), as well as blood and urine before ( $n = 27$ ) and after (urine,  $n = 26$ ; blood,  $n = 24$ ) a work shift were collected. **Results:** Toenail manganese, adjusted for age and dietary manganese, was significantly correlated with cumulative exposure in 7 to 9, 10 to 12, and 7 to 12 months before toenail clipping date, but not 1 to 6 months. Manganese exposure over a work shift was not correlated with changes in blood nor urine manganese. **Conclusions:** Toenails appeared to be a valid measure of cumulative manganese exposure 7 to 12 months earlier. Neither change in blood nor urine manganese appeared to be suitable indicators of exposure over a typical work shift.

There is growing interest in the health effects of manganese exposure. It has long been recognized that, in occupational settings, significant exposure to manganese (Mn) can occur among workers involved in ferroalloy production, iron, or steel foundries, and welding.<sup>1</sup> Mn in the forms of dust and fume is released in these processes, and enter the body primarily via inhalation and lead to adverse health effects. Short-term assessment of exposure to Mn among workers is generally conducted by measurement of airborne Mn from the worker's breathing zone, which assesses the estimated external dose for the duration of measurement. The general population is also exposed to manganese, although generally at lower levels than workers, largely through water, food, or air.<sup>1</sup>

In epidemiologic studies, biomarkers of exposure are useful to verify subject-specific exposures and as an index of internal dose. Blood manganese, urinary manganese, and less frequently nail and hair manganese, have been suggested as biomarkers of exposure.<sup>2</sup> Blood Mn and urinary Mn may reflect exposure over a short and recent period of time (hours to days), while nails and hair are thought to integrate exposure over longer periods of time and thus represent exposures over several months or longer.<sup>3-6</sup> These biological markers can provide important information on the relationship between exposure, the biologically effective dose of an agent at a susceptible site, and the eventual development of clinically apparent outcome.<sup>7,8</sup>

The utility of blood and urine Mn as biomarkers of Mn exposure is questionable according to the findings from several studies.<sup>2,5,9,10</sup> A recent study among occupationally exposed adults<sup>5</sup> found a complex and limited relation between exposure (as measured by total air Mn level and cumulative exposure indices) and blood Mn levels that may depend upon exposure attributes and the latency of blood measurements relative to exposure. Plasma and urine Mn

appeared even less useful as exposure biomarkers. In general, studies have found no or weak relations between blood or urine Mn and exposure, which could be explained by the high variability and short half-life of Mn in blood and urine.

Toenails and hair have been proposed as exposure biomarkers for longer-term cumulative exposure assessment. A number of previous studies have demonstrated that Mn can accumulate in toenails, fingernails, and hair,<sup>4,6,9,11,12</sup> and that Mn accumulation is greater in these matrices among high exposure groups than lower exposure groups.<sup>9,12</sup> These studies, however, have not examined the individual level correlation between external Mn exposure and hair or toenail Mn. A validated biomarker for cumulative exposure to Mn could be of tremendous use in studies of health effects of Mn exposure.

To determine how well toenail Mn reflects cumulative exposures in different exposure windows, we examined the correlation between Mn cumulative exposure indices and toenail Mn concentration among welders at a union welding school. We focused on toenails because hair is thought to be more subject to accumulation of metals due to external contamination, reflecting concentrations out of proportion to the body's actual internal exposure.<sup>4</sup> We studied welders because the determination of external exposures is difficult among the general population whereas specific exposure histories are more easily taken among welders, particularly those with a more limited range of welding jobs like those at the welding school. We also examined the correlation between toenail, blood, and urine Mn concentrations, and the change in blood and urine Mn following Mn exposure over a work shift.

