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INORGANIC COMPOUNDS

Persistence of deposited metals in the lungs after stainless steel and mild steel welding fume inhalation in rats

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Abstract Welding generates complex metal fumes that vary in composition. The objectives of this study were to compare the persistence of deposited metals and the inflammatory potential of stainless and mild steel welding fumes, the two most common fumes used in US industry. Sprague–Dawley rats were exposed to 40 mg/m^3 of stainless or mild steel welding fumes for 3 h/day for 3 days. Controls were exposed to filtered air. Generated fume was collected, and particle size and elemental composition were determined. Bronchoalveolar lavage was done on days 0, 8, 21, and 42 after the last exposure to assess lung injury/inflammation and to recover lung phagocytes. Non-lavaged lung samples were analyzed for total and specific metal content as a measure of metal persistence. Both welding fumes were similar in particle morphology and size. Following was the chemical composition of the fumes—stainless steel: 57% Fe, 20% Cr, 14% Mn, and 9% Ni; mild steel: 83% Fe and 15% Mn. There was no effect of the mild steel fume on lung injury/inflammation at any time point compared to air control. Lung injury and inflammation were significantly elevated at 8 and 21 days after exposure to the stainless steel fume compared to control. Stainless steel fume exposure was associated with greater recovery of welding fume-laden macrophages from the lungs at all time points compared with the mild steel fume. A higher concentration of total metal was observed in the lungs of the stainless steel welding fume at all time points compared with the mild steel fume. The specific metals present in the two fumes were cleared from the lungs at different rates. The potentially more toxic metals (e.g., Mn, Cr) present in the stainless steel fume were cleared from the lungs more quickly than Fe, likely increasing their translocation from the respiratory system to other organs.

Keywords Welding fume · Inhalation · Lung burden · Lung clearance · Pulmonary toxicity

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Introduction

Welding is a common industrial process used to join metals. Over 300,000 workers in the United States (Bureau of Labor Statistics 2007) and millions of workers worldwide are exposed to welding aerosols on a daily basis. Welding fume is a complex aerosol of different metals. The composition of the fume varies depending on the welding processes and materials used. Welding aerosols are mostly generated from the consumption of an electrode, wire, or rod. Two of the most common types of consumables used in welding are mild steel and stainless steel electrodes. Mild steel fume is composed of a complex of iron (Fe) with a smaller percentage of manganese (Mn), whereas stainless steel fume contains both Fe and Mn, but with significant amounts of chromium (Cr) and nickel (Ni) that are not present in mild steel fume.

The metals present in welding fumes are of interest toxicologically due to their potential effects on worker health. Significant amounts of Fe have been observed to deposit and persist in the lungs of full-time welders,

possibly leading to a condition called siderosis (Antonini 2003; Sferlazza and Beckett 1991). Manganese is a known neurotoxicant. Neurobehavioral changes have been reported in exposed welders (Bowler et al. 2007; Ellingsen et al. 2008). Welding fume has been classified as "possibly carcinogenic" by the International Agency for Research on Cancer (IARC) due to the presence of known human carcinogens, Cr and Ni, in stainless steel fume (IARC 1990). However, epidemiological studies have been unable to correlate chronic adverse lung effects, such as cancer, solely with exposure to stainless steel welding fume when compared with mild steel fume (Moulin et al. 1993; Moulin 1997; Langard 1994).

Numerous animal toxicology studies have been performed in recent years to evaluate the lung effects associated with welding fume exposure. Stainless steel welding fume was observed to induce a chronic lung inflammatory response with a trend for increased lung tumor incidence in a mouse lung tumor-susceptible model compared with a mild steel fume (Zeidler-Erdely et al. 2008). Short-term inhalation exposure to stainless steel, but not mild steel, fume caused lung injury and inflammation in rats (Antonini et al. 2007, 2009a). However, the same studies did show that both stainless and mild steel fumes suppressed lung immune responses after pulmonary inoculation with a bacterial pathogen. In a sub-chronic inhalation exposure study in rats, Yu et al. (2001) observed the appearance of interstitial fibrosis by 60 days that becomes prominent at 90 days after exposure to a high concentration of stainless steel welding fume (107 mg/m^3). Recovery studies indicated that the fibrosis observed in the rats exposed to the highest dose of stainless steel welding fume for 60–90 days did not fully resolve (Yu et al. 2003; Sung et al. 2004).

The objective of the current study was to compare the lung toxicity and persistence of the deposited particles after inhalation of different welding fumes using an animal model. A completely automated, robotic welding fume generation and inhalation exposure system has been constructed by NIOSH (Antonini et al. 2006). Unique to this current study was the examination of the persistence and clearance of individual metals associated with each type of fume. We hypothesize that one mechanism by which stainless steel fume may be more toxic than mild steel fume is that metal particles generated during stainless steel welding persist in the lungs longer than mild steel fume due to differences in metal composition. This potential increase in residence time of the metals associated with stainless steel fume in the lung would allow for a longer exposure period between the welding fume metals and pulmonary cells. Lung toxicity, pulmonary clearance of the metals associated with welding fume, and macrophage phagocytosis of inhaled particles were examined over a 42-day period after a 3-day inhalation of mild steel and stainless steel welding fumes by rats during gas metal arc welding.

Methods

Experimental design

Rats were exposed by inhalation for 3 h/day for 3 days to 40 mg/m^3 of fume generated during gas metal arc welding using either a mild steel or stainless steel welding electrode. Control animals were exposed to filtered air for 3 h/day for 3 days. At 1 h (day 0), 8, 21, and 42 days after the last exposure, lung injury, inflammation, and particle/metal lung deposition and clearance were assessed in the exposed animals.

Welding fume generation system

The welding fume generation system (Fig. 1) consisted of a welding power source (Power Wave 455, Lincoln Electric, Cleveland, OH), an automated, programmable six-axis robotic arm (Model 100 Bi, Lincoln Electric), a water-cooled arc welding torch (WC 650 amp, Lincoln Electric), a wire feeder that supplied the wire to the torch at a programmed rate up to 300 inches/min, and an automatic welding torch cleaner that kept the welding nozzle free of debris and spatter (Antonini et al. 2006). Gas metal arc welding was performed using either a mild steel electrode (carbon steel ER70S-6, Lincoln Electric) or a stainless steel electrode (Blue Max E308LSi wire, Lincoln Electric, Cleveland, OH). Welding took place on A36 carbon steel plates for daily exposures of 3 h at 25 V and 200 amps. During welding, a shielding gas combination of 95% Ar and 5% CO_2 (Airgas Co., Morgantown, WV) was continually delivered to the welding nozzle at an air flow rate of 20 l/min.

