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Inhalation Toxicology

International Forum for Respiratory Research

ISSN: 0895-8378 (Print) 1091-7691 (Online) Journal homepage: http://www.tandfonline.com/loi/iiht20

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To cite this article: James M. Antonini, Jenny R. Roberts, Sam Stone, Bean T. Chen, Diane Schwegler-Berry & David G. Frazer (2009) Short-Term Inhalation Exposure to Mild Steel Welding Fume had no Effect on Lung Inflammation and Injury but did Alter Defense Responses to Bacteria in Rats, Inhalation Toxicology, 21:3, 182-192, DOI: 10.1080/08958370802360661

To link to this article: https://doi.org/10.1080/08958370802360661

Inhalation Toxicology, 21:182–192, 2009

ISSN: 0895-8378 print / 1091-7691 online
DOI: 10.1080/08958370802360661

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Short-Term Inhalation Exposure to Mild Steel Welding Fume had no Effect on Lung Inflammation and Injury but did Alter Defense Responses to Bacteria in Rats

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Many workers worldwide are continually exposed to complex aerosols generated from welding processes. The objective was to assess the effect of inhalation exposure to mild steel (MS) welding fume on lung injury, inflammation, and defense responses. Male Sprague-Dawley rats were exposed to MS fume at a concentration of 40 mg/m^3 $\times$ 3 h/day $\times$ 3 or 10 days using a robotic welding fume generator. Controls were exposed to filtered air. To assess lung defense responses, a group of animals were intratracheally inoculated with 5 $\times$ 10^4 *Listeria monocytogenes* 1 day after the last daily exposure. Welding particles were collected during exposure, and chemical composition and particle size were determined. After exposure, lung injury, inflammation, and host defense (bacterial clearance) were measured. The particles were composed of iron (80.6 %) and manganese (14.7 %) with a mass median aerodynamic diameter of 0.31 μm. No significant difference was observed in lung injury or inflammation after MS fume inhalation at 1, 4, and 11 days after the last exposure. However, there were significantly more bacteria at 3 days after infection in the lungs of the animals exposed to MS fume compared to air controls. Acute exposure of rats to MS fume had no effect on injury and inflammation, but suppressed lung defense responses after infection. More chronic inhalation studies are needed to further examine the immune effects and to elucidate the possible mechanisms of the suppressed lung defense response to infection associated with the inhalation of MS welding fume.

INTRODUCTION

Million of workers worldwide are exposed on a daily basis to aerosols generated during welding processes. Many studies have indicated that welding fume exposure may cause respiratory effects. Bronchitis, metal fume fever, infection, siderosis, and cancer have all been reported in welders (as reviewed by Antonini, 2003; Sferlazza and Beckett, 1991). Unfortunately, health effects of welders have proven to be difficult to study because of diverse workplace setting and exposure to various aerosols generated from different processes. Welders may work in a number of settings that include enclosed, poorly-ventilated spaces (e.g., ship hull, building crawl space, holding tank, pipeline) or open, well-ventilated spaces (e.g., outdoors on a construction site). Animal models are needed that would use controlled welding exposures to investigate the possible mechanisms by which welding aerosols may induce adverse health effects.

Welding processes generate a complex mixture of aerosol and gaseous by-products. The generated fumes are composed of an array of metals (e.g., iron, manganese) volatilized from the welding electrode (Zimmer and Biswas, 2001). In addition, gases (e.g., ozone, carbon monoxide, nitrogen oxides) are formed during welding operations due to the use of shielding gases and ultraviolet irradiation of atmospheric elements and contaminants by the arc. Because of the diversity of the welding process and the need to continually generate welding fumes at a constant concentration over extended periods of time, very few welding fume inhalation exposure systems have been developed. A welding fume generation and inhalation exposure system has

Received 12 June 2008; accepted 22 July 2008.

The authors wish to acknowledge the expert technical assistance on the project of Michelle Donlin, Amy Frazer, and Jared Cumpston from the inhalation exposure team. The authors also wish to thank the National Toxicology Program and Dr. Richard Dey of West Virginia University for additional support and collaboration during the ongoing welding project.

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been constructed by NIOSH that simulates real workplace exposures and allows for continuous welding for extended periods of time without interruption (Antonini et al., 2006). The system is completely automated and uses a computer-controlled, robotic welder which welds and replaces materials as they are consumed during the operation.

