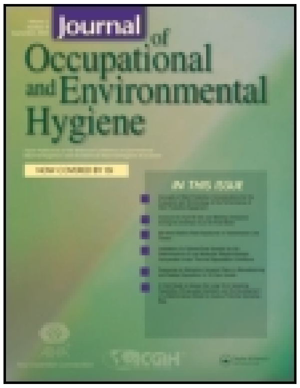

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Rachel Grashow[a], Jinming Zhang[a], Shona C. Fang[ab], Marc G. Weisskopf[ac], David C. Christiani[ac] & Jennifer M. Cavallari[ad]

[a] Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts

[b] New England Research Institutes, Watertown, Massachusetts

[c] Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts

[d] Division of Occupational and Environmental Medicine, University of Connecticut Health Center, Farmington, Connecticut

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Toenail Metal Concentration as a Biomarker of Occupational Welding Fume Exposure

Rachel Grashow,[1] Jinming Zhang,[1] Shona C. Fang,[1,2] Marc G. Weisskopf,[1,3] David C. Christiani,[1,3] and Jennifer M. Cavallari[1,4]

[1]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts
[2]New England Research Institutes, Watertown, Massachusetts
[3]Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts
[4]Division of Occupational and Environmental Medicine, University of Connecticut Health Center, Farmington, Connecticut

In populations exposed to heavy metals, there are few biomarkers that capture intermediate exposure windows. We sought to determine the correlation between toenail metal concentrations and prior 12-month work activity in welders with variable, metal-rich, welding fume exposures. Forty-eight participants, recruited through a local union, provided 69 sets of toenail clippings. Union-supplied and worker-verified personal work histories were used to quantify hours welded and respirator use. Toenail samples were digested and analyzed for lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni), and arsenic (As) using ICP-MS. Spearman correlation coefficients were used to examine the correlation between toenail metal concentrations. Using mixed models to account for multiple participation times, we divided hours welded into three-month intervals and examined how weld hours correlated with log-transformed toenail Pb, Mn, Cd, Ni, and As concentrations. Highest concentrations were found for Ni, followed by Mn, Pb and As, and Cd. All the metals were significantly correlated with one another (rho range = 0.28–0.51), with the exception of Ni and As (rho = 0.20, p = 0.17). Using mixed models adjusted for age, respirator use, smoking status, and BMI, we found that Mn was associated with weld hours 7–9 months prior to clipping (p = 0.003), Pb was associated with weld hours 10–12 months prior to clipping (p = 0.03) and over the entire year (p = 0.04). Cd was associated with weld hours 10–12 months prior to clipping (p = 0.05), and also with the previous year's total hours welded (p = 0.02). The association between Ni and weld hours 7–9 months prior to clipping approached significance (p = 0.06). Toenail metal concentrations were not associated with the long-term exposure metric, years as a welder. Results suggest Mn, Pb, and Cd may have particular windows of relevant exposure that reflect work activity. In a population with variable exposure, toenails may serve as useful biomarkers for occupational metal fume exposures to Mn, Pb, and Cd during distinct periods over the year prior to sample collection.

Keywords Toenail, welding fume, lead, manganese, cadmium, biomarker

Address correspondence to: Rachel Grashow, Department of Environmental Health, Harvard School of Public Health, Landmark Center, 401 Park Drive, 3rd Floor, Boston, MA 02215; e-mail: rgrashow@hsph.harvard.edu

INTRODUCTION

Welders are exposed to heavy metals including lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni), and arsenic (As) when molten metal from steel, electrodes, or wires is volatized. Small spherical particles (50–300 nm in diameter) contained in volatilized welding fume can reach deep into the alveolar region of the lung and initiate health effects.(1) Additionally, toxicological studies suggest that these small particles may bypass the blood-brain barrier by traveling through the olfactory nerves to brain areas, initiating a cascade of central nervous system effects.(2) Intermediate and long-term weld fume exposures have been shown to have cardiovascular,(3,4) pulmonary,(5,6) and neurological effects,(7–9) underscoring the need for biomarkers of long-term exposure that can be used in risk assessment.