Exposure chamber fume and gas determinations

A flexible trunk was positioned approximately 18 inches from the arc to collect the generated fume and transport it to the exposure chamber. The generated welding fume was mixed with dry HEPA-filtered air. Continuous records of chamber fume concentration, temperature, and humidity were maintained during welding fume generation. The mass concentration in the chamber was monitored by a real-time aerosol monitor (DataRAM, Thermo Electron Co., DR-4000, Franklin, MA). Depending on the desired concentration, the diluent air in this system was normally controlled between 20 and 80 l/min. Fume was collected onto 37-mm Teflon filters at a rate of 1 l/min, and the particle mass delivered to the exposure chamber was

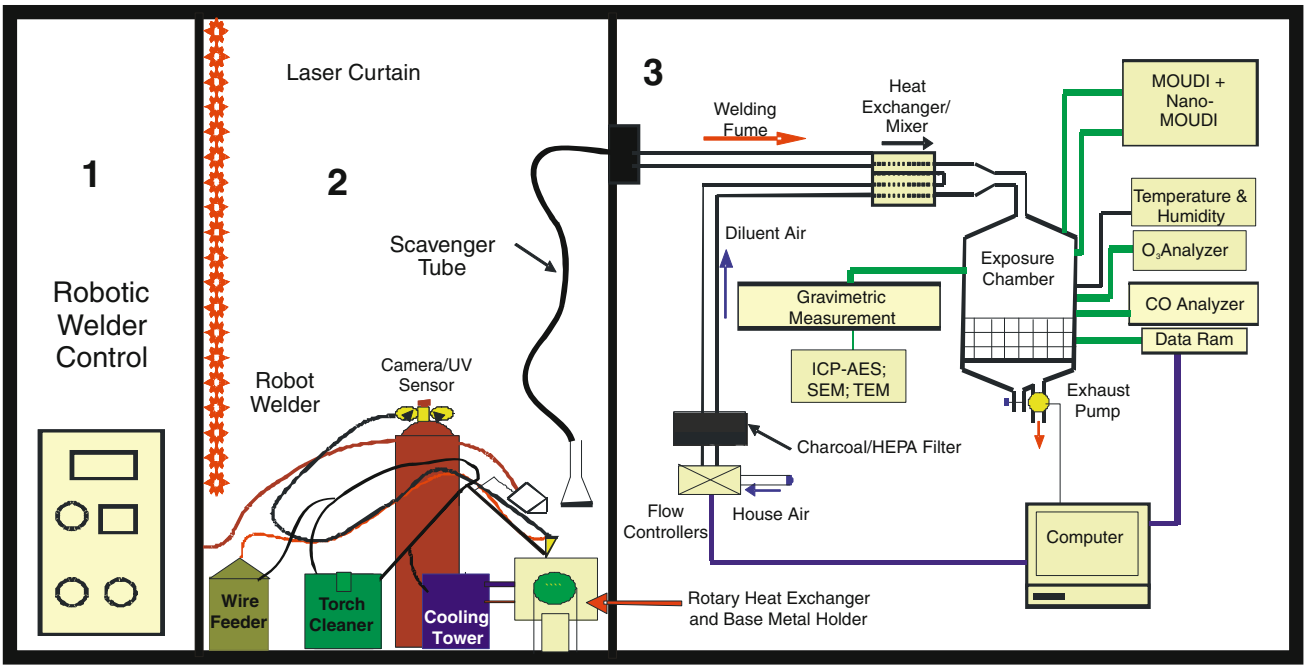

Fig. 1 Diagram of the NIOSH welding fume generation system including: [*1*] enclosed control room that contains the welding power source and controller; [*2*] robotic welding fume generator that contains the six-axis robotic arm, wire feeder, torch cleaner, coolers, and base metal holder; [*3*] animal exposure chamber with fume and gas characterization devices. *Abbreviations ICP-AES* inductively coupled plasma-atomic emission spectroscopy, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *Ram* real-time aerosol monitor, *MOUDI* Micro-Orifice Uniform Deposit Impactor. A modification of this figure was previously published (Antonini et al. 2006)

determined gravimetrically every 30 min in duplicate during the daily 3-h exposure in the breathing zone of the rats. In addition, particle samples were periodically collected gravimetrically onto grids from inside the exposure chamber for electron microscopy to assess particle-size distribution, particle morphology, and elemental composition. Gas samples were withdrawn from the exposure chamber through Teflon tubing with a protective particulate filter in the line during the period of welding, and ozone (ozone analyzer model #450, Advanced Pollution Instrumentation, Inc., San Diego, CA) and carbon monoxide (1312 Photo-acoustic Multi-Gas Monitor, Innova Air Tech Instruments, Ballerup, Denmark) were measured. Measured ozone and carbon monoxide levels were not significantly higher than background levels (data not shown).

Welding particle-size distribution

Particle-size distribution was determined in the exposure chamber in the breathing zone of the rats by using a Micro-Orifice Uniform Deposit Impactor (MOUDI, MSP Model 110, MSP Corporation, Shoreview, MN) that is intended for general purpose aerosol sampling, and a Nano-MOUDI (MSP Model 115) that is specifically designed for sampling aerosols in the size range down to 0.010 μm. Using the two MOUDI impactors in a series or in tandem, particles were collected in the size range from 0.010 to 18 μm that were separated into 15 fractions.

Welding particle composition

Welding particles were collected onto 5.0-μm polyvinyl chloride membrane filters in 37-mm cassettes during 30 min of welding. The particle samples were digested, and the metals analyzed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) by Clayton Group Services (A Bureau Veritas Company, Novi, MI), according to NIOSH method 7300 modified for microwave digestion (NIOSH 1994). The metals that compose stainless and mild steel electrodes were quantified: Fe, Mn, Cr, Ni, Cu, Al, Ti, and V. Metal content of blank filters was also analyzed for control purposes. Both the stainless and mild steel samples were found to be relatively insoluble in water with soluble-to-insoluble ratios 0.006 and 0.020, respectively.

