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RESEARCH ARTICLE

Pulmonary toxicity and extrapulmonary tissue distribution of metals after repeated exposure to different welding fumes

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Abstract

Welders are exposed to fumes with different metal profiles. The goals of this study were to compare lung responses in rats after treatment with chemically different welding fumes and to examine the extrapulmonary fate of metals after deposition in the lungs. Rats were treated by intratracheal instillation (0.5 mg/rat, once a week for 7 weeks) with gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fumes. Controls were treated with saline. At 1, 4, 35, and 105 days after the last treatment, lung injury and inflammation were measured, and elemental analysis of different organs was determined to assess metal clearance. The MMAW-HS fume was highly water-soluble and chemically more complex with higher levels of soluble Mn and Cr compared to the GMAW-MS fume. Treatments with the GMAW-MS fume had no effect on toxicity when compared with controls. The MMAW-HS fume induced significant lung damage early after treatment that remained elevated until 35 days. Metals associated with each fume sample was cleared at different rates from the lungs. Mn was cleared from the lungs at a faster rate and to a greater extent compared to the other metals over the 105-day recovery period. Mn and Cr in the MMAW-HS fume translocated from the respiratory tract and deposited in other organs. Importantly, increased deposition of Mn, but not other metals, was observed in discrete brain regions, including dopamine-rich areas (e.g., striatum and midbrain).

Keywords: *Biodistribution; brain; chromium; lung clearance; manganese; pulmonary toxicity; welding fume*

Background

Welding is a common industrial process used to join metals. Hundreds of thousands of workers are exposed to welding fume on daily basis in the United States (Bureau of Labor Statistics, 2007) and overseas. Due to extremely high temperatures, particles are generated from the consumption of an electrode, wire, or rod used during the welding process. Welding fume is the resulting vaporized metals that react with air to form complex metal oxides that are primarily of respirable size (Antonini et al., 2003). The physical and chemical properties of the fume can vary depending on the materials and welding processes used (Zimmer and Biswas, 2001; Keane et al., 2009).

The two most common types of welding processes are gas metal arc welding (GMAW) and shielded manual metal arc welding (MMAW). GMAW processes use shielding gases, usually different combinations of argon, helium, oxygen, or carbon dioxide, to protect the formed weld from potential weakening caused by oxidation from the environment. The fume generated during GMAW processes tend to be mostly water-insoluble and closely mimic the metal composition of the welding wire that is consumed during the process. In place of shielding gases, fluxes are incorporated into the welding rods used during MMAW to protect the welds from oxidation. Welding fumes formed during MMAW have been observed to be physically and chemically more complex than fumes generated during GMAW (Zimmer and Biswas, 2001; Jenkins and Eagar, 2005; Jenkins et al., 2005). Because of the presence of alkali metals (e.g., potassium and sodium) in the

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fluxes, the fumes from MMAW processes tend to be highly water-soluble (Antonini et al., 1999).

Metals commonly present in welding fume include iron (Fe), manganese (Mn), chromium (Cr), and nickel (Ni). These metals are of biological and toxicological interest because Fe induces siderosis in welders; Mn is a potential neurotoxicant; and Cr and Ni are both classified as human lung carcinogens (Antonini, 2003). Most animal toxicology studies have evaluated the pulmonary responses to welding fumes (White et al., 1982; Antonini et al., 1996; Yu et al., 2000, 2001; Taylor et al., 2003; Zeidler-Erdely et al., 2008), whereas fewer studies have examined the extrapulmonary effects, specifically the neurological effects (Park et al., 2007; Han et al., 2008). Moreover, little is known about the fate of metals that have deposited in the respiratory tract after welding fume inhalation. Employing an established animal model, the goal of our study was to assess the pulmonary responses and extrapulmonary fate of metals after treatment with welding fumes that have vastly different chemical characteristics. Rats were treated weekly with intratracheal instillations of either (1) GMAW–mild steel (MS) fume, which is water-insoluble and contains Fe and a smaller fraction of Mn; or (2) MMAW–hardsurfacing (HS) fume, which is highly water-soluble and contains elevated concentrations of Mn and soluble Cr. Lung injury and inflammation, metal deposition in the lungs, and extrapulmonary distribution and clearance of metals in different organs systems after pulmonary treatment were determined.

Methods

Welding fume collection and characterization

Bulk samples of different welding fumes were collected by the laboratory of Kenneth Brown of Lincoln Electric Co. (Cleveland, OH). The fumes were generated in a cubical open front fume chamber (volume = 1 m^3) by a skilled welder using a manual or semiautomatic technique appropriate to the electrode and collected on 0.2-μm Nuclepore filters (Nuclepore, Pleasanton, CA). The fume samples were generated using two different processes: (1) gas metal arc welding using a mild steel E70S-3 electrode (GMAW-MS; L-50 carbon steel electrode; Lincoln Electric Co.) and (2) manual metal arc welding using a flux-covered stainless steel hardsurfacing electrode (MMAW-HS; Wearshield 15CrMn; Lincoln Electric Co.) that contains elevated amounts of Mn compared to standard welding electrodes/rods/wires.

Welding fume samples (GMAW-MS and MMAW-HS) were suspended in distilled water, pH 7.4, and sonicated for 1 min with a Sonifier 450 Cell Disruptor (Branson Ultrasonics, Danbury, CT). The two particle suspensions (total samples) were incubated for 24 h at 37°C, and the samples were centrifuged at 12,000 × *g* for 30 min. The supernatant of the sample (soluble fraction) was recovered and filtered with a 0.22-μm filter (Millipore, Bedford, MA). The pellet (insoluble fraction) was resuspended in water. The sample suspensions (total, soluble, and insoluble fractions) were digested, and the metals analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-AES) by the Division of Applied Research and Technology (DART, Cincinnati, OH) according to National Institute for Occupational Safety and Health (NIOSH) method 7300 (NIOSH, 1994). The metals that were measured in the particle suspensions included Ag, Al, As, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, La, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Sr, Te, Ti, Tl, V, Zn, and Zr.

Animals

Male Sprague-Dawley [Hla:(SD) CVF] rats from Hilltop Lab Animals (Scottdale, PA), weighing 250–300 g, and free of viral pathogens, parasites, mycoplasmas, *Helicobacter*, and *Cilia*-associated respiratory (CAR) bacillus, were used for all exposures. The rats were acclimated for at least 6 days after arrival and were housed in ventilated polycarbonate cages on Alpha-Dri cellulose chips and hardwood Beta-chips as bedding, and provided HEPA-filtered air, irradiated Teklad 2918 diet, and tap water ad libitum when not being exposed. The animal facilities are specific pathogen-free, environmentally controlled, and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All animal procedures used during the study have been reviewed and approved by the NIOSH Animal Care and Use Committee.

Welding fume treatment

Rats were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1% solution of sodium methohexital (Brevital; Eli Lilly, Indianapolis, IN) and intratracheally instilled once a week for 7 weeks with 0.5 mg/rat of GMAW-MS and MMAW-HS welding fume in 300 μl of sterile phosphate-buffered saline (PBS). Vehicle control animals were intratracheally instilled with 300 μl of sterile PBS. The intratracheal instillation dose of 0.5 mg/rat was chosen based on results from a previous dose-response welding fume study (Antonini et al., 1996).