The choice of an appropriate biomarker is, in part, a reflection of the relationship between exposure and biomarker and the exposure-time period that the biomarker reflects. For example, a metal's half-life may be relevant when exposures are intermittent, but should exposure be constant, a steady state may be reached. In a study that examined the utility of blood Mn measurements in welders working on the California Bay Bridge, the authors found that blood Mn was associated with total air Mn in low and moderately exposed workers with constant exposure, but not for those exposed to the highest Mn levels.(10) Blood Pb has a half-life in blood of approximately 30 days,(11,12) making it a poor biomarker for intermediate exposure. For Cd the half-life is 12 years in urine(13) and 7–16 years in blood,(14,15) indicating that it better represents longer rather than intermediate exposures. Choosing an appropriate biomarker requires careful consideration of multiple factors related to both the biomarker as well as the exposure.

Toenail clippings collected from all ten toes are likely to reflect exposure integrated over the previous 6–12 months,[16] due to a growth rate of approximately 1.6 mm/month[17] and an average great toenail length of 20 mm.[18] Given that nails are non-invasively and painlessly collected, and easy to store and transport, nail metal concentration should be evaluated as a potential biomarker of internal dose for both occupational and environmental exposures. However, questions remain about what exposure window is captured by toenail samples, what exposures it may reflect, the ability to discriminate between the toxicants found in toenails and what external exposure measures are best for comparing to toenail metals.

Toenails have been evaluated as biomarkers in a variety of research settings, including environmental[19–21] and occupational[22–26] exposures, posthumously,[27,28] and in children.[29–31] Such studies have measured toenail metal concentrations of methylmercury,[32,33] Pb,[34,35] Cd,[36,37] As,[38–40] Mn,[24,41]and Ni[20] and more. Mn and Ni have been isolated in toenail samples from welders.[22,24] Cd and Pb were found in nail tissue samples in deceased copper smelter workers.[28] In Mortada et al.,[42] Pb was measured in toenail samples taken from police officers exposed to traffic pollution, which were significantly associated with increased markers of nephrotoxicity. Higher Ni concentrations have been found in the fingernails of welders[43] and other metal workers.[25] A recent study in rats found that exposure to Mn in welding fumes was correlated with manganese concentration in nails, as well as Mn accumulation in dopaminergic brain areas,[44] indicating that toenail metal concentration may reflect neurotoxicant deposition in the brain. Therefore it was reasonable to assume that Pb, Mn, Cd, Ni, and As would similarly be present in toenails of the welders in this study, but also might be accumulating in and affecting regions like the kidneys, lungs, and brain.

Previously, Laohaudomchok et al.[24] explored the utility of toenails as a biomarker of Mn exposure in a group of boilermaker welders with variable exposures. Boilermakers are welders trained to work on round vessels or pipes located within power plants. Such maintenance and repair work is largely seasonal, with most welding being performed during times of low energy need in the spring and fall months. Furthermore, union contracts can vary from one day to one year, adding additional variability to metal fume exposures. The high variability of occupational exposure for welders makes it an ideal population to explore the time window of exposure for biomarkers, since constant exposure is rare. In their study, Laohaudomchok et al.[24] used in-depth work history data to construct a cumulative exposure index over a work shift and over a year Mn (CEI-Mn). CEI-Mn was calculated using ambient air Mn concentration, type of welding performed, hours spent on each task, percentage of time working with respirator, and the protection factor associated with that respirator type. They found that after adjusting for age and dietary Mn, toenail Mn concentration was significantly associated with CEI-Mn for 7–9, 10–12, and 7–12 months prior to toenail clipping.