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 CAR Bacillus, were used for all exposures. The rats were acclimated for at least 6 days after arrival, were housed in ventilated polycarbonate cages on Alpha-Dri cellulose chips and hardwood Beta-chips as bedding, and were provided HEPA-filtered air, irradiated Teklad 2918 diet, and tap water ad libitum when not being exposed. During the daily 3-h exposures to welding fume or air in the inhalation chamber, food and water were withheld from the animals. Body weight was monitored before and after each exposure. No significant changes were observed in animal body weight from any treatment group during the exposure regimen used in the study (data not shown). Temperature and humidity were measured in the animal exposure chamber to be 21°C and 38%, respectively, and remained constant in the chamber during the exposure period.

During exposure to welding fume, no animal showed any outward signs or symptoms of labored breathing or respiratory distress. 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 institution's Animal Care and Use Committee.

Bronchoalveolar lavage

At different time points after exposure, control and welding fume-exposed rats were deeply anesthetized with an intraperitoneal injection of Sleepaway (>100 mg/kg body weight of sodium pentobarbital, Fort Dodge Animal Health, Fort Dodge, IA, USA) and then exsanguinated by severing the abdominal aorta. The left lungs were tied off to be saved for metal analysis, and the lungs were lavaged with a 1 ml/100 g body weight aliquot of calcium- and magnesium-free phosphate-buffered saline (PBS), pH 7.4. The first fraction of recovered bronchoalveolar lavage fluid (BALF) was centrifuged at $500\times g$ for 10 min, and the resultant cell-free supernatant was analyzed for various biochemical parameters and cytokine levels. The right lungs were further lavaged with 6 ml aliquots of PBS until 30 ml were collected. These samples were also centrifuged for 10 min at $500\times g$, 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 in the next section.

Cellular evaluation

Total cell numbers recovered by BAL were determined using a Coulter Multisizer II and AccuComp software (Coulter Electronics, Hialeah, FL, USA). 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, USA) as alveolar macrophages (AMs) and neutrophils (PMNs).

Another portion of the cells was preserved with Karnovsky's fixative for analysis by transmission electron microscopy (TEM). Cells were post-fixed in osmium tetroxide and embedded in epoxy resin. Welding particles within the lung macrophages were photographed on a JEOL 1220 transmission electron microscope (JEOL, Inc., Tokyo, Japan) at 80 kV.

Biochemical parameters of injury

Using the acellular first fraction of BALF collected from the right lungs, 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 bromcresol green using an albumin BCG diagnostic kit (Sigma Chemical Co., St. Louis, MO, USA). LDH activity was determined by measuring the oxidation of lactate to pyruvate coupled with the formation of NADH at 340 nm. Measurements were taken with a COBAS MIRA auto-analyzer (Roche Diagnostic Systems, Montclair, NJ, USA).

Pulmonary deposition and clearance of particles

To assess the clearance of deposited welding particles from the lungs, the percentage of recovered AMs that contained particles was counted on days 0, 8, 21, and 42 after BAL on cytospin-prepared slides. For air control and fume treatment groups, 200–300 AMs were counted for each animal using a Zeiss ICS Standard 25 light microscope (Carl Zeiss, Inc., Thornwood, NY, USA).

In addition, the metal content present in the left lungs on days 0, 8, 21, and 42 after exposure to the stainless steel and mild steel welding fumes was determined to assess particle clearance from the lungs. Non-lavaged left lungs from each animal were excised, weighed, and freeze-dried after an overnight lyophilization. The amount of Fe, Cr, Mn, and Ni deposited in the lung at each time point for each welding fume was determined at NIOSH-DART (Cincinnati, OH), according to NIOSH method 7300 (NIOSH 1994). The lung tissue samples were transferred to beakers for digestion. The sample containers were rinsed with concentrated nitric acid and three washings of deionized water, and the washings were transferred to the

sample. The samples were treated with 25 ml of concentrated nitric acid and 2 ml of concentrated perchloric acid, covered, and refluxed at 150°C until complete dissolution. The sample residues were dissolved in a dilute solution of 4% nitric acid/1% perchloric acid and then analyzed for trace metals by ICP-AES.

Statistical analysis

Results are expressed as means ± standard error of measurement. Statistical analysis was performed using JMP statistical software (SAS, Inc., Belmont, CA). Because we have only one welding fume generator and inhalation exposure system, animals were exposed to one type of fume at a time, either stainless steel or mild steel fume. Thus, exposures to stainless welding fume and mild steel welding fume were performed on different days. Corresponding control animals were exposed to filtered air during welding for both types of fume. For comparison of specific lung damage parameters (albumin and LDH activity) between the stainless steel and mild steel fume treatment groups, data are presented as a percentage of air control value for each parameter. 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 $P < 0.05$.

Results

The majority of the generated particles for each type of welding fume were in the fine size range with cut-off diameters of 0.10–1.0 μm for each fume (Fig. 2). The mass median aerodynamic diameter (MMAD) was calculated to be 0.30 μm for mild steel and 0.25 μm for stainless steel fume. In determining the metal profile for the two types of welding particles, the mild steel fume mostly contained Fe with a lesser amount of Mn, whereas the stainless steel fume contained Fe and Mn plus significant amounts of Cr and Ni (Table 1).

In the assessment of BALF for parameters of pneumotoxicity, there was a significant increase in lung injury at 8 and 21 days after stainless steel welding fume exposure compared with mild steel exposure and air control (Fig. 3). The increases in both BALF parameters observed for the stainless steel group returned to control levels by 42 days after exposure. Lung injury was not different after mild steel welding fume exposure compared with air control.

Cytospin-stained slides indicated that a significant number of both mild steel and stainless steel welding particles were engulfed by AMs within hours after exposure (data not shown). The stainless steel particles persist in