Arc welding processes can be quite complex. It is estimated that there are at least 80 types of welding processes, each with its own potential safety and health hazard. This study evaluates the pulmonary health effects associated with inhalation exposure to fumes generated from gas metal arc welding (GMAW) using a low alloy, mild steel (MS) electrode. MS electrodes are composed predominately of iron with varying amounts of manganese, and their use accounts for the majority of all welding (~90 %) performed in the USA (Beckett, 1996). Male Sprague-Dawley rats were exposed to GMAW-MS welding fumes for varying periods of time. Welding fumes were characterized both physically and chemically in the breathing zone of the exposed animals, and the resulting pulmonary responses after inhalation were examined.

MATERIALS AND METHODS

Experimental Design

Rats were exposed by inhalation to aerosols generated during gas metal arc welding using a MS electrode. During exposure, the generated welding particles were characterized. Three studies were performed that used different welding fume exposure regimens. See Table 1 for details of experimental design and exposure plan. At different time points after exposure, lung injury, inflammation, and defense responses to bacterial infection were assessed in the exposed animals. The welding fume generation and exposure system has been previously described in more detail and is divided into an enclosed control room; a robotic welding fume generator; and an animal exposure chamber with fume and gas characterization equipment (Antonini et al., 2006).

Animals were exposed to 40 mg/m^3 of MS welding fume in the current study. The rationale for the fume concentration was based on the results from a previous inhalation study using stainless steel welding (Antonini et al., 2007). Our calculation for dose of deposited particles in the alveolar region of the respiratory tract after exposure was based on the following equation, assuming that rat minute volume is 300 ml/min and particle deposition efficiency in the alveolar region is 10 % for rats:

$$\text{Fume concentration} \times \text{Min volume} \times \text{Exposure duration} \times \text{Deposition efficiency} = \text{Deposited dose}$$

$$40\ \text{mg/m}^3 \times (300\ \text{ml/min} \times 10^{-6}\ \text{m}^3\text{/ml}) \times (9\ \text{h} \times 60\ \text{min/h}) \times 0.10 = 0.648\ \text{mg deposited}$$

$$40\ \text{mg/m}^3 \times (300\ \text{ml/min} \times 10^{-6}\ \text{m}^3\text{/ml}) \times (30\ \text{h} \times 60\ \text{min/h}) \times 0.10 = 2.16\ \text{mg deposited}$$

Importantly, for rats exposed for 3 h/day × 10 days, the resulting deposited dose (2.16 mg) was comparable to the 2 mg/rat intratracheal instillation dose used in previously published welding fume studies (Antonini et al., 1996; Taylor et al., 2003; Antonini et al., 2004).

Welding Fume Generation System

The welding fume generation system 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. Gas metal arc welding was performed using a mild steel electrode (carbon steel ER70S-6, Lincoln Electric). 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.

TABLE 1
Experimental design and exposure regimen

Study	Fume concentration[a]	Exposure duration	Sacrifice	Pulmonary endpoints
Effect of time after exposure	Target: 40 mg/m^3 Actual: 39.8 ± 6.5	3 h/day × 3 days	1, 4, and 11 days post-exposure	Injury Inflammation
Effect of exposure duration	Target: 40 mg/m^3 Actual: 40.9 ± 7.6	3 h/day × 3 or 10 days	1 day post-exposure	Injury Inflammation
Infectivity	Target: 40 mg/m^3 Actual: 42.0 ± 6.3	3 h/day × 3 days + infection 1 day post-exposure	3 days post-infection	Bacterial clearance Injury Inflammation Cytokine response

Note.[a]Actual fume concentrations in exposure chamber are means ± standard deviations; measurements were made in duplicate every 30 minutes during the daily 3-h exposure.

To avoid disruption of welding fume exposure, a headstock was designed that holds and rotates a base metal plate holder in different programmed positions. The base metal plate holder has four sides and holds three carbon steel metal plates per side upon which the welding takes place. A computer-controlled water circulation unit was included within the base metal holder to reduce the temperature at the base metal surfaces where the welding takes place.

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. 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 determined gravimetrically every 30 minutes in duplicate during the daily 3-h exposure (see Table 1 for actual fume concentration measurements during exposure). In addition, particle samples were collected gravimetrically onto filters for scanning electron microscopy (SEM) and grids for transmission electron microscopy (TEM) to assess particle size distribution, particle morphology, and elemental composition.