## MATERIALS AND METHODS

### Study Population

The study was approved by the institutional review board of the Harvard School of Public Health. All individuals recruited for the study were informed of the study objectives before obtaining written consent for their participation. Forty-six welders were recruited from a local boilermaker union in June 2008 ( $n = 28$ ) and January 2009 ( $n = 18$ ). Many of them were apprentice welders with less than 3 years experience in welding while the others were more professional welders. These welders participated in our study at the boilermakers welding school where personal work history data and several biological samples were collected. In June 2008, 28 welders were recruited and completed questionnaires. Twenty-seven of these welders provided blood and urine samples at the beginning of the day, and 26 of them welded during the day during which they were monitored for Mn exposure. These 26 provided a second urine sample after their welding and 24 provided a second blood sample. In January 2009, another 14 welders were recruited, completed questionnaires, and provided toenail samples, but blood and urine samples were not collected. An additional 13 welders were recruited via mail and returned questionnaires and toenail samples.

The welding school consisted of a large, temperature-controlled room outfitted with 10 workstations with local exhaust ventilation where the welders received instruction and practiced welding, cutting, and grinding techniques. These welders primarily performed shielded metal arc welding or stick welding and gas metal arc welding or Mig welding, most commonly using base

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There is no competing interest for this research.

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metals of mild steel and stainless steel (manganese, chromium, and nickel alloys) with electrodes composed mainly of iron with variable amounts of manganese (1% to 5%). Plasma arc or acetylene torch cutting and grinding also occurred at the work site. Each participant completed a work log and an exposure diary, to assess the number of hours welded, type of welding done, and use of protective equipment and ventilation.

## Data Collection

### Questionnaires

All participants completed self-administered questionnaires that included items on work history, medical history, and life style variables. A standard food frequency questionnaire, which was slightly modified to focus on Mn-rich foods (tomato juice, grape juice, beer, wine, and liquor, and 15 food items such as wheat germ, pecans, oat meal, sweet potatoes, and spinach), was also used. Each participant was asked to consider a typical serving size of each (indicated in the food frequency questionnaire) for these foods and fill in the one frequency that best reflects his average use of each food in the last year (nine levels from never to 6 and more per day). Then, the amount of Mn intake from foods (mg Mn/month) was calculated for each participant based on nutrient tables for foods<sup>13,14</sup> and the consumption amounts reported on the questionnaire. A detailed work history (dates of specific jobs, welding tasks on that job, type of metal welded, total hours welded, and respirator use) was collected for the preceding 12 months. On days that participants welded, each also completed a work log and an exposure diary, to track the number of hours welded, type of welding done, and use of protective equipment and ventilation.

### Manganese Exposure Assessment

Personal gravimetric samples were collected over the duration of the work shift. We used KTL cyclones (GK2.05SH, BGI Inc, Waltham, MA) with aerodynamic diameter cutpoint of 2.5  $\mu\text{m}$ , in line with personal sampling pumps (APEX/VORTEX, Casella CEL, UK) drawing 3.5 L/min of air. The cyclone was secured to the participant's shoulder in the breathing zone area, and the pump was placed in a padded pouch that was carried by the participant for the entire workday. The cyclone was fitted with a cassette holding a 37 mm polytetrafluoroethylene membrane filter (Gelman Lab, Ann Arbor, MI). Before and after sampling, filters were weighed in a temperature- and humidity-controlled room using a standard protocol on an MT5 microbalance (Mettler-Toledo Inc, Columbus, OH). They were then analyzed for elemental components using a portable NITON XL3t Portable XRF (ThermoFisher, Billerica, MA), as previously described.<sup>15,16</sup> Air samples were collected to represent exposures around major tasks at the welding school. Repeated measurements were taken for most of these tasks and the average for a particular task (eg, stick welding of mild steel using 7018 electrode) was used in the construction of cumulative Mn exposure index (Mn-CEI) for each individual.<sup>17,18</sup>

On the basis of the growth rate of toenails,<sup>19,20</sup> clippings from all ten toenails would be expected to reflect exposure approximately 7 to 12 months before the clipping date. Therefore, we calculated Mn-CEIs from the work histories for the following exposure windows before the clipping date: months 1 to 6, months 7 to 9, months 10 to 12, months 7 to 12, and months 1 to 12, which were treated as measures of average exposure intensity across all exposed tasks in each specific exposure window for each participant.