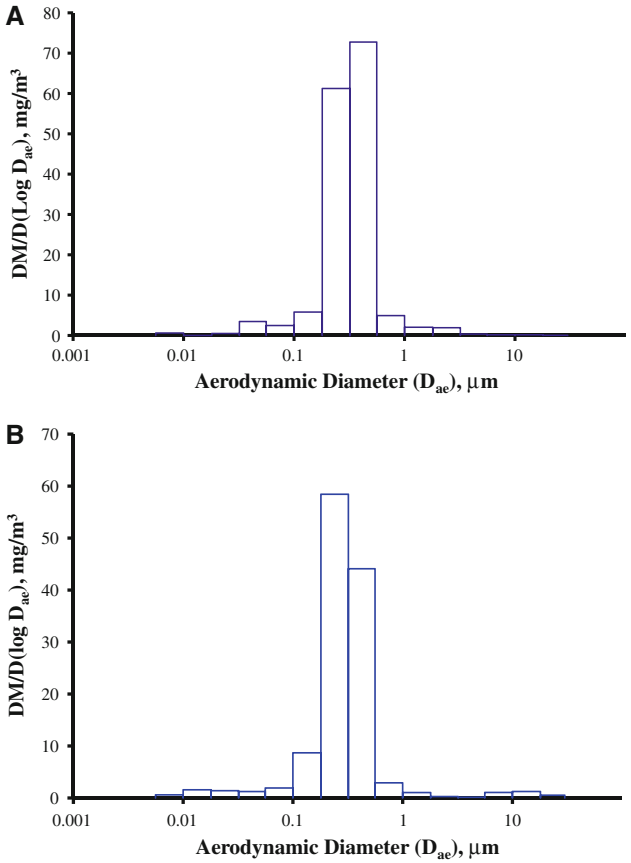

Fig. 2 Representative particle-size distribution graphs of generated **a** mild steel and **b** stainless steel welding particles comparing mass concentration, *M* versus particle size, aerodynamic diameter. Random daily measurements of particle-size distribution were made during exposures to each fume throughout the course of the study

AMs, in that the particles were present in AMs at all time points up to 42 days after exposure. Note examples of AMs that contain a significant amount of stainless steel welding particles at days 8 and 21 (Fig. 4b, d; arrows and arrowheads) and the presence of PMNs recovered from the lungs of stainless steel-exposed animals at day 8 (Fig. 4b; asterisks). The majority of the mild steel particles were cleared rather quickly. Very few AMs were found to contain mild steel particles at 8 and 21 days after exposure (Fig. 4a, c; arrows). Very few PMNs were observed in the cytospins at any time point after exposure to mild steel welding fume demonstrating a lack of an inflammatory response.

The number of AMs and of PMNs were counted from the cytospin-stained slides to determine lung inflammation (Fig. 5). There were significantly more AMs recovered from the lungs of rats exposed to stainless steel fume at 8, 21, and 42 days after exposure compared with mild steel (Fig. 5a). A significant increase in PMNs was observed in the stainless steel group at 8 and 21 days after exposure compared with the mild steel group, indicative of a delayed

Table 1 Metal composition of generated welding fumes

Metals analyzed	Mild steel (weight % of metal[a])	Stainless steel (weight % of metal[a])
Fe	82.8 ± 0.10	57.2 ± 1.20
Mn	15.2 ± 0.09	13.8 ± 0.47
Cr	n.d.	20.3 ± 1.60
Ni	n.d.	8.51 ± 0.40
Cu	1.84 ± 0.01	0.156 ± 0.01
Al	0.165 ± 0.01	0.085 ± 0.08

n.d. not detected

[a] Relative to the total metal content of metals analyzed; values are means ± standard error; $n = 4$ welding collection periods of 30 min

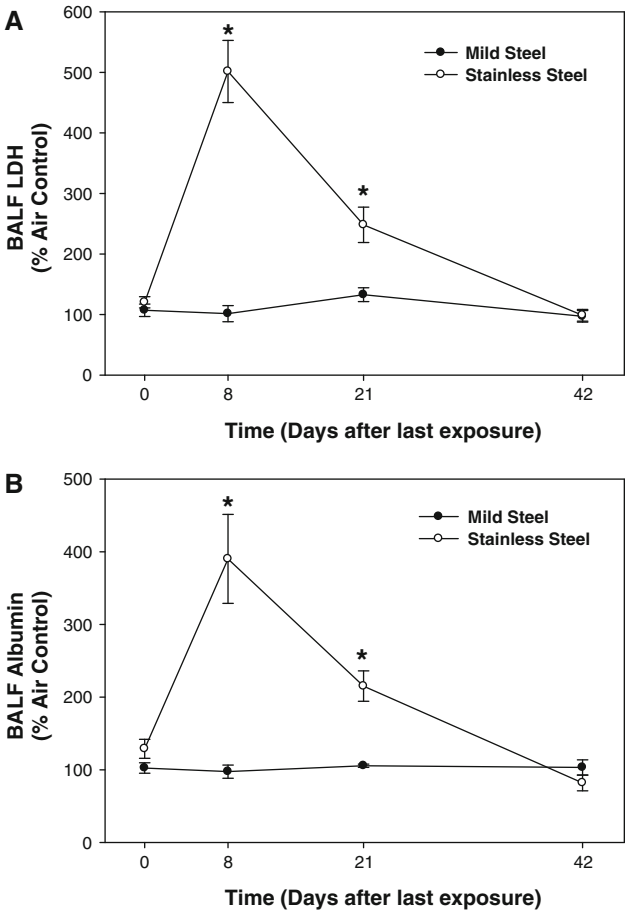

Fig. 3 Lung injury: **a** LDH activity (U/L) and **b** albumin (mg/ml) in acellular bronchoalveolar lavage fluid at days 0, 8, 21, and 42 after inhalation of 40 mg/m^3 of mild steel or stainless steel welding fume for 3 h/day for 3 days. Control animals were exposed to filtered air. Values are mean ± standard error ($n = 4$). *Significantly different from the other welding fume group, $P < 0.05$

inflammatory response (Fig. 5b). After counting the number of cells that contained welding particles after exposure, there were significantly more AMs that contained stainless steel particles compared with mild steel particles at each time point (Fig. 6). By 42 days, 29.8% of the AMs contained stainless steel particle, whereas only 10.2% of the AMs contained mild steel particles. Transmission electron microscopy indicated that stainless steel welding particles were still observed in AMs and resided in phagolysosomes at 42 days after exposure (Fig. 7).

In the assessment of metal persistence in the lungs after inhalation exposure to mild steel and stainless steel welding particles, concentrations of total and individual metals were measured in the lungs at different time points after exposure (Tables 2, 3). A higher concentration of metal was present in the lungs of the stainless steel welding fume at all time points compared with the mild steel fume. By 42 days after exposure, 47% of the metal for the stainless steel fume remained in the lungs compared with 30% of the metal for the mild steel fume (Fig. 8). The estimated elimination half-times ($t_{1/2}$) were 38.5 and 24.8 days for the stainless steel and mild steel groups, respectively. The specific metals appear to be cleared from the lungs at different rates after welding fume exposure. For the mild steel fume, nearly all of the Mn was cleared by 21 days, whereas Fe (after an initial quick clearance by 8 days) persisted, as 41% of Fe deposited at day 0 was still present in the lungs at 42 days after exposure (Table 2; Fig. 9a). The estimated $t_{1/2}$ for the mild steel metals were Fe (33.0 days) and Mn (6.93 days). For the stainless steel fume, Mn and Ni appeared to be cleared at a similar rate with 23 and 26% of each metal remaining in the lungs at 42 days after exposure, respectively (Table 3; Fig. 9b). A greater percentage of Cr was observed in the lungs at day 8 after stainless steel fume exposure compared with the other metals, with 39% of Cr deposited at day 0 still present in the lungs at day 42 (Fig. 9b). The complex of iron persisted in the lungs after stainless steel exposure as 57% of the deposited metal was still present in the lungs at the end of the exposure period (Fig. 9b). The estimated $t_{1/2}$ for the stainless steel metals were Fe (53.3 days), Mn (19.8 days), Cr (30.0 days), and Ni (21.7 days).