To estimate how the intratracheal instillation particle dose used in the study correlated with a "real world" worker exposure to welding fumes, the exposure was calculated two different ways: (1) total cumulative exposure over time and (2) daily exposure concentration. Importantly, the calculations made here do not account for particle clearance, but provide an estimate of the plausible welder exposure concentrations that our exposure paradigm mimics.

First, the daily lung burden of a welder was estimated, assuming 8 h of continuous welding, a worker minute ventilation of 20,000 ml/min, a particle deposition efficiency in the alveolar region of 15% (ICRP, 1994), and a fume concentration of 5 mg/m^3 (previous Threshold Limit Values for 8-h day for welding fume). The following calculations were used:

$$\text{Fume concentration} \times \text{minute volume} \times \text{exposure duration} \times \text{deposition efficiency} = \text{Daily deposited dose}$$

$$5\,\text{mg/m}^3 \times \left(20{,}000\,\text{ml/min} \times 10^{-6}\,\text{m}^3\text{/ml}\right) \times \left(8\,\text{h} \times 60\,\text{min/h}\right) \times 0.15 = 7.2\,\text{mg deposited per day}$$

Next, assuming an average worker weighed 75 kg and the average weight of the rats used in the study was 0.40 kg:

$$7.2\,\text{mg deposited}/75\,\text{kg worker} = x\,\text{mg}/0.40\,\text{kg rat}$$

$$x = 0.0384\,\text{mg deposited per day}$$

From the total cumulative doses used in the study, 0.5 mg × 7 doses = 3.5 mg, 3.5 mg/0.0384 mg = 91.1 days or ~4.6 months of exposure (based on 5-day work week).

Second, an estimate was performed to determine if the instillation dosing regimen used in the study was comparable to total welding fume concentration observed in a welder's work atmosphere. To do this, the approximate fume concentration was calculated necessary to achieve a daily lung burden in a welder that would be equivalent of the daily lung burden in rats exposed in the study.

We assumed the 0.5 mg/week intratracheal instillation exposure paradigm, on a divided daily dose basis (of a 5-day week), will give a daily lung burden of 0.1 mg in the rat (average body weight of 0.4 kg in this study).

$$\text{Daily lung burden}_{(\text{rat})} = \frac{\text{Dose per week}}{\text{Dose per week}} = \frac{0.5\text{mg/week}}{5\,\text{days/week}}$$

$$\text{Daily lung burden}_{(\text{rat})} = 0.1\,\text{mg/day}$$

The corresponding daily lung burden for total welding fume in a welder after adjusting for body mass (average human body weight of 75 kg) was calculated as follows:

$$\text{Daily lung burden}_{(\text{welder})} = \frac{\text{Lung burden}_{(\text{rat})} \times \text{Body mass}_{(\text{welder})}}{\text{Body mass}_{(\text{rat})}} = \frac{0.1\text{mg} \times 75\,\text{kg}}{0.4\text{kg}}$$

$$\text{Daily lung burden}_{(\text{welder})} = 18.75\,\text{mg/day}$$

Incorporating this value, the fume concentration was calculated using the following formula:

$$\text{Fume concentration} = \frac{\text{Daily lung burden}_{(\text{welder})}}{\text{minute volume}_{(\text{human})} \times \text{duration} \times \text{deposition efficiency}}$$

where human respiratory minute volume = 20,000 ml/min × 10^{-6} m^3/ml; exposure duration assuming a 8-h work schedule = 8 h/days × 60 min/h; and the deposition efficiency of particles in the alveolar region of 15%.

$$\text{Fume concentration} = \frac{18.75\text{mg}}{20{,}000\,\text{ml/min} \times 10^{-6}\,\text{m}^3/\text{ml} \times (8\,\text{hr} \times 60\,\text{min/h}) \times 0.15}$$

$$\text{Fume concentration} = 18.75\,\text{mg}/1.44\,\text{m}^3 = 13\,\text{mg}/\text{m}^3$$

Thus, our instillation treatment regimen would mimic a daily worker exposure to total welding fume concentration of about 13 mg/m^3, assuming 100% deposition in the lungs. Though this daily concentration is high, it is not unreasonable. Total welding fume levels measured in multiple industries (Korczynski, 2000; Susi et al., 2000), and especially in confined spaces (Harris et al., 2005), have been observed to exceed 13 mg/m^3.

Bronchoalveolar lavage

At 1, 4, 35, and 105 days after the last weekly intratracheal instillation, bronchoalveolar lavage (BAL) was performed to assess lung injury and inflammation. Animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (>100 mg/kg body weight; Sleepaway. Fort Dodge Animal Health; Wyeth, Madison, NJ) and then exsanguinated by severing the abdominal aorta. The left lungs were tied off to be used for metal analysis, and right lungs were first lavaged with a 1 ml/100 g body weight aliquot of calcium- and magnesium-free PBS, pH 7.4. The first fraction of recovered bronchoalveolar lavage fluid (BALF) was centrifuged at 500 × *g* for 10 min, and the resultant cell-free supernatant was analyzed for various biochemical parameters. The lungs were further lavaged with 6-ml aliquots of PBS until 30 ml were collected. These samples also were centrifuged for 10 min at 500 × *g* and the cell-free BALF discarded. The cell pellets from all washes for each rat were combined, washed, and resuspended in 1 ml of PBS buffer and evaluated as described below.

Cellular evaluation

Total cell numbers recovered by BAL were determined using Coulter Multisizer II and AccuComp software (Coulter Electronics, Hialeah, FL). Cells were differentiated using a Cytospin 3 centrifuge (Shandon Life Sciences International, Cheshire, England). Cell suspensions (5×10^4 cells) were spun for 5 min at 800 rpm and pelleted onto a slide. Cells (200/rat) were identified after labeling with Leukostat stain (Fisher Scientific, Pittsburgh, PA) as lung macrophages and neutrophils (polymorphonuclear neutrophils [PMNs]).

Biochemical parameters of injury

Using the acellular first fraction of BALF, albumin content, an index to quantify increased permeability of the bronchoalveolar-capillary barrier, and lactate dehydrogenase (LDH) activity, an indicator of general cytotoxicity, were measured. Albumin content was determined colorimetrically at 628 nm based on albumin binding to bromocresol green (BCG) using an albumin BCG diagnostic kit (Sigma Chemical, St. Louis, MO). LDH activity was determined by measuring the oxidation of lactate to pyruvate coupled with the formation of NADH at 340 nm. Measurements were performed with a COBAS MIRA autoanalyzer (Roche Diagnostic Systems, Montclair, NJ).