Recruiting from within the same base population of boilermakers, we sought to evaluate the association between total hours welded and toenail Mn concentrations over similar time periods as observed by Laohaudomchok et al.[24] Given a sample size of nearly 50 individuals, a detailed CEI-Mn could not be calculated, and as is common in occupational studies, a simplified exposure metric was used. Building upon the findings of Laohaudomchok et al.,[24] we wanted to explore whether in addition to Mn, other toenail metals (Cd, As, Pb, and Ni) were also related to welding exposures in this population, and to what extent the toenail metal concentrations correlated with one another. Specifically, we wished to use welding hours to identify the relevant window of exposure that toenail concentrations reflect. Given that the toenail clippings are easy to acquire, transport, store ,and analyze they may serve as an ideal biomarker of intermediate-term metal exposures.

METHODS

Study Population

Participants were recruited from members of a local boilermaker union located in Quincy, Massachusetts. Participants included journeymen and apprentice welders enrolled in a two-year training program, as well as retired welders. Union members, including retirees, were invited to participate in the study through letters sent by union leadership informing members of the study dates. In addition, current and apprentice welders were recruited on site. Recruitment occurred between January 2010 and June 2011 over four study site visits, resulting in a total of 73 welders recruited. Only participants who provided complete work history data, demographic data (age, height, weight, race, smoking status) and had complete toenail metal concentrations for all five metals were included in this analysis, totaling 48 welders. Welders were allowed to participate during each of the site visits. Therefore, some participants contributed multiple samples: eleven subjects provided two toenail samples over the 2010–2011 study period, and five participants provided three samples.

Work History and Questionnaire Data

Union-maintained work histories preceding toenail collection by 12 months were used by participants to reconstruct specific job activities and exposures. Specifically, participants reviewed job descriptions from union records from the previous year, providing specifics on respirator use, welding tasks performed, job dates, total hours welded, metal used, and location of work (indoor vs. outdoor, work site). Respirator use was reported as a percentage of time during which a full, half, or filterless mask was used for each job. These data were used during analysis to construct month-by-month total hours welded and percentage of hours used with a respirator for each participant over the preceding 12 months. This study focused on weld hours as the primary exposure measure.

The 12 months prior to toenail collection were divided into quarters. Q1 represents the first three months prior to toenail collection, Q2 represents the fourth to six months preceding toenail collection, Q3 represents months seven through nine, and Q4 represents months 10–12. Nail clippings are expected to represent exposure over the previous 6–12 months,[45] thus reflecting longer-term exposures than urine or blood, although individual toenail growth rates may vary. Welding hours were tabulated in each quarter,

Study participants also completed self-administered lifestyle questionnaires that included height, weight, smoking status, medical history, and number of years as a welder or boilermaker.

Toenail Metal Collection and Analysis

Study participants with adequate toenail growth clipped all 10 toenails at the study site, and placed them in a small envelope. Participants without adequate toenail growth were given pre-stamped addressed envelopes to be returned after the next toenail clipping, with the indicated clip date. Most of the toenail samples (68.1%) were collected on the same day as work history questionnaire completion. Subjects providing toenails from clippings 21 or more days after questionnaire information collection were excluded. Sensitivity analyses using main models were performed on only subjects with clipping lag times of one day or less to confirm that longer lag times did not bias results.

Toenail samples were analyzed for concentrations of lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni), and arsenic (As) at the Harvard School of Public Health Trace Metals Laboratory, using a dynamic reaction cell-inductively coupled plasma mass spectrometer (DRC-ICP-MS, Elan 6100, Perkin Elmer, Norwalk, Conn.). Quality control measures performed in the laboratory include analysis of initial calibration verification standards (NIST SRM 1643d trace elements in water), continuous calibration standards, procedural blanks, duplicate samples, spiked samples, quality control standards, and certified reference material.

Toenail clippings from all ten toes were combined for each sample and analyzed as previously described.[38] Briefly, prior to ICP-MS, external contaminants were removed by sonication using 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., Billlerica, Mass.), dried, weighed, and digested in nitric acid. Each subject sample underwent five replicate analyses. The net averaged concentration for each metal was calculated by subtracting detectable laboratory blank concentrations within each batch.