To maintain welding fume concentrations during animal exposures, fume was collected through an aerosol delivery line above the welding system at a flow rate of 5 L/min drawn from an in-line peristaltic pump. Downstream from the pump, a mass-flow controller was installed as an air dilution system. Dilution airflow rate was adjusted by a solenoid valve regulator using a feedback signal to provide a desired mass concentration in the exposure chamber. The mass concentration in the chamber was monitored in real time by a real time aerosol monitor (DataRAM, Thermo Electron Co., DR-4000, Franklin, MA), and the obtained mean electrical signal was compared with a pre-calibrated signal according to the desired concentration. Based on the difference between the two signals, the solenoid valve was regulated to adjust a dilution airflow to the desired concentration in the exposure chamber. Depending on the desired concentration, the diluent air in this system was normally controlled between 20 and 80 L/min.

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. Ozone and carbon monoxide levels that were measured in the breathing zone of the animals during exposures were not significantly higher than background levels (data not shown). Temperature and humidity were measured to be 21°C and 38%, respectively, and remained constant in the chamber during the exposure period.

Welding Particle Morphology

Scanning Electron Microscopy (SEM)

Welding fume samples were collected onto 47-mm Nuclepore polycarbonate filters (Whatman, Clinton, PA). The filters were cut into equal sections and mounted onto aluminum stubs with silver paste. The deposited welding particles were viewed using a JEOL 6400 scanning electron microscope (JEOL, Inc., Tokyo, Japan).

Transmission Electron Microscopy (TEM)

Welding fume samples were collected at 30-min intervals during 3 h of welding directly onto formvar-coated TEM grids and viewed using a JEOL 1220 transmission electron microscope (JEOL, Inc.).

Welding Particle Size Distribution

Particle size distribution was determined 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, particles were collected in the size range from 0.010 to 18 μm that were separated into 15 fractions.

Welding Particle Composition

Mild steel 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) as coordinated with the Division of Applied Research and Technology (DART, Cincinnati, OH) according to NIOSH method 7300 modified for microwave digestion (NIOSH, 1994). Metal content of blank filters also were analyzed for control purposes.

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 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. 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 either treatment group during any of the exposure regimens used in the study (data not shown).

During exposure to welding fume, no animal showed any outward signs or symptoms of labored breathing or respiratory distress. Respiratory rate was measured and found not to be different when comparing fume-exposed and air control animals (data not shown). 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, exposed rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (>100 mg/kg body weight, Butler Co., Columbus, OH, USA) and then exsanguinated by severing the abdominal aorta. The lungs were first 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 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 $\times$ *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 below.

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) as alveolar macrophages (AMs) and 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 bromcresol green using an albumin BCG diagnostic kit (Sigma Chemical Co., 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 auto-analyzer (Roche Diagnostic Systems, Montclair, NJ).

Intratracheal Bacterial Inoculation

Listeria monocytogenes (strain 10403S, serotype 1) was cultured overnight in brain heart infusion broth (Difco Laboratories, Detroit, MI) at 37°C in a shaking incubator. Following incubation, the bacterial concentration was determined at an optical density of 600 nm using a spectrophotometric method. Bacterial cultures were diluted with sterile PBS to a concentration of 5×10^4 of *L. monocytogenes*. At 1 day after welding fume exposure, a separate set of animals that were exposed to MS welding fume (40 mg/m^3 $\times$ 3 h/day $\times$ 3 days) or air were inoculated with 5×10^4 of *L. monocytogenes* by intratracheal instillation. In a previous pilot study, this bacterial dose gave a uniform pulmonary lung infection and did not have an effect on animal morbidity and mortality in untreated naive Sprague-Dawley rats (Antonini et al., 2001).

Pulmonary Burden of *L. Monocytogenes*

At 3 days after infection, the left lungs were removed from rats in each treatment group. The excised tissues were suspended in 10 ml of sterile water, homogenized using a Polytron 2100 homogenizer (Brinkmann Instruments, Westbury, NY, USA), and cultured on brain heart infusion agar plates (Becton Dickinson and Co., Cockeysville, MD, USA). The number of viable colony-forming units (CFUs) was counted after an overnight incubation at 37°C. Body weight was monitored daily after infection to assess the general health status of the treated animals. BAL was performed on right lungs of infected animals to assess injury, inflammation, and cytokine levels in the recovered acellular BALF.