Tissue deposition of metals

Nonlavaged left lungs, lung-associated lymph nodes (LALNs), heart, liver, kidney, spleen, and whole blood were collected at 1, 35, and 105 days after the last treatment and freeze-dried after lyophilization. The dried tissue

and blood samples were digested in a microwave in the presence of nitric acid. The amount of the most common metals found in the fume samples (Fe, Cr, Mn, Ni, Cu, Al, Ti, V, and Zn) were determined by ICP-AES at NIOSH Division of Applied Research and Technology (DART) (Cincinnati, OH), according to NIOSH method 7300 (NIOSH, 1994). In addition, discrete brain regions (olfactory bulb, striatum, frontal cortex, hippocampus, thalamus, midbrain, and cerebellum) from treated animals were recovered at 1, 4, 35, and 105 days after the last welding fume treatment and sent to the National Health and Environmental Effects Research Laboratory of the US Environmental Protection Agency (EPA) for metal analysis. To determine metal content in discrete brain areas, 1 ml of 3 N hydrochloric acid/10% trichloroacetic acid solution was added to preweighed wet brain tissue and heated at 70°C for 18 h to digest the tissue. After centrifugation at 600 × *g* for 10 min, concentrations of the metal in the supernatant were quantified using inductively coupled plasma spectroscopy (ICP-OES; Model Optima 4300D; Perkin Elmer, Norwalk, CT).

Statistical analysis

Results are expressed as mean ± standard error of measurement. Statistical analysis was performed using JMP statistical software (SAS, Belmont, CA). The significance of difference between treatment groups within a time point was analyzed using a one-way analysis of variance (ANOVA) and the Tukey-Kramer post hoc test. For all analyses, the criterion of significance was set at $p < .05$.

Results

Metal composition was determined for the two welding fume samples (Table 1). The GMAW-MS fume was relatively insoluble in water and composed of mostly Fe (72.2%) and Mn (21.7%). Of the small amount of soluble metals measured, potassium (K) and Mn were present in the highest concentrations in the GMAW-MS fume. The MMAW-HS fume was more complex, highly water-soluble, and composed of a greater number of metals, predominately Mn, Fe, and Cr and alkali metals, K and Na. The percentage of total Mn was more than double (50.9%) in the MMAW-HS fume compared to GMAW-HS. Significant amounts of soluble K and Na, components of the flux shield, were present in the MMAW-HS fume. Soluble Cr was absent in the GMAW-MS fume.

Table 1. Metal composition and solubility properties of welding fume samples.

Fume samples	Weight % total metals[a]	Soluble/insoluble ratio	% soluble/total metals[a]
GMAW-MS	Fe 72.2% Mn 21.7%	0.0139	1.35% (Fe 0.482%, Mn 47.3%, K 42.9%)
MMAW-HS	Fe 19.3% Mn 50.9% Cr 8.46% K 12.1% Na 6.73% Ni 0.09%	0.2182	19.4% (Fe 1.39%, Mn 1.43%, Cr 15.1%, K 56.0%, Na 25.4%)

Note. Metal composition was determined in particles suspended in saline by ICP-AES. Soluble metal composition was determined in the filtered soluble fraction (supernatant) of the particle suspension after an overnight incubation at 37° C.

[a]Relative to all metals analyzed.

For examination of pulmonary toxicity, animals were treated with the different welding fumes by intratracheal instillation once a week for 7 weeks, and pulmonary responses were measured at 1, 4, 35, and 105 days after the last treatment. BALF LDH activity was significantly elevated at 1 and 4 days after the last treatment with MMAW-HS (Figure 1A). Similarly, BALF albumin levels were elevated at 1 and 4 days (Figure 1B). These indices of lung injury returned to control values by 35 days posttreatment. Lung macrophages were significantly elevated at 1 and 4 days following MMAW-HS treatment compared to the other groups (Figure 2A), indicative of an inflammatory response. Consistent with this, lung PMN numbers were also elevated at 1 and 4 days (Figure 2B). At 35 days post MMAW-HS exposure, lung macrophage number was still significantly elevated. On the other hand, GMAW-MS

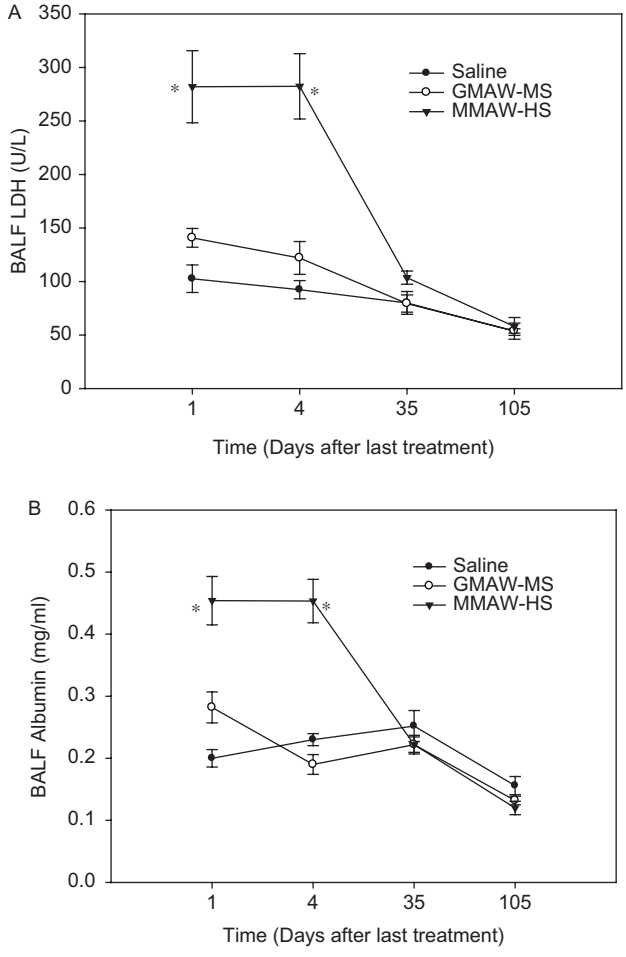

Figure 1. Lung injury. (A) LDH activity and (B) albumin in acellular bronchoalveolar lavage fluid (BALF) at 1, 4, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Values are means ± standard error ($n = 5$). *Significantly greater than other groups within a time point ($p < .05$).

treatment did not alter any of the toxicity or inflammatory measures at all time points examined. Interestingly, lung macrophages were significantly decreased in the MMAW-HS and GMAW-MS groups compared to control at 105 days (Figure 2A).

The metal content in the lungs was examined at 1, 35, and 105 days following 7 weeks of intratracheal instillations to the two fumes (Figure 3). Fe was significantly elevated in the lungs of MMAW-HS and GMAW-MS groups compared to saline at all time points (Figure 3A). Consistent with the elemental composition of the fumes, there was significantly more Fe in the lungs of the GMAW-MS group at days 1 and 35 compared to the MMAW-HS group. However, by 105 days, no significant difference in lung Fe content between the two welding fume groups was observed (Figure 3A). Lung Mn levels also increased significantly above control levels at all time points following MMAW-HS exposure and at 1 and 35 days following GMAW-MS (Figure 3B). At 1 day, significantly higher levels of Mn were observed in the lungs of MMAW-HS group compared to GMAW-MS group. Cr and Ni concentrations were similarly elevated in the lungs of MMAW-HS group at all time points compared to other groups (Figure 3C and D). Cu levels significantly increased in the lungs of GMAW-MS group at 1 and 35 days compared to MMAW-HS (Figure 3E).