Discussion

Previously, gas metal arc-stainless steel fume, like the one used in the current study, was observed to induce significantly greater lung inflammation and injury that persisted up to 35 days after treatment compared with gas metal arc-mild steel fume after a single intratracheal instillation (Antonini et al. 1996). Elevations in two inflammatory cytokines, tumor necrosis factor-α and interleukin-1β, were observed in recovered BALF 1 day after treatment with the stainless steel fume, likely due to the presence of Cr and Ni, two potentially pneumotoxic metals not present in mild steel fumes. In addition, our previous magnetometry study

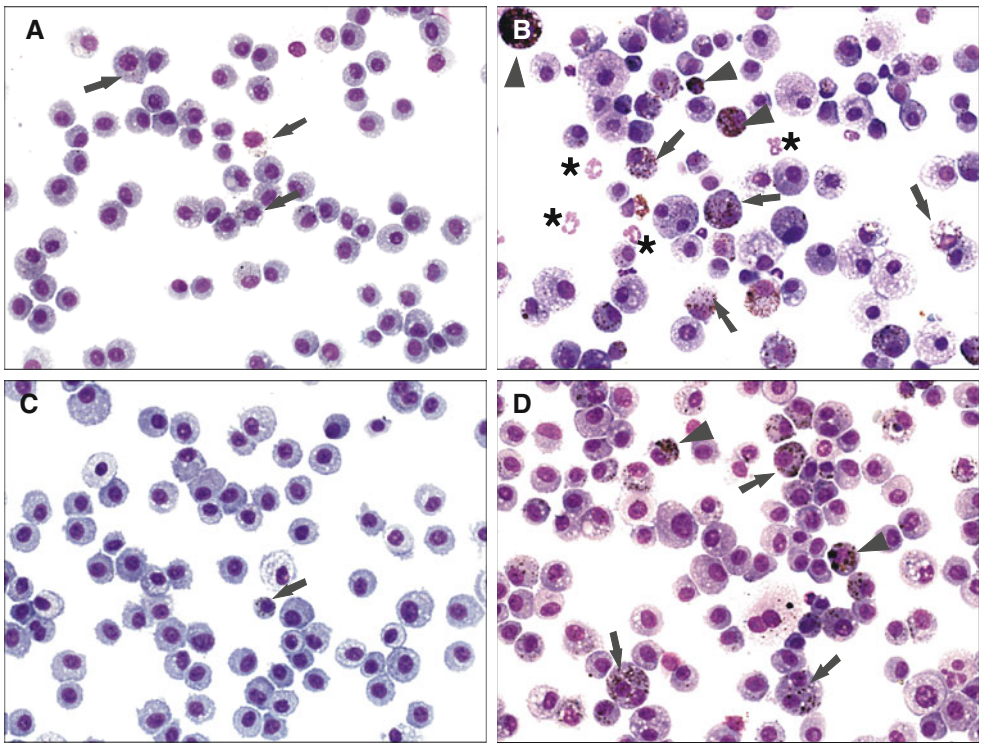

Fig. 4 Representative images of cytopsin-stained lung cells recovered from animals at 8 (**a** mild steel; **b** stainless steel) and 21 (**c** mild steel; **d** stainless steel) days after exposure to 40 mg/m^3 of welding fume for 3 h/day for 3 days. *Arrows* highlight examples of alveolar macrophages that contain welding particles; *arrowheads* highlight examples of alveolar macrophages that contain elevated amounts of welding particles; and *asterisks* highlight neutrophils that were recovered from stainless steel-exposed animals

indicated that stainless steel particles persist in the lungs longer than the mild steel particles (Antonini et al. 1996). It was estimated that the elimination half-time for stainless steel particles in the lungs was 47 days compared with only 18 days for the mild steel particles after a single, bolus intratracheal instillation dose of 1 mg/100 g animal body weight.

An important drawback of our previous study was the limitations of method that was used to treat the animals with the welding fume samples. The use of intratracheal instillation to treat animals with particles is not recommended for lung-particle retention and clearance studies (Driscoll et al. 2000). Pulmonary instillation of particles has been shown to result in heavier, more centralized particle deposition, likely due to a bolus delivery of particles, whereas inhalation of similarly sized and shaped particles resulted in a wider, more even lung distribution (Brain et al. 1976). These differences in particle distribution in the lungs may overwhelm lung clearance pathways and influence dose exposures to lung defense and clearance cells and the degree and site of systemic absorption (Driscoll et al. 2000). These factors may influence deposition, retention, and toxicological responses after exposure to welding fumes. Thus, an automated, robotic welding fume generation and inhalation exposure system has been developed that is capable of continuously generating a consistent concentration of welding fume for extended periods of time for animal exposure studies (Antonini et al. 2006, 2007, 2009a).

Before lung responses induced by each of the welding fumes could be compared, characterization of the physical properties of the welding particles was needed to ensure that any potential differences in size or morphology between the two types of welding fumes did not influence the responses. Fumes formed during stainless steel and mild steel particles were both relatively insoluble in water and morphologically similar. They were arranged as homogeneous, chain-like aggregates of nanometer-sized primary particles that are typical for gas metal arc welding fume. In a comparison of size, the majority of the mass of both the mild steel and stainless steel particles fell in the fine size range with the aerosols having a MMAD between 0.20 and 0.30 μm. Differences did exist in the chemical composition of the two fumes. As expected, Fe and Mn were prominent in each fume, whereas stainless steel particles also contained significant amounts of Cr and Ni.