Cumulative Mn exposure indexes for exposure windows of interest were calculated using each welder's report of specific welding tasks and total hours of welding for that task during the given exposure window, and the reported use of respiratory protection, collected from the work histories. Averaged Mn concentration from air measurements for each specific task was used in the Mn-CEI

calculations. Occasionally welders reported a task for which we did not have air measurements from the school itself; in this case, we used typical values from the literature. The Mn-CEI for an exposure window in the past 12 months was derived by summing (Equation 1) the products of the average Mn exposure intensity, the number of hours worked on that particular task, and an adjustment for the effect of respiratory protection used for each welding task the participant performed during that exposure window (Equation 2).

$$\text{CEI}_w \text{ (mg/m}^3\text{-hr)} = \text{CEI}_{w_1} + \text{CEI}_{w_2} + \text{CEI}_{w_3} + \dots + \text{CEI}_{w_n} \quad [1]$$

$$\text{CEI}_{w_i} = (\text{MnA}_i) (\text{Time}_{w_i}) \left[ \left( \frac{\% \text{respirator}_{w_{ij}}}{\text{APF}_j/2} \right) + \left( \frac{\% \text{respirator}_{w_{ij}}}{\text{APF}_j/2} \right) + \dots \right] \quad [2]$$

Where  $\text{MnA}_i$  is the average air Mn concentration for the  $i$ th task performed;  $\text{Time}_{w_i}$  is the total hours spent for the  $i$ th task performed in the  $w$ th exposure window;  $\% \text{respirator}_{w_{ij}}$  is the estimated percentage of time the participant used the  $j$ th respirator in the  $i$ th task performed in the  $w$ th exposure window; and  $\text{APF}_j$  is the assigned protection factor for the  $j$ th respirator used.<sup>21-23</sup> This yields a measure of cumulative exposure across all exposed jobs in that exposure window, in  $\text{mg/m}^3\text{-hr}$ .

### Biomarkers of Mn Exposure

Pre- and postwork shift blood samples were collected by phlebotomists in trace metal Vacutainers (Becton-Dickinson and Company, Franklin Lakes, NJ) containing EDTA. Pre- and postwork shift urine samples were collected in plastic cups and were aliquoted into 15-mL tubes for metals analysis. Toenail clippings from all toes were collected from each participant in small envelopes. Participants who could not give toenail samples at the time of our visit were asked to mail them in using prestamped envelopes. Participants recruited by mail also mailed in their toenail samples. In each of these cases, the participant indicated the toenail clipping date.

The biological samples were analyzed in the Harvard School of Public Health Trace Metals laboratory, which is equipped with a class-100 clean-room facility and a dynamic reaction cell-inductively coupled plasma mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, CT). Quality control measures are routinely performed in the laboratory and included analysis of initial calibration verification standards (National Institute for Standards and Technology Standard Reference Material 1643d trace elements in water), continuous calibration standards, procedural blanks, duplicate samples, spiked samples, quality control standards, and certified reference material.

Toenail clippings were prepared and analyzed as described by Kile et al, in 2007.<sup>24</sup> Briefly, external contamination was removed from nails by sonicating samples in a 1% Triton X-100 solution (Sigma-Aldrich, Inc, St Louis, MO) for 20 minutes. Toenails were then rinsed repeatedly in Milli-Q water (Millipore Corp, Billerica, MA), dried, weighed, digested in nitric acid, and analyzed using inductively coupled plasma mass spectrometer. Each sample was subjected to five replicate analyses. The net averaged concentration was calculated by subtracting detectable laboratory blank concentrations within each batch.

### Data Analysis

Spearman correlations were used when considering toenail Mn measurements because these concentrations were not normally distributed. When toenail Mn was not involved, Pearson's correlations were used as the other measures were adequately normally

distributed. Correlation coefficients, adjusted for age (year) and dietary Mn (mg/month), were computed to assess the associations between toenail Mn and Mn-CEI for the exposure windows of interest (partial correlation). Correlations between Mn exposure over a work shift and changes in blood Mn or urinary Mn, and the correlations among these three biomarkers of Mn exposure were also determined.