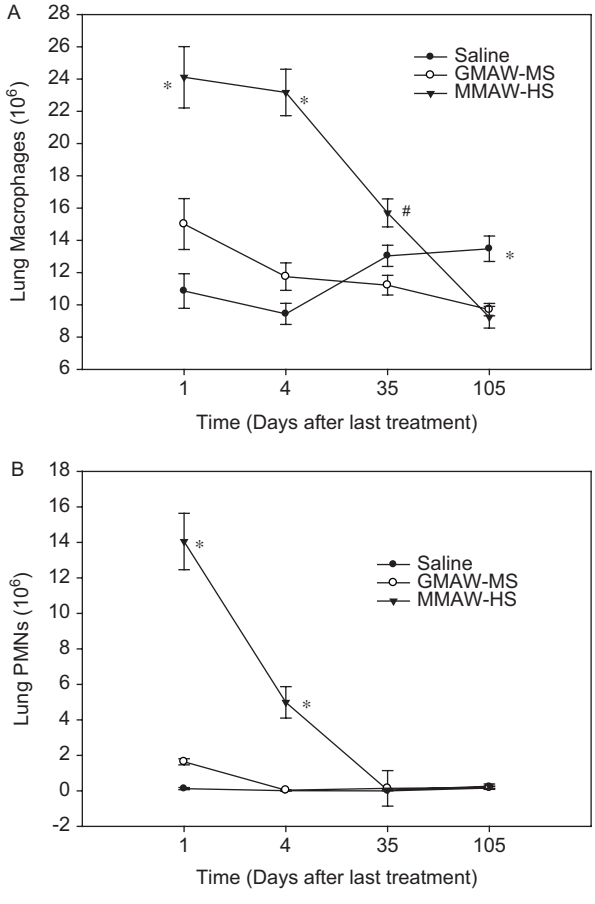

Figure 2. Lung inflammation. (A) macrophages and (B) PMNs from bronchoalveolar lavage fluid (BALF) at 1, 4, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Values are means ± standard error ($n = 5$). *Significantly greater than other groups within a time point; #MMAW-HS and GMAW-MS groups are significantly different within a time point ($p < .05$).

The rate at which the individual metals were cleared from the lungs after welding fume treatment was vastly different. Mn was cleared at a faster rate and to a greater extent from the lungs compared to the other metals (Table 2). There was no significant difference in the clearance rate of Mn for the two welding fume groups. By 105 days, over 80% of lung Mn was cleared after treatment with either MMAW-HS or GMAW-MS (Table 2). On the other hand, lung Fe was cleared much slower in the MMAW-HS group (38% clearance by 105 days) compared to nearly 70% of Fe being cleared after GMAW-MS treatment (Table 2). Cr and Ni were cleared from the lungs after MMAW-HS treatment at comparable rates and to a similar extent.

To determine if pulmonary deposition of welding fumes caused specific metal constituents to translocate into systemic circulation, elemental analysis of blood was performed at 1, 35, and 105 days after treatment with either MMAW-HS or GMAW-MS. There were significant elevations in both Mn and Cr levels in whole blood at 1 day after the last treatment with the MMAW-HS, whereas GMAW-MS did not alter the levels of either of these metals (Figure 4). No significant differences in blood Mn and Cr content were observed at 35 and 105 days after treatment with either fumes (Figure 4).

To assess translocation and biodistribution of metals from the lungs to other organ systems, elemental analysis of various organs and brain areas was performed at 1, 35, and 105 days after treatment with either MMAW-HS or GMAW-MS. Fe, Mn, and Cr were measured in LALNs, heart, liver, kidney, and spleen at 1, 35, and 105 days posttreatment. Fe was significantly increased in the LALNs at 1 and 35 days after treatment with MMAW-HS fume (Table 3). Similarly, GMAW-MS treatment increased Fe levels in the LALNs at 1 and 35 days (Table 3). LALN Fe values for the two fume groups returned to control value by 105 days. There were no significant differences in Fe levels in the heart, liver, and kidney at any time point examined (Table 3). At 1 day following MMAW-HS, Mn levels were significantly elevated in the LALNs, heart, kidney, and spleen (Table 4). By 35 days, Mn levels in all organs except LALNs were comparable to saline control. GMAW-MS treatment did not alter Mn levels in any of the organs at all time points examined. At 1 day following MMAW-HS, Cr levels were significantly increased in LALNs, liver, kidney, and spleen (Table 5). Wheras the liver and kidney Cr levels returned to normal by 35 days, LALN Cr levels remained significantly elevated at 35 days. Spleen Cr levels were significantly elevated at all three time points examined.

Metal concentrations also were measured in discrete brain regions in treated animals at 1, 4, 35, and 105 days after treatment with the two welding fumes. Following MMAW-HS treatment, Mn levels were significantly elevated in the striatum at 1 day and in the midbrain at 1 and 4 days, two dopamine-rich regions that may be potential targets for Mn neurotoxicity (Figure 5). Increased levels of Mn were