Lung Cytokines and Chemokines

Levels of cytokines, tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), and interleukin-12p70 (IL-12), and chemokines, monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2), were assayed in the first fraction of BALF recovered at 3 days after pulmonary infection with 5×10^4 *L. monocytogenes* or without (non-infected controls) in rats exposed to 40 mg/m^3 of MS welding fume for 3 h/day for 3 days. The selection of the cytokines/chemokines to be assayed was based on their potential role in lung inflammatory and immune responses after particulate exposure. Cytokine/chemokine protein concentrations were determined using enzyme-linked immunoabsorbent assay (ELISA) kits (Biosource International, Inc., Camarillo, CA). The results of the colorimetric assay were obtained with a Spectramax 250 plate spectrophotometer using Softmax Pro 2.6 software (Molecular Devices Corp., Sunnyvale, CA).

Statistical Analysis

Results are expressed as means $\pm$ standard error of measurement. Statistical analysis was performed using JMP statistical software (SAS, Inc., Belmont, CA). The significance of

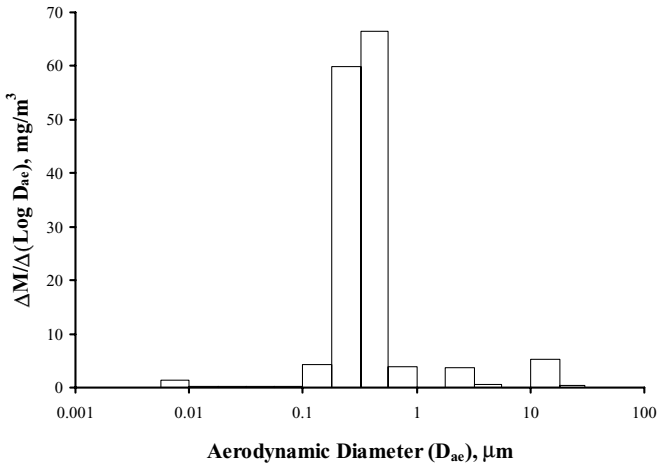

FIG. 1. A representative particle size distribution graph of generated mild steel welding fume comparing mass concentration versus particle size. Random daily measurements of particle size distribution were made during exposures throughout the course of the study.

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

Welding Particle Characterization

The majority of the collected particles was observed to be in the fine size range with cut-off diameters of 0.10–1.0 μm (Figure 1). Additional nanometer-sized particles in the range of 0.010–0.10 μm, as well as larger, coarse particles with diameters > 1.0 μm in size also were collected. The mass median aerodynamic diameter was calculated to be approximately 0.31 μm. SEM and TEM analysis of particles collected on different stages of the MOUDI samplers demonstrated that most of the aerosols generated were arranged in homogeneous, chain-like agglomerates of nanometer-sized primary particles (Figure 2). Table 2 illustrates the metal profile of the generated MS welding fume collected in the breathing zone of the animals. The particles were composed primarily of iron (80.6%) and manganese (14.7%). Small amounts of silicon and copper also were measured.

Lung Injury and Inflammation

In examining lung responses at different time points after exposure, no significant differences were observed in any of the lung injury or inflammation parameters when comparing the MS welding fume group with the air control group within each time point (Figure 3A-D). In addition, no significant differences were observed in LDH, albumin, PMN number, and AM number in animals one day after exposure to MS welding fume for 3 h/day for either 3 or 10 days when compared to the corresponding air control group (Figure 4A-D).