Statistical analyses were performed with Statistical Analysis System (SAS Institute Inc, NC) version 9.1.3. Statistical significance was set at  $\alpha = 0.05$ .

## RESULTS

From the personal exposure monitoring over their work shifts, the concentrations of airborne Mn from welding ranged from 2.02 to 137.41  $\mu\text{g}/\text{m}^3$  (median = 12.89  $\mu\text{g}/\text{m}^3$ ). Cumulative Mn exposure index (median) ranged from 0 to 6.88 (0.52)  $\text{mg}/\text{m}^3\text{-hr}$  for the 7- to 9-month window, 0 to 5.05 (0.44)  $\text{mg}/\text{m}^3\text{-hr}$  for the 10 to 12 month window, 0 to 21.88 (1.23)  $\text{mg}/\text{m}^3\text{-hr}$  for the 1 to 6 month window, 0 to 10.09 (1.15)  $\text{mg}/\text{m}^3\text{-hr}$  for the 7 to 12 month window, and 0 to 24.14 (3.41)  $\text{mg}/\text{m}^3\text{-hr}$  for the past 12 months. Blood Mn ( $n = 27$ ) collected at the beginning of the day ranged from 15.29 to 28.61  $\text{ng}/\text{mL}$  (mean = 18.88  $\text{ng}/\text{mL}$ ) and beginning of the day urinary Mn ( $n = 27$ ) ranged from 0.45 to 3.02  $\text{ng}/\text{mL}$  (mean = 1.50  $\text{ng}/\text{mL}$ ). Collected toenail samples ( $n = 49$ ) had a skewed distribution ranging from 0.05 to 10.41  $\mu\text{g}/\text{g}$  with a median of 0.80  $\mu\text{g}/\text{g}$  (25th to 75th percentiles: 0.45 to 1.49). The distribution of toenail Mn by different characteristics and Mn-CEI for the different exposure windows, are presented in Table 1. Toenail Mn tended to increase with age, was higher in whites, and increased with the Mn-CEIs for the exposure windows between 7 and 12 months prior to the toenail clipping date. However, toenail Mn did not appear to be higher with increased dietary Mn intake. There was little relation between either blood Mn or urinary Mn, and any of the variables (data not shown).

The Spearman correlations between toenail Mn and past Mn-CEI, adjusted for age and dietary Mn intake, were significant for the three Mn-CEI windows encompassing months 7 to 12 before the toenail clipping date (7th to 9th months, 10th to 12th months, and 7th to 12th months), ranging from 0.32 to 0.35 (Table 2). The unadjusted correlations were similar but slightly weaker. Pearson's correlation analyses on square root transformed toenail Mn and Mn-CEI also yielded similar coefficients. There was little correlation between toenail Mn and any of the other Mn-CEI exposure windows, nor either prework shift blood Mn or urinary Mn. In contrast, the adjusted correlation between prework shift blood Mn and urinary Mn was reasonably high ( $r = 0.33$ ) and close to significant ( $P = 0.10$ ). There was little correlation between postwork shift blood and urine ( $r = 0.06$ ;  $P = 0.78$ ), or between the change in each ( $r = 0.03$ ;  $P = 0.88$ ).

There was no correlation between air Mn exposure over a work shift (mean duration = 5.83 hours; SD = 1.02 hours) and either postshift blood Mn concentration or change in blood Mn concentration over the work shift (Table 3). Air Mn exposure over a work shift was slightly more correlated with postwork shift urinary Mn and change in urinary Mn over the work shift, although these did not reach statistical significance. Pre- and postwork shift concentrations of both blood Mn and urinary Mn, however, were significantly correlated. Prework shift blood Mn and urinary Mn were also each correlated with the change in those measures, although inversely and only statistically significantly for change in blood Mn (Table 3).