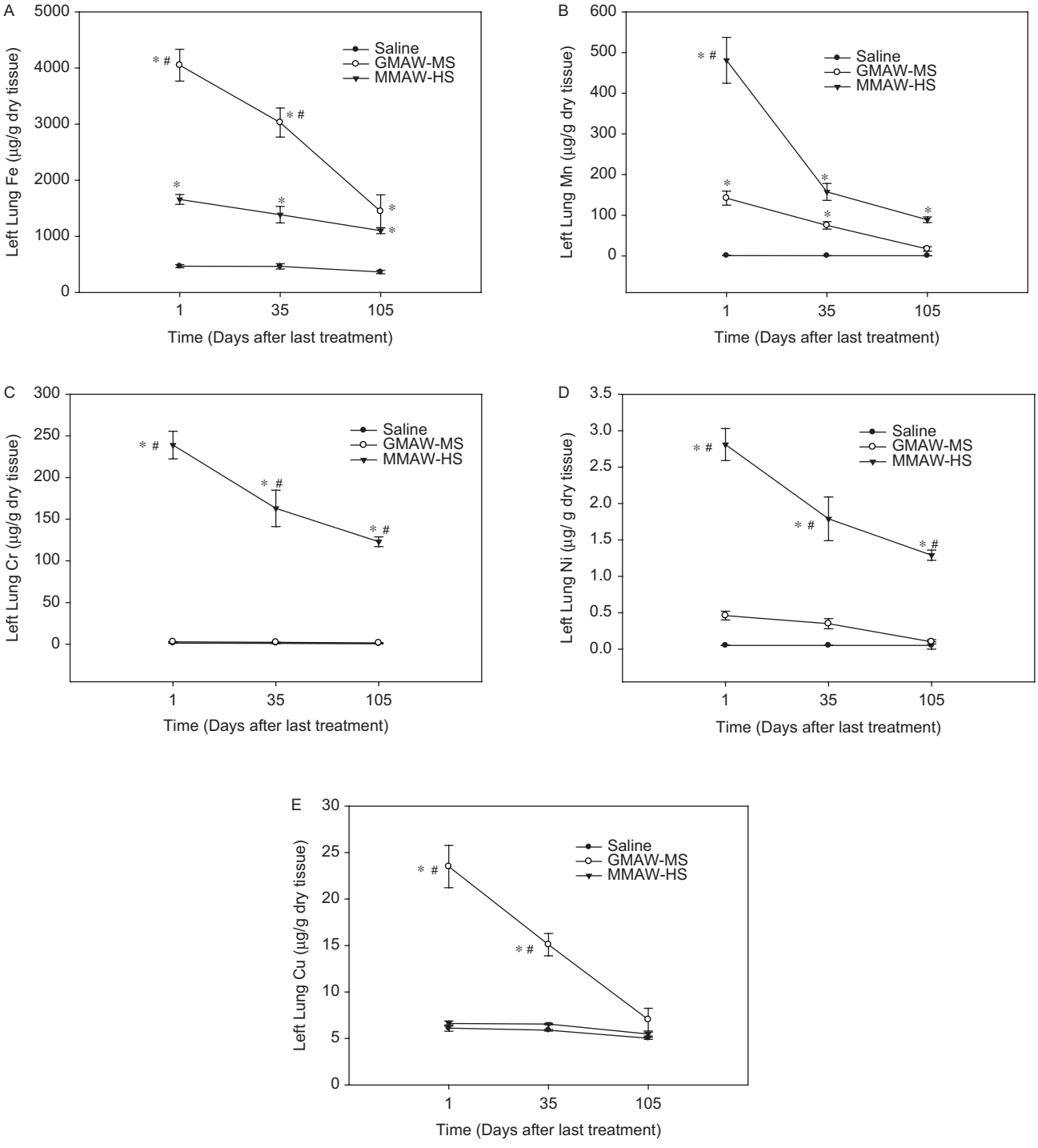

Figure 3. Concentrations of (A) Fe, (B) Mn, (C) Cr, (D) Ni, and (E) Cu in lungs at 1, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations were measured by ICP-AES. Values are means ± standard error ($n = 4$–5). *Significantly greater than saline control within a time point; #MMAW-HS and GMAW-MS groups are significantly different within a time point ($p < 0.05$).

also observed in other brain areas, including olfactory bulb, frontal cortex, hippocampus, thalamus, and cerebellum, 1 day after treatment with MMAW-HS fume (Table 6). Mn levels remained significantly elevated in the hippocampus at day 4 and in the cerebellum at days 4 and 35 following MMAW-HS treatment. Although GMAW-MS treatment did not significantly alter the levels of Mn in the dopaminergic targets, small increases were observed in the frontal cortex and thalamus at 1 day posttreatment. By 105 days after the last treatment, there were no significant changes in Mn levels in any brain region. The levels of Fe, Cr, or Cu in different brain regions remained unaltered following treatment with either MMAW-HS or GMAW-MS fumes (data not shown), indicating selective translocation of Mn to the brain.

Table 2. Percentage of specific metals cleared from lungs.

Fume groups	Fe—saline[a] (μg/g dry tissue)	% Fe cleared	Mn—saline[a] (μg/g dry tissue)	% Mn cleared	Cr—saline[a] (μg/g dry tissue)	% Cr cleared	Ni—saline[a] (μg/g dry tissue)	% Ni cleared
GMAW-MS								
Day 1	3582		141		n.d.	n.d.	n.d.	n.d.
Day 35	2563	28.4%	75	46.8%				
Day 105	1080	69.8%	17	87.9%				
MMAW-HS								
Day 1	1190		480		238		2.81	
Day 35	922	22.5%	157	67.3%	162	31.9%	1.75	37.7%
Day 105	737	38.1%	89	81.5%	123	48.3%	1.24	55.9%

[a]Mean saline control lung tissue values were subtracted from mean values for each metal at a given time point. n.d. = not determined because Cr and Ni levels were not significantly different from control values for the GMAW-MS sample.

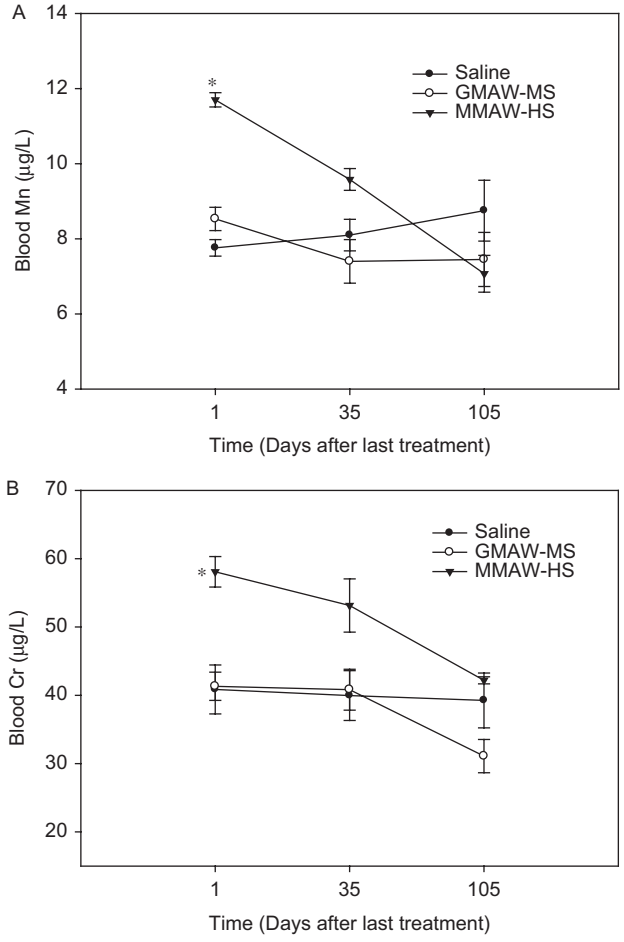

Figure 4. Concentrations of (A) Mn and (B) Cr in blood at 1, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations were measured by ICP-AES. Values are means ± standard error (n = 4–5). *Significantly greater than other groups within a time point ($p < .05$).

Discussion

Two welding fumes were used in the study, GMAW-MS and MMAW-HS. GMAW using MS wire is one of the most common types of welding processes used in US industries. It has been estimated that 90% of all welding uses MS consumables (Beckett, 1996). HS welding involves the deposition of special alloys on a metallic part, using specific welding processes (Monroe, 2006). HS electrodes generally contain much higher concentrations of Mn than more conventional welding electrodes or rods. Mn is an essential element in most welding fumes. Mn increases hardness and strength, prevents steel from cracking during manufacture, improves metallurgical properties, and acts as a deoxidizing agent to remove iron oxide from the weld pool to form a stable weld (Harris, 2002). The most common reason for HS welding is to increase equipment's resistance to abrasion and impact in order to extend its service life. HS welding is becoming more common and occurs in many industries (e.g., mining, cement, chemical, power, and railroad) for mechanical process engineering such as in crushing, conveying, mixing, and separating (Wahl, 2006). Thus, there is potential for a greater number of workers being exposed to HS fumes.