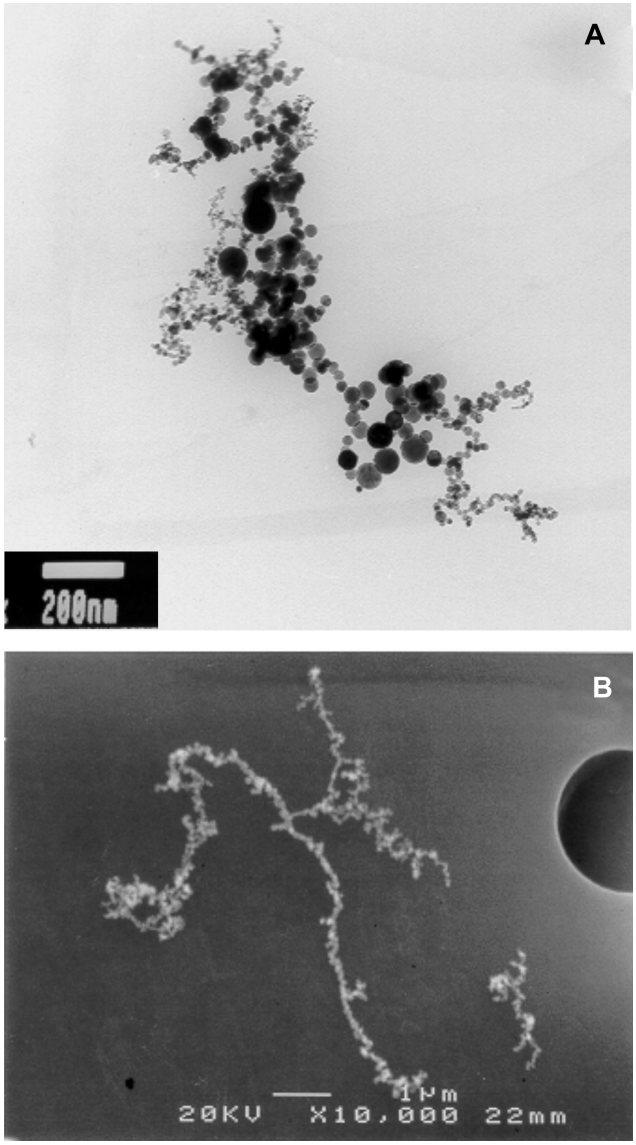

FIG. 2. Representative (A) TEM and (B) SEM micrographs of mild steel welding particles. Welding particles are chain-like agglomerates formed after aggregation of nanometer-sized primary particles.

Lung Defense Responses to Infection

To assess the effect of MS welding exposure on defense responses to bacterial challenge, the lungs of the animals were inoculated with *L. monocytogenes* one day after inhalation of MS welding fume for 3 days. There were significantly more bacteria in the lungs of the animals pre-exposed to MS welding fume before infection compared to the air control group (Figure 5A). A highly significant increase, 27-fold, in lung CFUs was observed for the MS welding fume group at 3 days after infection. Also, the same MS-exposed animals lost significantly more weight than air control animals 3 days after bacterial infection (Figure 5B).

TABLE 2
Metal composition of generated mild steel welding fume

Metals	μg/sample	Weight % of metals[a]
Fe	776 ± 9.8	80.6 ± 0.17
Mn	142 ± 2.0	14.7 ± 0.11
Si	26.6 ± 2.9	2.75 ± 0.28
Cu	17.2 ± 0.20	1.79 ± 0.02

Note. Values are means $\pm$ standard error; n = 5 welding collection periods of 30 min.
[a]Relative to all metals analyzed. Trace amounts (< 0.02 wt. %) of Al, Cr, Ni, and Ti also were present.

In the assessment of injury and inflammation, MS-exposed animals infected with *L. monocytogenes* had a significant increase in albumin, LDH, and PMN number compared to the infected-air control group and both non-infected groups (Figure 6A-C). Lung inflammation and injury, as measured by elevated PMN numbers and albumin levels, were significantly elevated for the infected-air control group compared to the two non-infected groups (Figure 6A and C). No significant differences were observed in AM number among any of the groups (Figure 6D).

No significant differences were observed for any of the cytokines or chemokines measured when comparing the non-infected animals exposed to MS welding fume or filtered air (Table 3). At 3 days after infection, significant increases in TNF-α and MIP-2 were observed in the recovered BALF of MS-exposed animals compared to all other groups. Infection caused significant elevations in BALF IL-12 and MCP-1 for both the air and MS-exposed groups when compared to the non-infected groups, whereas BALF IL-6 was significantly elevated for the infected-air control group compared to the two non-infected groups.

DISCUSSION

Previously, our group demonstrated that a single intratracheal instillation of MS welding fume, $\geq$1.0 mg/100 g body weight, induced an initial, but transient inflammatory response in the