## DISCUSSION

Our study demonstrates that toenail Mn, averaged over clippings from all 10 toes, is correlated with Mn-CEI windows encompassing months 7 to 12 before the toenail clipping date, which is the exposure window it would be expected to reflect on the basis of toenail growth rates. One of the first estimates reported a toenail growth

**TABLE 1.** Distributions of Toenail Mn by Different Characteristics of the Participants and Their Cumulative Exposure Index Levels (Mn-CEI) Over Different Windows

Characteristics	n	Mean (SD)	Toenail Mn ( $\mu\text{g}/\text{g}$ )	
			Median	25th—75th Percentiles
<b>Age</b>				
<35 (years)	17	26.9 (4.6)	0.46	0.26–1.49
35–50 (years)	15	41.1 (4.5)	0.79	0.50–1.17
>50 (years)	17	55.5 (3.6)	1.10	0.56–1.95
<b>Years of Welding</b>				
<5 (years)	19	1.66 (0.8)	0.69	0.30–1.59
5–10 (years)	15	7.58 (1.8)	0.80	0.54–1.13
>10 (years)	15	25.6 (7.1)	0.81	0.35–1.61
<b>Race</b>				
White	40		0.91	0.51–1.54
Nonwhite	9		0.46	0.11–0.46
<b>Body mass index</b>				
$\leq 25$	7	23.15 (1.0)	0.56	0.30–1.40
25–30	26	27.18 (1.2)	0.81	0.45–1.26
>30	14	34.12 (3.1)	0.74	0.46–1.44
<b>Dietary Mn</b>				
$\leq 40$ mg/month	15	25.55 (12.2)	0.65	0.37–1.76
40–85 mg/month	17	58.57 (11.5)	0.86	0.58–1.24
>85 mg/month	17	145.4 (51.7)	0.79	0.44–1.27
<b>Mn-CEI</b>				
For months 1–6				
<0.7 $\text{mg}/\text{m}^3\text{-hr}$	19	0.17 (0.24)	0.79	0.27–1.49
0.7–4.0 $\text{mg}/\text{m}^3\text{-hr}$	14	1.75 (0.98)	0.93	0.32–1.63
>4.0 $\text{mg}/\text{m}^3\text{-hr}$	16	7.92 (4.30)	0.76	0.54–1.21
For months 7–9				
<0.3 $\text{mg}/\text{m}^3\text{-hr}$	16	0.06 (0.09)	0.39	0.19–0.93
0.3–1.0 $\text{mg}/\text{m}^3\text{-hr}$	16	0.52 (0.20)	0.84	0.57–1.49
>1.0 $\text{mg}/\text{m}^3\text{-hr}$	17	2.28 (1.03)	1.10	0.51–1.70
For months 10–12				
<0.3 $\text{mg}/\text{m}^3\text{-hr}$	18	0.05 (0.07)	0.58	0.27–0.81
0.3–1.0 $\text{mg}/\text{m}^3\text{-hr}$	18	0.57 (0.24)	0.98	0.40–1.49
>1.0 $\text{mg}/\text{m}^3\text{-hr}$	13	2.63 (1.16)	1.19	0.56–2.13
For months 7–12				
< 0.7 $\text{mg}/\text{m}^3\text{-hr}$	19	0.20 (0.26)	0.46	0.21–0.81
0.7–2.0 $\text{mg}/\text{m}^3\text{-hr}$	15	1.29 (0.36)	1.07	0.54–1.59
>2.0 $\text{mg}/\text{m}^3\text{-hr}$	15	4.61 (2.12)	1.10	0.54–1.70
For Past 12 Months				
<1.5 $\text{mg}/\text{m}^3\text{-hr}$	20	0.64 (0.76)	0.62	0.21–1.37
1.5–7.0 $\text{mg}/\text{m}^3\text{-hr}$	14	3.99 (1.42)	1.17	0.53–1.76
>7.0 $\text{mg}/\text{m}^3\text{-hr}$	15	11.86 (4.84)	0.80	0.53–1.23