Solubility measurements revealed that the GMAW-MS fume was mostly water-insoluble and was composed of Fe (72.2%) and Mn (21.7%). However, of the small fraction of metals that was water-soluble in the GMAW-MS fume, Mn was a major component. MMAW-HS fume, on the other hand, was highly-water soluble and chemically more complex. The MMAW-HS fume was composed of a greater percentage of Mn (50.9%) compared to the GMAW-MS fume. A significant amount of soluble Cr and alkali metals (K, Na) present in the

Table 3. Iron concentration (μg/g dry tissue) in different organs.

Time	LALN	Heart	Liver	Kidney
Day 1				
Saline	156 ± 22.2	354 ± 18.9	283 ± 9.38	220 ± 9.48
GMAW-MS	322 ± 44.7*	459 ± 48.4	313 ± 7.66	236 ± 4.13
MMAW-HS	426 ± 68.2*	458 ± 56.6	356 ± 18.1	271 ± 31.0
Day 35				
Saline	155 ± 19.6	373 ± 22.1	294 ± 12.9	254 ± 14.4
GMAW-MS	452 ± 131*	343 ± 4.70	310 ± 29.2	279 ± 12.3
MMAW-HS	484 ± 94.2*	361 ± 24.2	284 ± 13.1	288 ± 10.9
Day 105				
Saline	188 ± 24.1	400 ± 15.8	350 ± 13.3	337 ± 20.2
GMAW-MS	263 ± 33.9	390 ± 19.8	359 ± 15.5	295 ± 20.2
MMAW-HS	284 ± 41.3	462 ± 72.2	388 ± 22.8	271 ± 34.2

Note. Organs were excised at 1, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations in digested organs were measured by ICP-AES. LALN = lung-associated lymph node. Values are mean ± standard error. *Significantly greater than saline control within a time point (n = 4–5).

Table 4. Manganese concentration (μg/g dry tissue) in different organs.

Time	LALN	Heart	Liver	Kidney	Spleen
Day 1					
Saline	0.82 ± 0.12	1.47 ± 0.05	5.33 ± 0.29	2.98 ± 0.12	0.73 ± 0.04
GMAW-MS	6.05 ± 1.47	1.56 ± 0.05	5.60 ± 0.31	3.31 ± 0.04	0.78 ± 0.06
MMAW-HS	47.0 ± 15.9*#	2.38 ± 0.15*#	6.08 ± 0.22	4.89 ± 0.18*#	1.00 ± 0.07*#
Day 35					
Saline	1.65 ± 1.03	1.77 ± 0.09	4.54 ± 0.33	2.99 ± 0.08	0.74 ± 0.03
GMAW-MS	11.0 ± 6.57	1.63 ± 0.04	5.05 ± 0.41	3.30 ± 0.07	0.67 ± 0.05
MMAW-HS	36.6 ± 11.3*	1.80 ± 0.07	4.90 ± 0.22	3.47 ± 0.09	0.74 ± 0.01
Day 105					
Saline	0.58 ± 0.07	1.70 ± 0.04	4.75 ± 0.14	2.84 ± 0.10	0.70 ± 0.03
GMAW-MS	5.98 ± 1.94	1.68 ± 0.07	5.39 ± 0.25	2.69 ± 0.18	0.66 ± 0.02
MMAW-HS	14.7 ± 4.28	1.55 ± 0.06	5.08 ± 0.30	2.64 ± 0.36	0.68 ± 0.02

Note. Organs were excised at 1, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations in digested organs were measured by ICP-AES. LALN = lung-associated lymph node. Values are mean ± standard error. *Significantly greater than saline control within a time point; #MMAW-HS and GMAW-MS groups are significantly different within a time point (n = 4–5).

Table 5. Chromium concentration (μg/g dry tissue) in different organs.

Time	LALN	Heart	Liver	Kidney	Spleen
Day 1					
Saline	9.80 ± 1.07	0.66 ± 0.03	0.27 ± 0.01	0.45 ± 0.01	0.86 ± 0.05
GMAW-MS	9.81 ± 1.40	0.59 ± 0.04	0.29 ± 0.01	0.43 ± 0.02	0.86 ± 0.04
MMAW-HS	44.0 ± 11.1*#	0.74 ± 0.03	0.39 ± 0.01*#	1.08 ± 0.09*#	1.47 ± 0.07*#
Day 35					
Saline	6.53 ± 0.96	0.72 ± 0.03	0.23 ± 0.02	0.41 ± 0.01	0.79 ± 0.10
GMAW-MS	8.70 ± 1.98	0.50 ± 0.11	0.30 ± 0.04	0.45 ± 0.04	0.72 ± 0.07
MMAW-HS	66.5 ± 16.2*#	0.66 ± 0.11	0.27 ± 0.01	0.59 ± 0.05	1.82 ± 0.11*#
Day 105					
Saline	5.93 ± 0.41	0.60 ± 0.06	0.27 ± 0.01	0.43 ± 0.03	0.61 ± 0.09
GMAW-MS	7.50 ± 1.07	0.65 ± 0.10	0.31 ± 0.01	0.39 ± 0.03	0.69 ± 0.08
MMAW-HS	41.5 ± 13.1	0.58 ± 0.08	0.32 ± 0.02	0.47 ± 0.06	1.24 ± 0.06*#

Note. Organs were excised at 1, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations in digested organs were measured by ICP-AES. LALN = lung-associated lymph node. Values are mean ± standard error. *Significantly greater than saline control within a time point; #MMAW-HS and GMAW-MS groups are significantly different within a time point (n = 4–5).

flux material were also present in this particular MMAW-HS fume. The alkali metals tend to stabilize the presence of Cr(VI), a lung carcinogen, in fumes generated during MMAW (Fiore, 2006). The incorporation of Cr into welding consumables increases tensile strength, hardenability, and corrosion resistance. Because the MMAW-HS fume is more water-soluble, it is predicted that the metals associated with this particular fume will be more bioavailable and likely will distribute more freely to other organ systems after pulmonary deposition compared to the less water-soluble GMAW-MS fume.

Animals were treated by repeated weekly intratracheal instillations of the fumes to simulate the long-term, continuous exposure to welding fumes typically encountered in a welding occupation. Intratracheal instillation is a widely used procedure to deliver materials into lungs of laboratory animals (Driscoll et al., 2000). Some reasons for employing this method instead of the more physiologic inhalation exposure include simplicity, relative low cost, and the delivery of a well-defined dose of particles (Brain et al., 1976). Most intratracheal instillation studies utilize a single, large bolus of instillate to treat laboratory animals (Driscoll et al., 2000; Henderson et al., 1995). However, multiple instillations at smaller doses may be more representative of an inhalation exposure than a large, single instillation. In determining how the dose used in the current study related to "real world" worker exposure to welding fumes, we estimated that the 7-week animal exposure regimen was equivalent to ~4.6 months of continuous exposure to the fumes with the assumption that the worker welded for 8 h a day for 5 days a week.