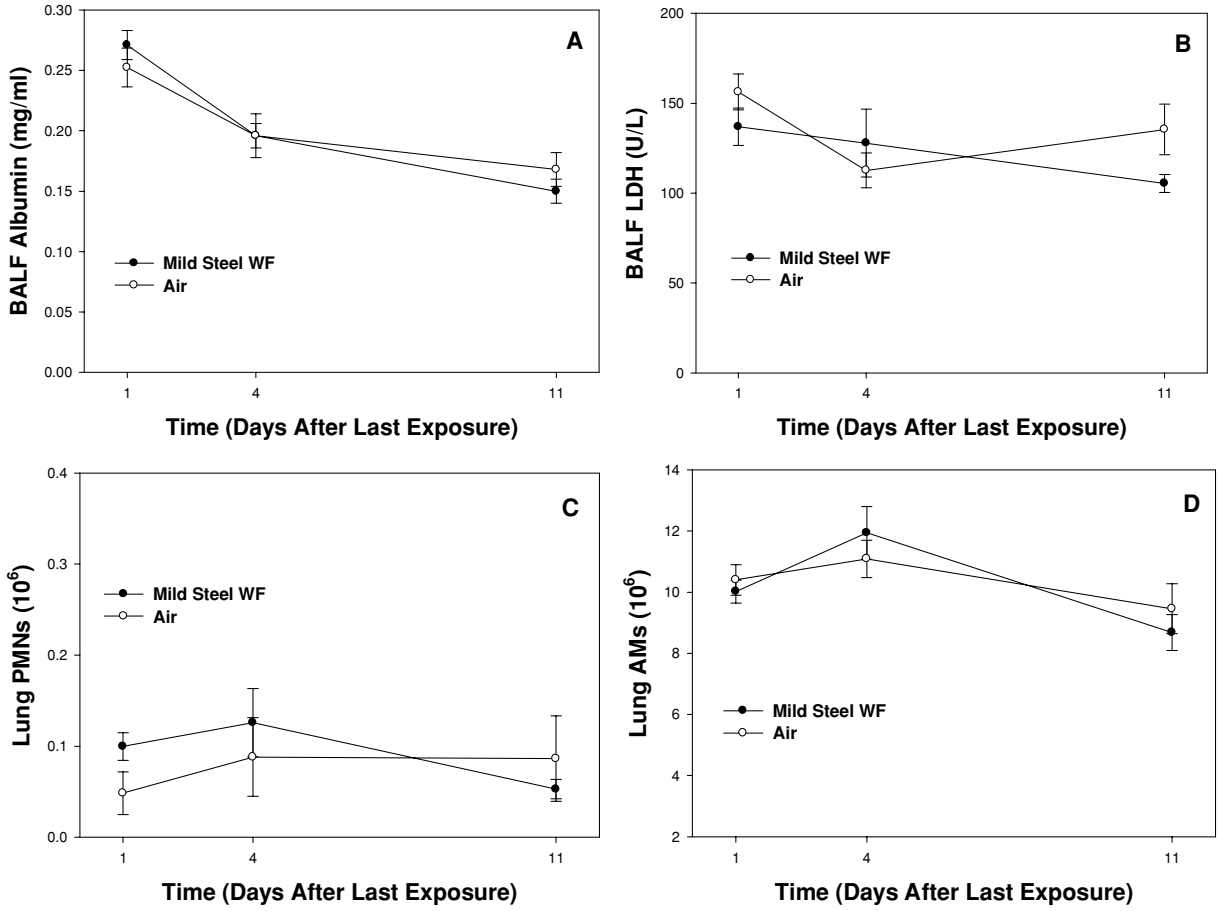

FIG. 3. (A) Albumin; (B) lactate dehydrogenase (LDH); (C) neutrophil (PMN) number; and (D) alveolar macrophage (AM) number at 1, 4, and 11 days after inhalation of 40 mg/m^3 of mild steel welding fume (WF) for 3 h/day for 3 days. Control animals were exposed to filtered air; n = 5–8 rats/treatment group.