Statistical Analysis

Toenail metal concentrations were not normally distributed so Spearman correlations were used in metal concentration comparisons, and geometric mean calculated to describe overall toenail concentration values. Nonparametric one way analysis of variance Kruskal-Wallis tests were used on the weld hour summary data to compare across time intervals. We used linear mixed models to estimate the associations between weld hours and toenail metal concentrations due to the presence of multiple toenail measurements for some participants. Toenail metal concentrations were skewed, so all toenail values used in models were log-transformed. To determine whether there was a relationship between toenail metal concentration and hours welded, we separately modeled the logarithm of each toenail metal as a function of weld hours for each quarter, as well as across the entire year in a separate model that encompassed all work history data for that sample. All models were adjusted for BMI, age, respirator use, and smoking status.

Percentage of hours welded while wearing a full or half-face respirator was combined into a single variable, while use of a dust mask was considered equivalent to unprotected welding. Percentage of time with a respirator was modeled as a continuous variable. A separate sensitivity analysis used a logit-transformed percentage of respirator weld hours variable. Participants with missing respirator data were assigned 0 for the respirator use variable in an additional analysis, thus assuming maximum exposure to weld fume.

RESULTS

The study sample included 47 men and one woman. The average age at first participation in the study was 39 years (standard deviation [SD] = 12.1). Additional participant characteristics are shown in Table I. On average, the participants had 11.2 (8.6) years of experience as a welder, and 8.6 (8.8) years as a boilermaker. This difference between these numbers may be due to the fact that some of the participants may have entered the welding apprentice program with prior welding experience. As expected, years as a boilermaker and years as a welder were highly correlated in this population ($\rho = 0.60$, $p < 0.0001$). Twelve out of 69 (17.3%) toenail samples were missing respirator use data. Percentage of weld hours performed with a respirator was not associated with age, nor was percentage of respirator hours associated with number of hours welded across any of the time intervals.

TABLE I. Participant (n = 48) Demographics and Characteristics

Characteristic	Mean	SD
Age at first participation	39.0	12.1
Body mass index (BMI)	27.9	4.7
Years as a boilermaker	8.6	8.8
Years as a welder	11.2	8.6
Respirator use over full year (%)	40.2	31.2
	n	%
White	39	81
Current smokers	18	38
Male	47	98

TABLE II. Hours Worked Prior to Toenail Clipping

Time period	Median	GM	GSD	IQR
1–3 months (Q1)	90.0	91.0	3.1	172.0
3–6 months (Q2)	38.0	57.8	4.0	142.0
6–9 months (Q3)	38.4	67.1	4.4	110.8
9–12 months (Q4)	3.6	40.0	5.3	60.0
1–12 months	279.3	256.1	2.7	424.4

Notes: Data include multiple samples for some participants (n = 69 observations, n = 48 participants); GM = geometric mean; GSD = geometric standard deviation; IQR = interquartile range.

Work hours corresponding with each toenail sample and quarter were averaged across participants (Table II). Using non-parametric ANOVAs, we found that the distributions of logged hours worked across each quarter were not the same (p = 0.04). This is likely due to seasonal differences in work activity, as well as the timing of subject testing: work hours were lowest in the period 10–12 months prior to subject testing, which is carried out in the early summer and winter. This indicates that hours worked were less during November–January and April– June.

To determine whether long-term exposure to metals correlated with shorter-term exposure, we ran regression models that compared years as a welder or boilermaker to average hours worked over each of the four three-month intervals. There were no significant correlations between years as a boilermaker or welder and average hours worked across any of the quarters (data not shown). Overall, these results indicate that lifetime cumulative exposures are not related to hours welded in the past year, minimizing the possibility of confounding by years at work.

Toenail metal concentrations of lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni), and arsenic (As) were taken from each participants' first study visit and used to calculate summary statistics (Table III). Cd was found at the lowest concentration, with 4.3% of samples falling below the limit of detection. Ni had the highest concentration in toenail samples. When toenail metal concentration values from multiple visits were included, the resulting geometric mean and other summary statistics remained similar (data not shown).