Despite the relatively high exposure for an extended period of time and significant metal deposition in the lungs of the treated animals, the GMAW-MS fume had no effect on lung injury and inflammation. This finding correlates well with our earlier studies that examined the pulmonary effects of GMAW-MS fume after a single intratracheal instillation (Antonini et al., 1996; Taylor et al., 2003) or after exposure to 40 mg/m^3 of fume by inhalation for 10 days (Antonini et al., 2009). These findings suggest that the pneumotoxic potential of GMAW-MS fume is low. However, repeated lung treatments with the MMAW-HS fume induced significant elevations in lung injury and inflammation at 1 day postexposure. By 35 days after the last treatment with MMAW-HS fume, most indices of toxicity returned to normal. The increase in lung toxicity early after MMAW-HS treatment is likely due to the presence of Cr and an increased concentration of Mn in the MMAW-HS fume. Animal studies have established that both

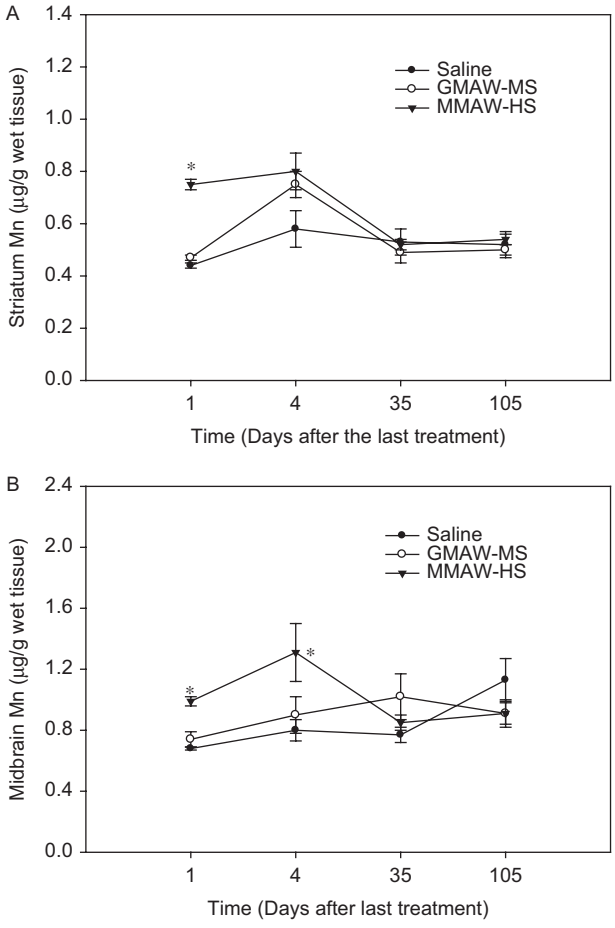

Figure 5. Concentrations of Mn in (A) striatum and (B) midbrain at 1, 4, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations were measured by ICP-OES. Values are means ± standard error ($n = 4$–5). *Significantly greater than other groups within a time point ($p < .05$).

Mn (Lison et al., 1997; Rice et al., 2001; Dorman et al., 2005) and Cr (Glaser et al., 1990; Cohen et al., 1998; Derelenko et al., 1999) are pulmonary irritants and can induce inflammation. The current study is the first that we are aware of that has examined the pulmonary responses to HS fumes in a biological model.

Another important aspect of this study was to examine the fate of welding fume metals after deposition in the alveolar regions of the lungs. Mechanisms of inhaled particle/metal translocation that have been examined in recent years include uptake and transport along olfactory and sensory neurons, transcellular transport across respiratory epithelium to the circulation, and lymphatic clearance (Oberdorster et al., 2005). The use of intratracheal instillation in the current study allowed direct delivery of the welding particles to the lungs, thus bypassing exposure to olfactory areas and the uptake of metals along olfactory neurons that may occur after inhalation exposure to particles. As expected, significant elevations in Fe, Mn, and Cu were observed in the lungs treated with GMAW-MS, consistent with the elemental composition of the fumes. Although lung Fe was efficiently cleared (~70% clearance by 105 days) following GMAW-MS exposure, its levels remained elevated above controls throughout the recovery period. Lung concentrations of Mn and Cu following GMAW-MS treatment also remained elevated at 35 days, but were not significantly different from controls at 105 days. On the other hand, Fe, Mn, and Cr all remained significantly elevated in the lungs at all time points after MMAW-HS treatment, suggesting that a large fraction of these metals were potentially bioavailable for a prolonged duration, even after cessation of exposure.

The rate at which specific deposited metals were cleared from the lungs after welding fume treatment was different. Mn was cleared from the lungs faster and to a greater extent when compared to the other metals, thus making Mn potentially more bioavailable to other organ systems. Using

Table 6. Manganese concentrations (µg/g wet tissue) in specific brain region.

Time	Olfactory bulb	Frontal cortex	Hippocampus	Thalamus	Cerebellum
Day 1					
Saline	0.39 ± 0.03	0.35 ± 0.00	0.35 ± 0.01	0.62 ± 0.03	0.50 ± 0.05
GMAW-MS	0.44 ± 0.01	0.39 ± 0.01*	0.39 ± 0.01	0.77 ± 0.03*	0.45 ± 0.01
MMAW-HS	0.68 ± 0.04*#	0.50 ± 0.01*#	0.52 ± 0.01*#	1.12 ± 0.04*#	0.63 ± 0.01*#
Day 4					
Saline	0.58 ± 0.06	0.55 ± 0.05	0.45 ± 0.02	0.64 ± 0.10	0.48 ± 0.02
GMAW-MS	0.68 ± 0.08	0.61 ± 0.05	0.56 ± 0.06	0.81 ± 0.10	0.54 ± 0.04
MMAW-HS	0.79 ± 0.05	0.77 ± 0.07	0.72 ± 0.03*	0.87 ± 0.06	0.64 ± 0.04*
Day 35					
Saline	0.54 ± 0.03	0.45 ± 0.03	0.47 ± 0.01	0.72 ± 0.04	0.42 ± 0.02
GMAW-MS	0.51 ± 0.05	0.48 ± 0.03	0.42 ± 0.02	0.70 ± 0.03	0.46 ± 0.01
MMAW-HS	0.63 ± 0.02	0.53 ± 0.03	0.47 ± 0.02	0.71 ± 0.05	0.53 ± 0.04*
Day 105					
Saline	0.66 ± 0.04	0.53 ± 0.02	0.52 ± 0.04	0.78 ± 0.07	0.52 ± 0.01
GMAW-MS	0.63 ± 0.03	0.51 ± 0.03	0.51 ± 0.04	0.62 ± 0.06	0.51 ± 0.03
MMAW-HS	0.65 ± 0.04	0.59 ± 0.03	0.55 ± 0.03	0.63 ± 0.03	0.51 ± 0.02

Note. Discrete brain regions were excised at 1, 4, 35, and 105 days after intratracheal instillation of 0.5 mg gas metal arc–mild steel (GMAW-MS) or manual metal arc–hardsurfacing (MMAW-HS) welding fume/rat once a week for 7 weeks. Control animals were treated with sterile saline. Metal concentrations in digested organs were measured by ICP-OES. Values are mean ± standard error. *Significantly greater than saline control within a time point; #MMAW-HS and GMAW-MS groups are significantly different within a time point ($n = 4$–5).

neutron-activated welding fume, Lam et al. (1979) indicated that the metal constituents of welding fume are cleared in different phases. In the initial phases, intact welding particles were cleared from the body within days up to a week by mucociliary and lung macrophage clearance. The clearance rates of each element of the fume were similar during this initial phase, indicating that the eliminated particles were transported in their entirety, without separation of the constituents. The later clearance phase was a much slower process, with the welding constituents having biological half-times of several weeks. Our observations are in agreement with the findings of Lam et al. (1979) in that the various elements of a particular fume were cleared from the lungs at very different rates, suggestive of material separation, which is likely attributable to the tissue solubility of each metal present in the fume.