Stainless steel welding fume induced more lung injury and inflammation than mild steel fume after short-term inhalation exposure in the current study. Compared to their air controls, elevations in lung damage persisted for 3 weeks and had returned to control levels by 6 weeks. No elevations in any lung toxicity parameter were observed after exposure to mild steel fume. In addition, the rate of AM clearance of stainless steel particles was significantly slower compared with mild steel particles, thus causing a longer retention time of the stainless steel particles and their associated metals in the lungs. Previous studies by our group have indicated that stainless steel fume samples were

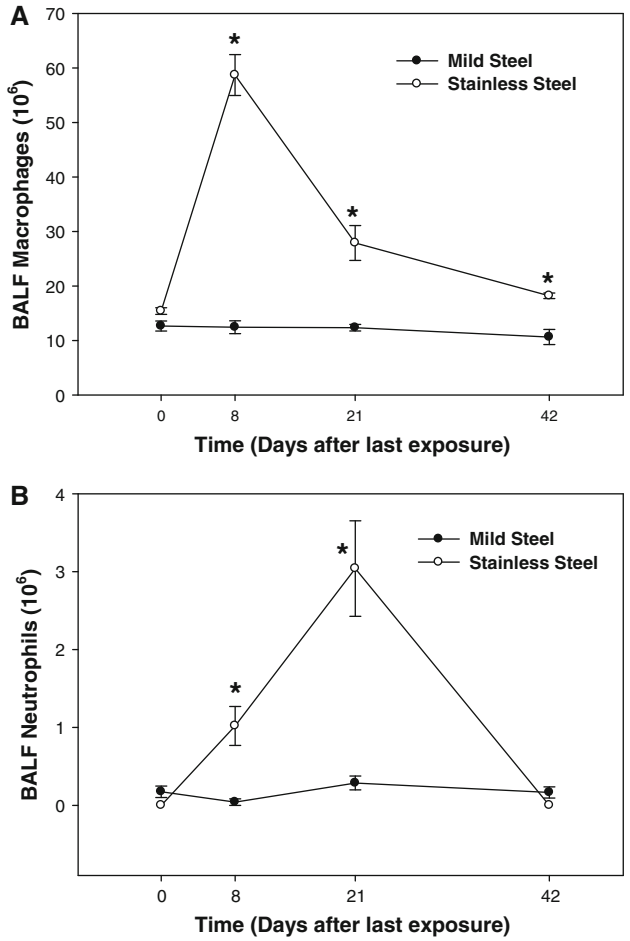

Fig. 5 Lung inflammation: **a** AMs and **b** PMNs recovered from bronchoalveolar lavage fluid at days 0, 8, 21, and 42 after inhalation of 40 mg/m^3 of mild steel or stainless steel welding fume for 3 h/day for 3 days. Values are mean ± standard error ($n = 4$). *Significantly different from the other welding fume group, $P < 0.05$

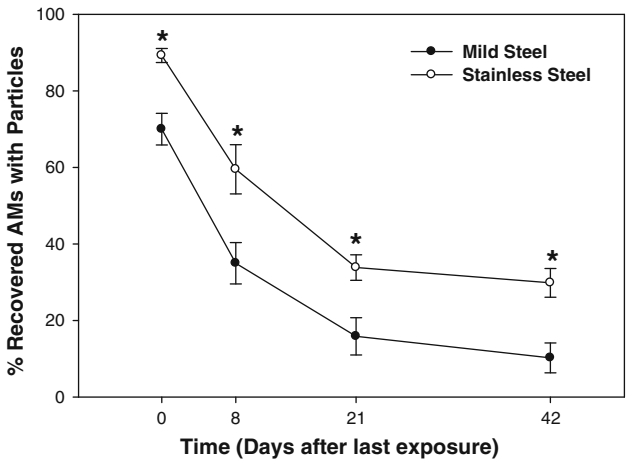

Fig. 6 Percentage of alveolar macrophages that contain particles at days 0, 8, 21, and 42 after inhalation of 40 mg/m^3 of mild steel or stainless steel welding fume for 3 h/day for 3 days. Values are mean ± standard error ($n = 4$–6). *Significantly different from the other welding fume group, $P < 0.05$

more cytotoxic to AMs in vitro compared with mild steel samples (Antonini et al. 1999), and the metals present in stainless steel fumes, specifically Cr, may suppress AM function and alter lung defense responses to bacterial challenge (Antonini et al. 2004; Antonini and Roberts 2007).

In the measurement of metal persistence after welding fume inhalation, a significantly greater amount of total metal was present in the lungs at all time points after exposure to stainless steel fume compared with mild steel fume, confirming the observations of our previous intratracheal instillation study (Antonini et al. 1996). Chemically, gas metal arc welding fumes are complex mixtures of different metal oxides that have been shown to be highly insoluble in water (Minni et al. 1984; Antonini et al. 1999; Jenkins and Eagar 2005). Although complexed together upon fume formation, specific metals were cleared from the lungs at different rates after exposure to each type of welding fume. After an initial quick clearance phase, ~40 and 50–60% of total deposited Fe persisted in the lungs during the recovery period after exposure to mild steel and stainless fumes, respectively. Interestingly, more than 80% of total deposited Mn after mild steel fume inhalation had been cleared by 8 days after exposure and was nearly all cleared from the lungs by 21 days. For the stainless steel fume, the clearance patterns among Mn, Cr, and Ni were similar to each other, but were more linear when compared with the clearance of Fe. Cr and Ni were cleared from the lungs less effectively than Fe at early time points, which may help explain the observed early elevations in lung injury and inflammation for the stainless steel fume compared with the mild steel fume, but were more completely cleared than Fe by 42 days. The rate of clearance of Mn was faster and more complete after mild steel fume exposure compared with stainless steel fume exposure, likely due to a chemically less complex metal composition of the mild steel fume. In agreement with our findings, Lam et al. (1979), using neutron-activated welding fume, observed that the clearance of each element was similar during an initial mucociliary and macrophage clearance phase (up to 1 week after exposure), but at a later phase, various elements of specific fumes were cleared at much different rates, indicative of tissue solubilization of the deposited particle. Moreover, Han et al. (2008) observed tissue solubilization of Mn deposited in the lungs of non-human primates after long-term (33 weeks) stainless steel welding fume inhalation to be an important determinant of Mn translocation from the lungs to blood to specific brain regions.