Mn-CEI, manganese-cumulative exposure index.

rate of approximately 0.050 mm per day,<sup>19</sup> which would suggest that clippings should reflect exposures, on average, 8 to 9 months before the clipping date. A recent study<sup>20</sup> found slightly faster nail growth rates, and suggested that the great toenails grow faster than other toenails—at the estimated rate of 2.09 mm/month. Since it is much longer than other toenails, great toenails (average length of 2 cm) may represent exposures more than 10 months before clipping.<sup>20</sup> The average exposure window reflected would be 1 to 2 months earlier than this if nails from all 10 toes were used. These are consistent

**TABLE 2.** Associations Between Toenail Mn ( $\mu\text{g/g}$ ), Prework Shift Blood and Urine Mn (ng/mL), and Mn-CEI ( $\text{mg/m}^3\text{-hr}$ ) Exposure Windows

	Partial Correlation*					
	Toenail Mn ( $\mu\text{g/g}$ )		Blood Mn (ng/mL)		Urinary Mn (ng/mL)	
	$\rho$	P	$\rho$	P	$\rho$	P
Blood Mn (ng/mL)	(n = 21)					
	0.11	0.66	1.00			
Urinary Mn (ng/mL)	(n = 21)		(n = 27)			
	-0.18	0.46	0.33	0.10	1.00	
Mn-CEI, $\text{mg/m}^3\text{-hr}$	(n = 49)		(n = 27)		(n = 27)	
Months 1–6	0.07	0.62	0.05	0.82	-0.04	0.84
Months 7–9	0.35	0.016	0.12	0.58	0.02	0.94
Months 10–12	0.32	0.031	0.17	0.42	0.06	0.77
Months 7–12	0.32	0.027	0.07	0.75	0.01	0.97
Past 12 months	0.19	0.19	0.15	0.46	-0.01	0.98

Mn, manganese; CEI, cumulative exposure index.

\*Adjusted for age (year) and dietary Mn (mg/month). Spearman correlations were used when toenail Mn or CEI was involved.

**TABLE 3.** Correlations Between Work Shift Mn Exposure ( $\mu\text{g/m}^3$ ), and Blood/Urinary Mn (ng/mL)

	n	Pearson's Correlation $\rho$ (P value)	
		Postshift	Change*
Blood			
Preshift	27	0.63 (<0.01)	-0.66 (< 0.01)
Air Mn exposure	26	-0.07 (0.75)	-0.06 (0.80)
Urine			
Preshift	27	0.45 (0.02)	-0.22 (0.30)
Air Mn exposure	26	0.14 (0.52)	0.21 (0.32)

Mn, manganese.

\*Change over work shift: Postshift concentration minus preshift concentration.

with the findings from our study that the correlation between toenail Mn and Mn-CEI was strongest for exposure windows in the range of 7 to 12 months before clipping. This is also consistent with a previous study of toenails as markers of selenium intake.<sup>25</sup> Toenail Se concentration was unaffected by dietary intake in the 3 months before nail clipping and appeared to provide a time-integrated measure of intake over a period of 26 to 52 weeks (~6.5 to 12 months) before nail clipping, which is essentially the same exposure window as ours.

A few studies have suggested that the human toenail could be used as a biomarker of exposure to other elements such as arsenic<sup>24</sup> and selenium.<sup>25,26</sup> However, we are aware of only two studies that looked at toenails as biomarkers of Mn exposure. One study compared toenail Mn to whole blood Mn and fecal Mn collected at the same time among exposed workers.<sup>6</sup> No assessment of individual level external exposures, however, was made in this study. They observed poor correlations between the different biomarkers and concluded that toenails might not be a good biomarker for Mn. Another study in a general population sample compared toenail Mn among multivitamin users to matched controls and found that toenail Mn was not significantly higher among Mn supplement users.<sup>4</sup> This is consistent with our finding that toenail Mn did not tend to increase with dietary Mn, although we did observe slightly stronger

correlations with Mn-CEIs after adjusting for age and dietary Mn. Unlike other elements (eg, As),<sup>24</sup> Mn from food intake may not make a significant contribution to internal dose represented by toenail Mn. It is also possible, however, that increases in toenail Mn from dietary sources could have been masked by a relatively larger amount of occupational exposure to Mn by inhalation.