Some of the deposited metals were cleared rather quickly from the lungs after treatment with the fumes. There were significant elevations of Mn and Cr in the blood and lung-draining lymph nodes at 1 day after MMAW-HS fume exposure. Fe was significantly elevated in the lung-draining lymph nodes at 1 day after the last treatment with either of the fumes, remaining elevated up to 35 days. Interestingly, despite the significant increase in Mn concentration in the lungs of GMAW-MS–exposed animals, there was no observed increase in blood Mn levels. Animal studies have indicated that Mn is quickly eliminated from the blood and possesses a short half-life, but a prolonged tissue half-life, after exposure, thus rendering blood Mn levels as an unreliable indicator of total body burden of Mn (Newland et al., 1987; Zheng et al., 2000). A study among welders indicated that career welders had higher serum Mn levels compared to controls; however, this increase in serum Mn among welders was not associated with welders' length of employment (Lu et al., 2005). The increase in blood Mn observed in the welder study may possibly indicate recent, but not historical, exposure in welders.

Some metals were observed to translocate from the lungs to other organs of animals treated with the MMAW-HS fume, but not after treatment with the GMAW-MS fume. Mn was significantly elevated in the heart, kidney, and spleen at 1 day after the last treatment with the MMAW-HS fume. Recently, significant elevations in Mn levels have been observed in the kidneys, spleen, and liver after 33-week inhalation exposure of cynomolgus monkeys to a MMAW–stainless steel welding fume, also demonstrating Mn translocation (Han et al., 2008). Cr was significantly elevated in the kidney and spleen after exposure to the MMAW-HS fume in the current study. Spleen Cr levels remained elevated throughout the 105-day recovery period after treatment. In previous studies, Cr-containing MMAW–stainless steel welding fumes have been shown to suppress both local and systemic immune responses, which is likely associated with the bioavailability of soluble Cr and the interaction of Cr with local lung and systemic splenic immune cells (Antonini et al., 2004, 2007; Antonini and Roberts, 2007). Despite the significant elevations in Fe in the lungs after treatment with both fumes, Fe was not significantly elevated in the heart, liver, and kidney.

There have been questions as to whether or not Mn in welding fumes reaches specific areas of the brain after pulmonary exposure, which could potentially lead to adverse neurological responses. Concerns exist regarding a causal relationship between exposures to Mn-containing welding fume and the development of Parkinson-like neurological disorders (Racette et al., 2001, 2005). By 1 day after MMAW-HS treatment, we observed Mn deposition in the striatum and midbrain, two dopamine-rich regions of the brain that may be potential targets for Mn neurotoxicity. Elevations in Mn also were observed in olfactory bulb, frontal cortex, hippocampus, thalamus, and cerebellum after MMAW-HS fume treatment. The mechanism by which Mn translocates from the lungs to the brain is likely by solublization of Mn and transport via systemic circulation. Once in circulation, Mn can reach the brain either via transport across the cerebral capillaries or the choroid plexus (Murphy et al., 1991; Rabin et al., 1993) and is perhaps carrier-mediated (Aschner and Aschner, 1990; Crossgrove et al., 2003). There is also a suggestion that the divalent metal transporter 1 (DMT-1) is involved in the transport of Mn to the brain (Gunshin et al., 1997; Conrad et al., 2000), although others argue against its involvement (Crossgrove and Yokel, 2004). Thus, although a consensus remains to be reached regarding the possible transport mechanisms for Mn to the brain, it is nevertheless evident that Mn may accumulate in the brain following welding fume exposure.

Concerning the clearance of Mn from the brain, the observed elevated Mn levels in the specific brain areas after MMAW-HS treatment returned to control levels by 35 days, except for in the cerebellum. It also has been shown that Mn elevations in affected brain regions in welders tend to decrease after removal from the exposure. In the assessment of magnetic resonance imaging (MRI) T1 basal ganglia hyperintensity, Josephs et al. (2005) performed follow-up MRI head scans in workers after stopping welding and observed a trend for the hyperintense T1 signal to fade with increasing time from exposure. In regards to a mechanism, limited information exists about how Mn is cleared from the brain. The brain efflux of Mn across the brain blood barrier does not appear to be carrier-mediated and likely occurs slowly by diffusion (Yokel et al., 2003).

Animals were treated with the fume samples by intratracheal instillation, thus preventing particle deposition in olfactory regions of the upper airways. This is important because olfactory uptake and transport of metals into the central nervous system after particle inhalation are significant in laboratory animals, but may be less so in humans because of significant species differences in nasal anatomy and respiratory physiology (Dorman et al., 1997). The selective translocation of Mn from the lung is perhaps influenced by its dissolution rate, which promotes pulmonary clearance and extrapulmonary transport. Indeed, exposure to soluble forms of Mn resulted in

higher brain Mn concentrations, suggesting that particle solubility is a critical determinant for Mn delivery to the brain (Dorman et al., 2001). Our observations with the water-soluble MMAW-HS fume are consistent with the findings of Dorman et al. (2001). Of interest, deposition of Mn in the frontal cortex and thalamus was also observed after lung treatment with the GMAW-MS, a fume that is less soluble and contains less Mn compared to MMAW-HS. It must be noted that GMAW-MS is the most common type of fume generated in the United States. Although no significant Mn accumulation was seen in the dopaminergic brain areas, striatum and midbrain, the frontal cortex and thalamus are involved in dopamine signaling and perhaps have a possible role in manifesting some of the neurological effects associated with Mn exposure. The neurological consequence of the presence of Mn in specific brain regions, including dopaminergic brain areas, following exposure to different welding fumes is currently being investigated by our group.

In summary, MMAW-HS fume was observed to be highly water-soluble and chemically more complex than the GMAW-MS fume, which is more often used in industry. Weekly lung treatments with the MMAW-HS fume induced significantly more lung injury and inflammation compared to the GMAW-MS fume. This increase in pneumotoxicity is likely due to the presence of Cr and increased concentrations of Mn in the MMAW-HS fume. Thus, workers in specific industries who are exposed to MMAW-HS fumes may be at a greater risk for the development of lung disease. In addition, the metals associated with the welding fumes appear to be cleared at different rates from the lungs. Mn was observed to be cleared from the lungs at a faster rate and to a greater extent compared to the other metals over the 105-day recovery period. Also, Mn in the MMAW-HS fume translocated from the respiratory tract and deposited in other organs and discrete brain regions, in particular dopamine-rich areas, that may be potential targets for adverse neurological responses. Because of this, animal studies are ongoing to evaluate the potential toxic effects of welding fumes on different organ systems.

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Declarations of interest

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