TABLE III. Summary Statistics for Toenail Metal Concentrations (μg/g toenail) at First Participation (n = 48)

Metal	DL	Below DL (%)	GM	GSD	Median	IQR
Pb	0.002	0	0.39	3.00	0.35	0.57
Mn	0.003	0	1.03	2.84	0.81	1.31
Cd	0.002	4	0.02	2.40	0.02	0.02
Ni	0.019	0	2.53	3.50	2.19	2.04
As	0.009	0	0.19	1.91	0.17	0.19

Note: DL = detection limit; GM = geometric mean; GSD = geometric standard deviation; IQR = interquartile range.

TABLE IV. Spearman Correlations (ρ) and Statistical Significance (P) Between Toenail Metals at First Participation (n = 48)

Metal	Mn	Cd	Ni	As
Pb	$\rho = 0.32$	$\rho = 0.51$	$\rho = 0.34$	$\rho = 0.49$
	$p = 0.03$	$p < 0.001$	$p = 0.02$	$p < 0.001$
Mn		$\rho = 0.60$	$\rho = 0.31$	$\rho = 0.37$
		$p < 0.001$	$p = 0.03$	$p = 0.01$
Cd			$\rho = 0.28$	$\rho = 0.37$
			$p = 0.05$	$p = 0.01$
Ni				$\rho = 0.20$
				$p = 0.17$

Correlating biological outcomes with specific metal exposure depends on the ability to distinguish the concentrations of one metal from another. Using Spearman correlations, we calculated the associations between each of the five metals (Table IV). We found that all the metals were significantly correlated with one another, with the exception of Ni and As which were not significantly associated with one another.

Using mixed effects models, we evaluated the association between weld hours and log-transformed toenail metal concentrations after adjusting for age, respirator use, smoking status, and BMI (Table V). Individual models were run for each metal and quarterly time period as well as yearly time period (sum of Q1–Q4). No associations were seen with any of the metals and the first two quarters (Q1–Q2, representing the most recent 0–6 months of exposure). This is to be expected; nail samples included in the clippings were mostly likely laid down much earlier than 0–6 months, given toenail growth rates.

Pb toenail concentration was associated with hours welded for the fourth quarter (10–12 months prior to toenail collection) and across the entire year (Table V), although this may be due to the highly correlated relationship between Q1 and Q1–Q4 (rho = 0.495, p < 0.0001). Toenail Mn concentration was only associated with weld hours for the third quarter (months 7–9). Cd concentration was significantly associated with weld hours during the fourth quarter, and summed weld hours across the entire year. Toenail Ni concentration was marginally associated with hours welded in the 4th quarter (p = 0.06). As was not significantly associated with weld hours over any time interval. Age and smoking were not significant in any of the models. The association between BMI and toenail As was significant for Q2 ($\beta = -0.0492$, 95% CI = -0.0966, -0.00178, p = 0.0433), and approached significance for Q1 and Q4. In these models, percentage of respirator weld hours was associated with toenail metal concentration for Mn in the Q4 only ($\beta = -0.1877$, 95% CI: -0.34, -0.04, p = 0.02), but not for any other metal/time interval model combination.

TABLE V. Mixed Model Results Showing Associations Between Weld Hours and Log-Transformed Toenail Metal Concentrations (μg/g toenail), Adjusted for Age, Respirator Use, Smoking Status, and BMI