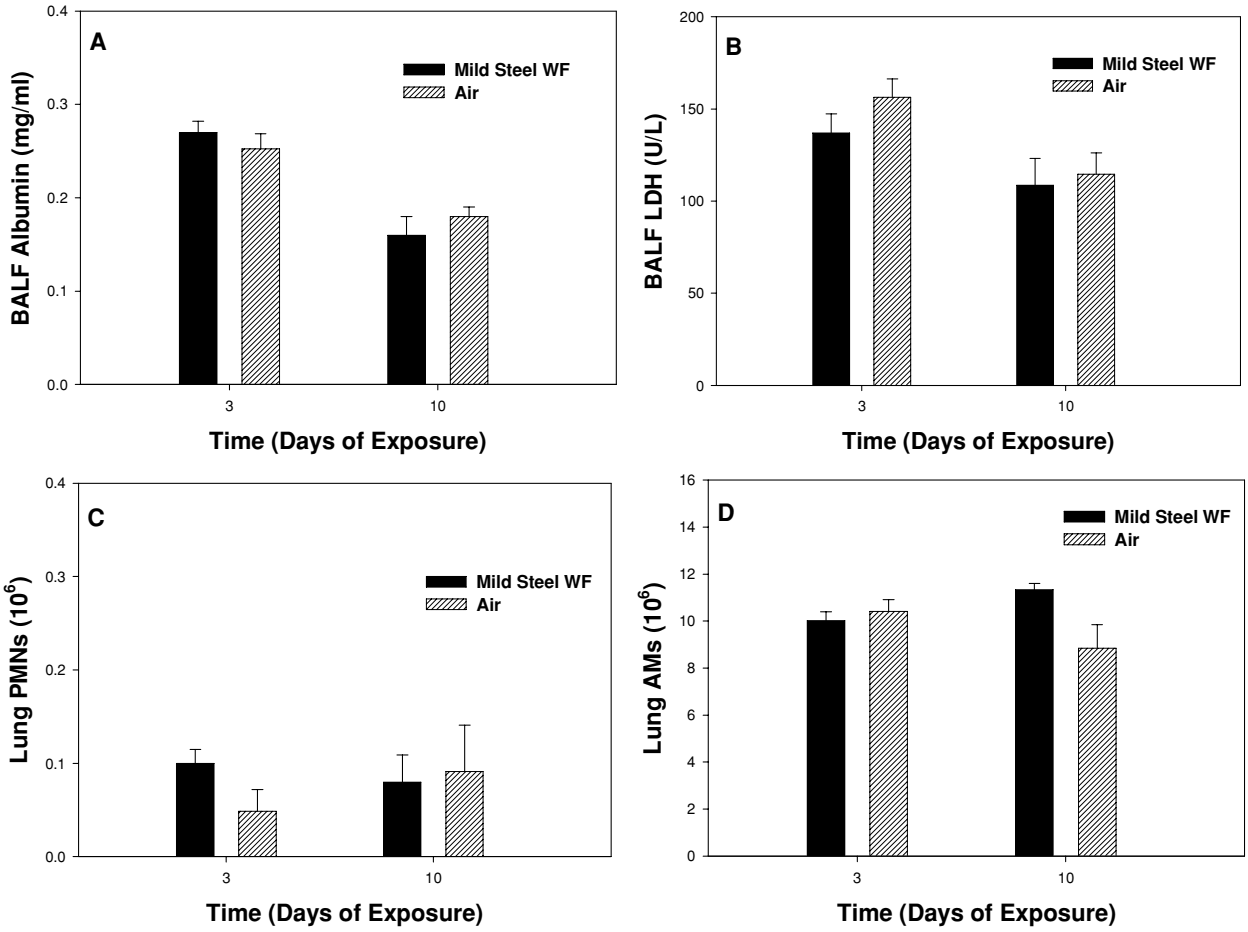

FIG. 4. (A) Albumin; (B) lactate dehydrogenase (LDH); (C) neutrophil (PMN) number; and (D) alveolar macrophage (AM) number at 1 day after inhalation of 40 mg/m^3 of mild steel welding fume (WF) for 3 h/day for 3 and 10 days. Control animals were exposed to filtered air; n = 5–8 rats/treatment group.

lungs that subsided by three days after treatment (Antonini et al., 1996; Taylor et al., 2003). This initial increase in PMN influx in the lungs observed after MS treatment may have been caused by the non-physiologic introduction of a large bolus of MS fume that often is associated with the intratracheal method of particle delivery. A study was needed to assess the effect of MS fume on lung inflammation and injury after inhalation, whereby the welding particles accumulate in the lungs gradually over time. Also, pre-collected MS welding particles used for intratracheal instillation studies may not be representative of actual fumes

TABLE 3
profile after infection in lungs exposed to MS welding fume

Group	Listeria	TNF-α (pg/ml)	IL-2 (pg/ml)	IL-6 (pg/ml)	IL-10 (pg/ml)	IL-12 (pg/ml)	MCP-1 (pg/ml)	MIP-2 (pg/ml)
Air	No	47.1 ± 24.3	115 ± 23.2	40.2 ± 12.4	165 ± 17.5	1.92 ± 1.56	18.3 ± 11.7	474 ± 26.5
MS WF	No	39.0 ± 14.6	116 ± 22.0	47.4 ± 5.96	131 ± 13.7	1.13 ± 1.13	12.4 ± 7.62	399 ± 27.4
Air	Yes	88.9 ± 14.9	93.3 ± 12.5	194 ± 60.7$^{\#}$	172 ± 33.8	63.6 ± 7.22$^{\#}$	513 ± 80.8$^{\#}$	403 ± 20.7
MS WF	Yes	323 ± 50.3*	82.4 ± 14.1	106 ± 51.7	220 ± 79.3	102 ± 17.2$^{\#}$	590 ± 53.1$^{\#}$	615 ± 72.1*