The observation that specific metals are cleared from the lungs at different rates after welding fume inhalation is a significant finding. After particle deposition, Fe appears to be bound up and persists in the lungs as significant amounts

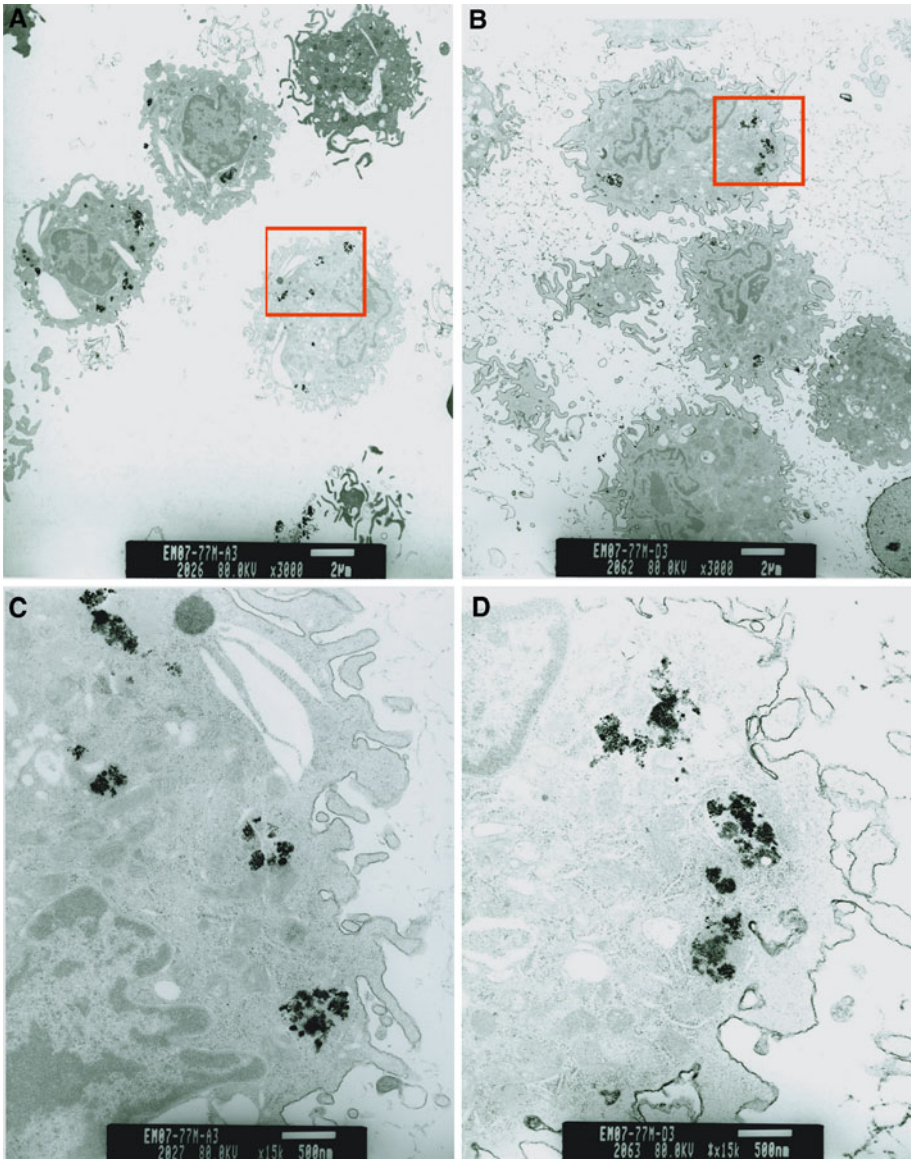

Fig. 7 Transmission electron micrographs of welding particles within AM phagolysosomes at **a** 1 and **b** 42 days after inhalation of 40 mg/m^3 of stainless steel welding fume for 3 h/day for 3 days. The images in **c** and **d** are higher magnifications of the *boxed*, *highlighted areas* from images in (**a**) and (**b**), respectively

Table 2 Lung concentration of metals after mild steel welding

Time (days)	Total metal (μg/g dry tissue)	Iron (μg/g dry tissue)	Manganese (μg/g dry tissue)
0	818 ± 34	698 ± 34	81.4 ± 5.4
8	310 ± 31	301 ± 26	11.3 ± 1.1
21	268 ± 25	288 ± 28	3.07 ± 0.6
42	252 ± 20	289 ± 18	1.03 ± 0.3

Lung deposition of total and individual metals at 0, 8, 21, and 42 days after inhalation of 40 mg/m^3 of mild steel welding fume for 3 h/day for 3 days. Control animals were exposed to filtered air. Control baseline values for metals were subtracted from metal values of the treatment group and are displayed as mean ± standard error ($n = 4$)

of Fe remained in the lungs at 42 days after exposure. A consistent observation in the examination of lungs of long-time welders is the presence of multiple iron oxide deposits, a benign form of pneumoconiosis referred to as siderosis (Kleinfeld et al. 1969; Morgan 1989). In addition, other studies have observed a slower pulmonary clearance of Fe after exposure to different welding fumes by intratracheal instillation (Lam et al. 1979; Kalliomaki et al. 1986). However, Mn was cleared more completely at the end of the exposure period compared with Fe, enhancing its elimination from the body or increasing its bioavailability and the likelihood of its translocation from the respiratory system to the circulation and other organ systems. Indeed, after repeated weekly intratracheal treatments with high doses of different welding fumes, Fe levels were not significantly increased in heart, liver, kidney, and specific brain regions, whereas elevations in Mn were observed in the kidney and spleen (Antonini et al. 2010). Importantly, after both inhalation and intratracheal instillation exposure,

Table 3 Lung concentration of metals after stainless steel welding

Time (days)	Total metal (μg/g dry tissue)	Iron (μg/g dry tissue)	Manganese (μg/g dry tissue)	Chromium (μg/g dry tissue)	Nickel (μg/g dry tissue)
0	943 ± 29	552 ± 16	108 ± 4.9	199 ± 8.2	65.5 ± 2.9
8	504 ± 22	259 ± 15	66.9 ± 2.5	153 ± 5.1	43.5 ± 2.0
21	524 ± 48	314 ± 34	50.1 ± 4.9	137 ± 10	35.0 ± 2.9
42	440 ± 63	316 ± 58	24.6 ± 1.7	76.5 ± 4.3	17.2 ± 1.2

Lung deposition of total and individual metals at 0, 8, 21, and 42 days after inhalation of 40 mg/m^3 of stainless steel welding fume for 3 h/day for 3 days. Control animals were exposed to filtered air. Control baseline values for metals were subtracted from metal values of the treatment group and are displayed as mean ± standard error ($n = 4$)