Metal	Q1 β [95% CI]	Q2 β [95% CI]	Q3 β [95% CI]	Q4 β [95% CI]	Q1–Q4 β [95% CI]
Pb	0.002 [−0.001, 0.004]	0.0005 [−0.002, 0.003]	0.0001 [−0.002, 0.002]	*0.003 [0.0004, 0.006]	*0.001 [0.0001, 0.002]
Mn	−0.0004 [−0.003, 0.003]	0.0002 [−0.002, 0.003]	**0.0032 [0.0014, 0.0051]	0.0006 [−0.002, 0.003]	0.0006 [−0.0004, 0.002]
Cd	0.0015 [−0.0009, 0.004]	0.0006 [−0.001, 0.003]	0.001 [−0.0005, 0.003]	*0.002 [0.000, 0.004]	*0.001 [0.0001, 0.002]
Ni	−0.001 [−0.005, 0.004]	0.001 [−0.002, 0.004]	−0.0001 [−0.003, 0.002]	0.003 [0.0009, 0.007]	0.0004 [−0.0008, 0.002]
As	0.0006 [−0.001, 0.003]	−0.0002 [−0.002, 0.001]	0.0002 [−0.001, 0.001]	0.0006 [−0.001, 0.002]	0.0002 [−0.0004, 0.0009]

Note: Each cell contains the parameter estimate (β) and 95% confidence interval [95% CI] (n = 69).
*indicates $p \leq 0.05$, **indicates $p < 0.001$.

We ran an additional analysis that used percentage of respirator weld hours as an interaction term with weld hours, in case the presence of a respirator changed the slope of the association between weld hours and toenail metal concentration. None of the interaction terms were significant and they were therefore excluded from the final model. For the toenail samples that lacked respirator information, we ran a series of models that assigned 0% respirator use to overestimate weld fume exposure and thus bias results toward the null, and saw no substantive changes in results (data not shown).

When subjects with greater than a 24-hour lag time between providing the work history questionnaire data and toenail sample collection were excluded, Pb was no longer associated with Q4, with all other results essentially unchanged (data not shown). Models were also run that additionally adjusted for total years as a welder. The years as a welder term were not statistically significant in all models, with negligible changes to the model parameters for weld hours and other covariates and was therefore excluded from the final model.

DISCUSSION AND CONCLUSION

Among a population of construction workers occupationally exposed to welding fume, we observed detectable levels of lead (Pb), manganese (Mn), cadmium (Cd), nickel (Ni), and arsenic (As) in toenail clippings. All toenail metal concentrations were significantly correlated with one another, with the exception of Ni and As. After adjusting for age, respirator use, smoking status, and BMI, we found that weld hours 7–9 months prior to toenail clipping were a statistically significant predictor of toenail Mn concentration. Weld hours 10–12 months prior to toenail clipping as well as summed over the previous year were statistically significant predictors of toenail Pb and Cd concentrations. No associations were observed between toenail Ni or As concentrations and welding hours. Furthermore, long-term exposure, expressed as total yeas as a welder, was not associated with toenail metal concentrations.

Median Mn levels in toenails reported here are similar to those seen in an earlier study with the same population (median of 0.80 μg/g),[24] yet lower than toenail Mn measured in Portuguese miners (mean [SD]: 2.51[0.70] μg/g).[26] Higher toenail metal concentrations were reported in a study from an industrialized area with high levels of environmental exposures from air pollution and dust.[35] However, those results may have been skewed by a small number of extremely high exposures, and were calculated using adults and children, where children tend to have higher toenail metal concentrations than adults.[31,33]

A non-occupational study of elderly men in the Boston area measured toenail metal concentrations much lower than the welders from the current study (in participants under the age of 72: (mean (SD) As: 0.08 (0.06); Cd: 0.01 (0.02); Mn: 0.3 (0.41); Pb: 0.28 (0.47)).[37] While some of these differences are due to age and other factors, the relative geographic similarity of that population with ours suggests there would be similar background level of environmental exposure to these metals, indicating that some portion of the discrepancy is due to occupational exposure to welding fumes.

Information on the relative levels of metal fume exposure among this population can be gleaned from a study of welders taken from the same base population. Among a cohort using similar welding techniques, personal $PM_{2.5}$ exposure to welding fume was predominately comprised of iron, followed by Mn, Al, Zn, Cr, Pb, and Ni (Cd was not identified[46]). If the metabolism and distribution throughout the body were equal for each metal, we would expect the relative concentration of toenail metals to follow the relative air metal concentrations. However, results indicate toenails were highest in Ni, followed by Mn, Pb, As, and Cd. The dominance of Mn in air[46] and Ni in toenails within the current study is of interest.