It was not unexpected that blood Mn and urinary Mn were not well correlated with toenail Mn, nor any of the Mn-CEI given the relatively short half-life (several days) of Mn in blood and urine.<sup>7,27</sup> A number of previous studies suggested that blood Mn and urinary Mn may be used to discriminate groups of exposed and nonexposed subjects, but not differences in exposure on an individual basis.<sup>2,5,9–10</sup> Morning blood and urine samples were reasonably well correlated with each other, suggesting that the exposure windows they reflect are similar. Because of the shorter time frames of blood Mn and urinary Mn as exposure biomarkers, we were interested in how well these biomarkers reflected Mn exposure across a work shift. However, changes in both blood Mn and urinary Mn over a work shift were not well correlated with air Mn exposure over that time. Intriguingly, there was a strong significant inverse correlation between prework shift blood Mn and change in blood Mn over the work shift. This suggests that there may be homeostatic pressures that limit the rise in blood Mn such that those who have a high blood Mn concentration before the work shift do not increase blood Mn concentration as much as those whose blood Mn is lower before the work shift. There was a similar although less strong and significant trend for urinary Mn. Small sample size for blood and urine may limit our ability to observe such relationship.

A strength of our study is that the population was recruited from a welding school, which meant that the range of their welding exposures to Mn over the prior year was more limited than in many other settings and we were able to take air measurements during most of the types of welding tasks they performed over the past year. These factors should have helped increase the accuracy of our Mn-CEI. Nonetheless, Mn-CEI created on the basis of retrospective work histories is likely to have some error that would reduce the correlation between toenail Mn and true Mn exposure for a given window. Thus, the correlations we found may still underestimate correlations with actual air Mn exposures.

A broader implication of our results relate to the use of toenails as a biomarker of Mn exposure in nonoccupational settings. In

our occupational study setting air Mn exposures are likely far more variable than in the general population because the exposures depend heavily on job or training-related exposure and these can vary substantially from month to month (welders often have periods of intense work activity followed by periods of no work activity and these can vary month to month). As a result air Mn exposure in months 1 to 6 before toenail clipping are less correlated with air Mn exposure 7 to 12 months before clipping than they might be in a general population sample. Thus, the utility of toenail Mn as an exposure metric in an occupational setting is likely restricted to a period of 7 to 12 months before the clipping date. However, to the extent that external exposures are more consistent, as might be expected in a general population setting, toenail Mn may be a better biomarker for more general cumulative exposure. Furthermore, toenails have important practical advantages over other samples such as blood and urine in that they are easier to collect (eg, they can be collected by mail with no special mailing requirements) and store.

Hair and fingernail tissues are similar to toenail and have also been explored to some extent as biomarkers of Mn exposures, although they are generally thought to be more subject to external contamination because they are more frequently open to the external environment than toenails. This may explain in part why the few studies that have explored these tissues as biomarkers of Mn exposure suffered from a relatively great variation as well as from analytical problems.<sup>9,11</sup>

## CONCLUSIONS

Our study is, to our knowledge, the first to assess the relation between external exposure to manganese and toenail Mn concentration on an individual basis. Among this population of boilermaker welders, neither blood nor urine was reflective of cumulative exposure in the prior year and changes in Mn concentration in these media over a welding work shift did not correlate well with Mn exposure over that time. Toenail Mn, however, was well correlated with exposures 7 to 12 months earlier. While we conducted this study among occupationally exposed workers to better assess the external exposures, the results may be applicable to the general population, where toenail Mn may well be reflective of a more extended exposure window since external exposures in the ambient settings are likely to be more consistent than in occupational settings.

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