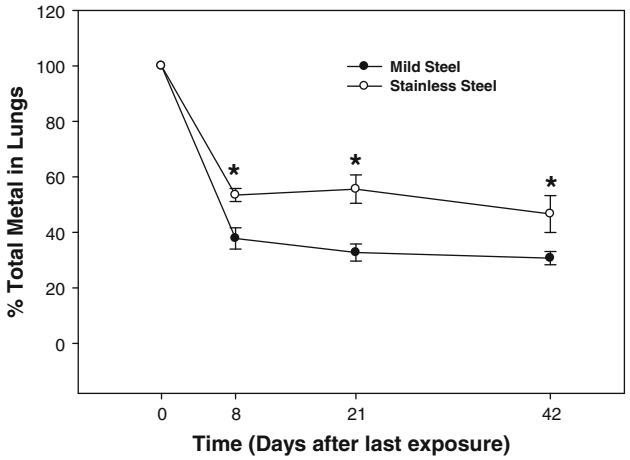

Fig. 8 Percentage of total metal measured in the lungs at days 0, 8, 21, and 42 after inhalation of 40 mg/m^3 of mild steel or stainless steel welding fume for 3 h/day for 3 days. Values are mean ± standard error ($n = 4$). *Significantly different from the other welding fume group, $P < 0.05$

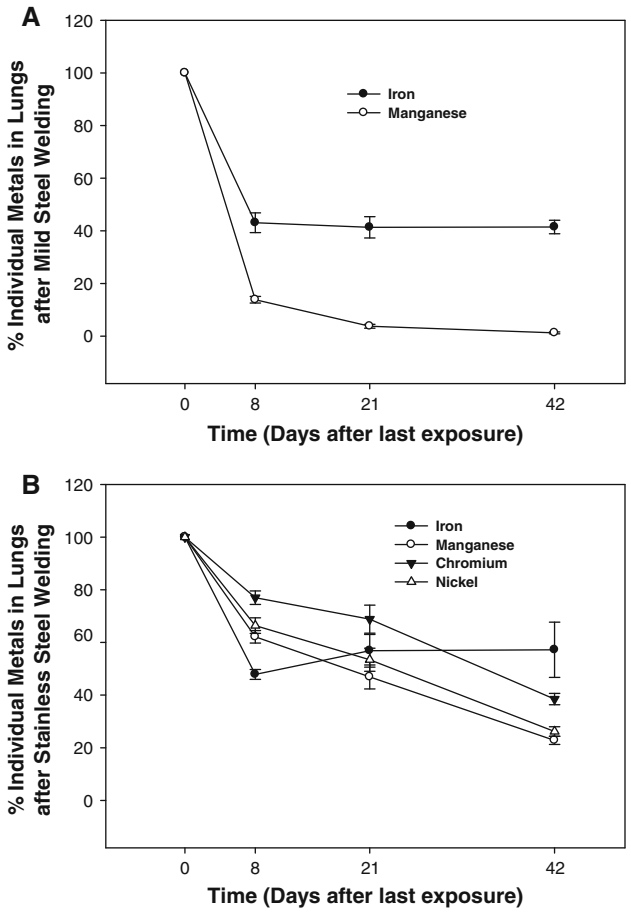

Fig. 9 Percentage of specific metals measured in the lungs at days 0, 8, 21, and 42 after inhalation of 40 mg/m^3 of **a** mild steel or **b** stainless steel welding fume for 3 h/day for 3 days. Values are mean ± standard error ($n = 4$)

Mn, unlike any of the other metals, was observed to accumulate in discrete brain regions, such as the olfactory bulb, striatum, and midbrain (Antonini et al. 2009b, 2010; Sriram et al. 2010). There is an emerging concern among occupational health investigators about the potential neurological effects associated with the exposure to Mn in welding fumes. A positive brain MRI TI hyperintensity, indicative of Mn overexposure, has been observed in welders in multiple case reports (Kim et al. 1999; Sadek et al. 2003; Josephs et al. 2005).

The mechanisms involved in the uptake of metals by lung cells and the subsequent transport of the metals from the lungs to the circulation are not completely understood. A complex interplay exists between the regulation of metal transporters by Fe status and competition for different metal transport processes among metals. Due to chemical similarities of Mn and Fe, these metals and their different valence states can compete for binding to metal transporters, e.g., divalent metal transporter-1 (DMT-1), and Fe-binding proteins, e.g., transferrin (Tf), ferritin, and lactoferritin. It has been hypothesized that the fate and toxicological implications of Mn in welding fumes may be influenced by the systemic Fe storage or by the presence of increased amounts of Fe that have been deposited in the lungs after inhalation (Brain et al. 2006). In agreement with the findings of the current study in which Mn and Fe are differentially cleared from the lungs after welding fume

inhalation, recent studies have indicated that the in vivo pharmacokinetics and the mechanisms by which intratracheally instilled Mn and Fe cross the pulmonary epithelium are remarkably different (Heilig et al. 2005, 2006). Solubilized Fe was readily oxidized to bind to transferrin, and the elements of the Tf/Tf receptor/DMT-1 pathway were involved in its transport from the lungs. In contrast, depending on valence state, the transport of Mn may involve L-type Ca2+ channels and TRPM7, a member of the transient receptor potential melastatin subfamily.

In summary, the two welding fumes generated for the study were nearly identical in particle size, solubility, and morphology. Stainless steel welding fume was observed to induce more lung injury and inflammation compared with mild steel fume after a short-term inhalation exposure in rats. No evidence of lung toxicity was observed after exposure to the mild steel fume. As we hypothesized, the rate of pulmonary clearance of deposited stainless steel particles was significantly slower compared with mild steel particles, thus causing a longer retention time of the stainless steel particles and their associated metals in the lungs. However, the specific metals present in the two fumes were cleared from the lungs at different rates after welding fume inhalation. The potentially more toxic metals (e.g., Mn, Cr, Ni) present in the stainless steel fume were cleared more completely than Fe by the end of the 42-day exposure period, likely increasing their translocation from the respiratory system to other organs. Ongoing studies in the NIOSH welding laboratory are examining the effects of these metals and their transport/uptake mechanisms after welding fume inhalation in other organs, specifically the central nervous and cardiovascular systems.

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Conflict of interest The authors declare that there are no conflicts of interest.

Disclaimer The findings and conclusions of this paper have not been formally disseminated by NIOSH and should not be construed to represent any agency determination or policy.

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