We cannot rule out the possibility that this difference was seen because the welders in the current population were predominantly exposed to Ni, not Mn. However, it is unlikely that the metal exposure profile of the welders in the current study varies greatly from the Cavallari study from 2008, given that no large-scale changes have occurred in welding techniques, job locations, or source materials. More likely, the deposition of metal in toenail in our study participants, as in other toenail studies, is a complex interaction between weld fume exposure, rate of nail growth, age,[47] kinetic models for peripheral tissues,[16] and how the body regulates and excretes essential nutrient metals like Mn[44] or selenium,[48] and non-essential metals like As. The availability of metal ions to bind with sulfhydryl nails will in part depend on the metal concentration in the blood, so blood half-lives of different metals may also factor into toenail metal concentrations. For example, the half-life of Pb in blood is 30–36 days,[49] which is much longer than arsenic, with a half-life of 10–24 hours.[50] In addition, the ionic structure of each metal may impact absorption; studies have shown that Pb may be substituted for calcium in the body,[51,52] and be more likely to be incorporated into tissue. Uptake of different metals may be further enhanced by nutritional deficiencies[53,54] or by individual genotypes.[55,56]

We saw high correlations between toenail metals measured during each participant's first participation date. The highest correlation was between Mn and Cd ($\rho = 0.60$, $p < 0.001$). Significant correlations were found between all metals, with the exception of Ni and As, and with the Cd-Ni relationship being just slightly over the significance threshold. In personal $PM_{2.5}$ air exposure measured within a similar population of welders performing training at an apprentice welding school,[46] the correlation between Pb and Mn was 0.63, whereas toenail correlation for these metals was 0.31. Similarly, the relationship between toenail Pb and Ni was 0.34, whereas air exposure showed a correlation of 0.49. These changes in correlations between the air and internal dose are likely due in part to the different accumulation patterns of these metals, as previously discussed. Notably, the correlation between personal exposures to air metal concentrations in other environments among these participants, such as power plants where these welders primarily work, is unknown.

To date, only one occupational study among carpenters found similar correlations between toenail Pb and Cd and Ni.[23] In the study of Boston-area elderly men previously cited, most correlations between metals were similar with the exception of Mn-Pb and Mn-As which showed higher correlations in our study.[37] Correlational similarities may be due to the way that these metals are co-regulated in the body regardless of absolute concentration values, and reflect similar geographical environmental exposures. It is unclear whether higher welder correlations are due only to occupational exposure to welding fume, and in general the extent to which correlations seen in one occupational setting will be comparable to another occupational study or to an environmental exposure. Regardless of the study type, however, such correlations imply that it may be difficult to disentangle one exposure from another, making it difficult to assign health outcomes to specific metals.

In models of toenail metal concentration and hours welded with adjustments for age, BMI, respirator use, and smoking status, Mn and Pb showed the most robust associations. Specifically, Pb was significantly associated with hours welded in Q4 and the full year summary, Q1–Q4. However, the high correlation between Q1 and Q1–Q4 for Pb makes it difficult to disentangle the true relationship. Hours welded over Q3 were significantly associated with Cd toenail concentration. Total years as a welder was not significantly associated with toenail concentration for any metal or time period, indicating that toenail metals in this group better reflected exposures occurring over the previous year, as opposed to cumulative exposures over many years.

Mn was associated with hours welded over a 3-month period with a lag of 7–9 months prior to clipping, which replicates previous findings in this group.[24] Laohaudomchok et al. used a detailed algorithm that included respirator type and use, welding task performed, and air Mn concentration in models that adjusted for dietary Mn intake. Therefore, it is of note that the association between toenail Mn and the exposure measure persisted despite using weld hours, a simplified measure of exposure. Using this simplified exposure metric allowed us to expand the sample size of the population. Furthermore, assumptions about exposure levels specific to different welding techniques and situations were not used. Despite potential exposure misclassification by using a simplified exposure metric, we were still able to observe significant exposure-response associations. Furthermore, our techniques remained sensitive to identifying associations with quarterly exposures across the yearly exposure window.

No associations were seen with any of the metals analyzed and the more recent work history windows, Q1 and Q2. This was to be expected, given that rates of toenail growth indicate that exposures occurring 0–6 months prior to clipping would be closer to the nail bed, and thus not incorporated into the clipping. However, we feel that these results serve as an important control. If the relationships between toenail metal concentrations and weld hours were spurious, we would have seen associations in these incongruent time windows (Q1 and/or Q2). Likewise, if within this population metal exposures were constant or invariant from occupational or environmental sources (e.g., water, smoking, etc.), the body metal burden would reach a steady state and associations of similar magnitudes would have been observed across all time windows (Q1–Q4).

There are a number of limitations associated with using toenail metal concentration as a biomarker of weld fume exposure. Toenail growth rate is variable across individuals, and studies have identified average growth rates to be between 1.5–1.6 mm/month.[17,57] These values suggest that toenail concentrations should reflect exposures 8–10 months prior to the clip date based on average toenail growth rates and lengths.[18] A recent study found slightly faster growth rates of 2.43 mm/month in the largest toenail in men, approximately the same average age as the population described,[17] indicating that toenail clippings reflect exposures more recent than 8–12 months. However, any variation in toenail growth or clipping length would not be correlated with hours welded and therefore would not affect the internal validity of the study results. Each of the metals included in this study has different accumulation patterns in the body which occur over different time scales. For example, Cd tends to accumulate in the kidney cortex and bone[58] while Pb will remain in blood by binding to erythrocytes, and also accumulate in bone and teeth preferentially.[34] Therefore, in comparing concentrations between metals, lower toenail concentrations of a certain metal may not necessarily mean lower occupational or environmental exposure, but rather could be a reflection of differences in distribution within the body.

Additionally, non-differential exposure misclassification of the crude exposure measure welding hours may have limited the ability to detect an associations with the metals that showed no relationship between weld hours and toenail metal concentration and may have biased significant effect estimates towards the null. Furthermore, we didn't account for dietary sources of Mn and other metals, but these sources are unlikely to be correlated with working patterns and workplace exposure histories. In a previous toenail study on the same population, estimated dietary Mn intake was not correlated with toenail, blood, or urine Mn levels.[24] Since dietary sources of exposure are unlikely to be linked to welding hours, any exposure misclassification due to dietary sources would be non-differential, would bias estimates to the null, and would not explain the associations we observed. Welders may also be exposed when on the job site but not actively welding. Likewise, we did not account for type of welding performed or location of work site (indoor vs. outdoor). Both would lead to exposure misclassification. Finally, weld type—a variable not considered here—could account for relatively low levels of Ni and Cd if stainless steel welding was less common than mild steel welding and soldering, which produce relatively higher levels of Mn and Pb.

The validation of a biomarker is an iterative process including evaluation of the relationship between exposure, biomarker concentrations, and health outcomes.[45] The current study sheds light on the relevant exposure time period for toenail metals. Using weld hours as a surrogate measure for metal exposure, we were able to assess associations between specific time periods of exposure to weld fume and toenail metal concentrations. Our ability to detect an association is in large part due to the variability of exposure within the population. Rather than reaching a steady state, exposure varied within and across months for each individual.

Future studies in other metal exposed populations should continue to explore the relevant exposure windows for toenail metal concentrations as well as intra- and inter-individual variability. Overall, the data presented here support the hypothesis that toenail metal concentrations capture internal exposure over specific time intervals and reflect longer-term exposure. Toenail samples are painless during collection, and are easy to store and transport and therefore have great potential for use in occupational and environmental risk assessment and exposure studies.

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