Note. Values are means ± standard error (n = 4–5); *significantly different from all other groups for a specific cytokine; $^{\#}$significantly different from both non-infected groups for a specific cytokine, $p < 0.05$. Cytokines were measured by ELISA in BALF recovered at 3 days after pulmonary infection with 5×10^4 *L. monocytogenes* or without (non-infected controls) in rats pre-exposed to 40 mg/m^3 of mild steel (MS) welding fume for 3 h/day for 3 days. Control animals were exposed to filtered air.

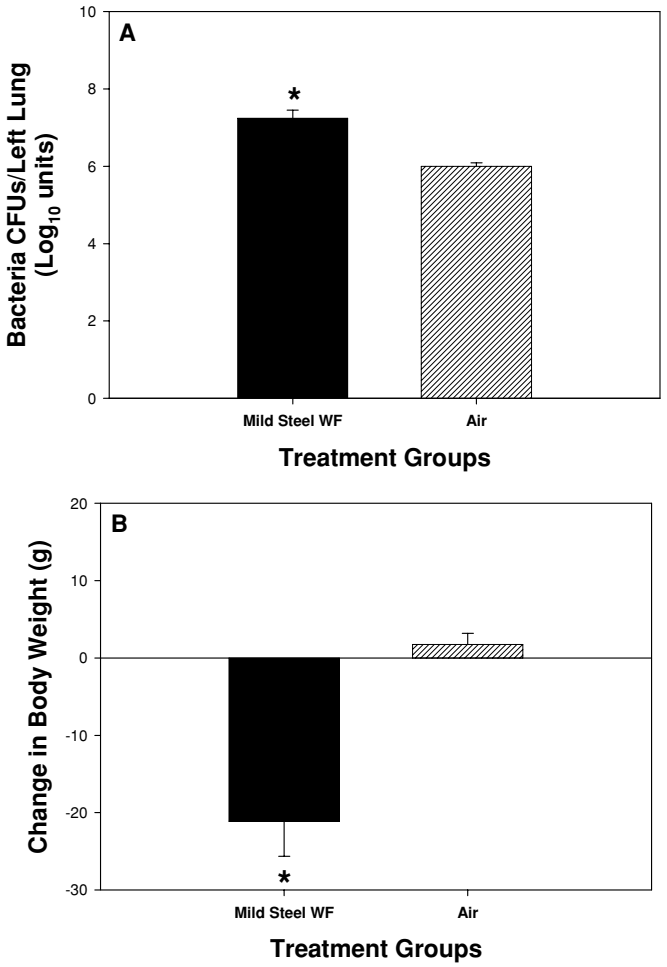

FIG. 5. Infectivity study: (A) pulmonary bacterial load and (B) change in body weight at 3 days after pulmonary infection with 5×10^4 *L. monocytogenes* in rats pre-exposed to 40 mg/m^3 of mild steel welding fume (WF) for 3 h/day for 3 days. Control animals were exposed to filtered air before infection; n = 8 rats/treatment group. *significantly different from air control, $p < 0.05$.

that are freshly formed during welding. We have observed that freshly generated stainless steel welding fume had an increase in free radicals on particle surfaces and was more inflammatory to lungs compared to aged fumes (Antonini et al., 1998).

An automated robotic welding fume generator and exposure system has been constructed and characterized by NIOSH. Initial studies have shown that the system can continuously generate a consistent concentration of fume from GMAW processes for extended periods of time (Antonini et al., 2006; Antonini et al., 2007). Characterization of the generated MS aerosol in the current study has indicated that particle morphology, size distribution, and chemical composition are comparable to welding fume studied by other investigators (Zimmer and Biswas, 2001; Jenkins and Eagar, 2005; Jenkins et al., 2005). The MS fume generated by the NIOSH robotic welder was found to be insoluble in water and nearly identical in chemical composition to the MS fume used in previous intratracheal instillation studies (Antonini et al., 1996; Taylor et al., 2003). The generated MS aerosols were composed of iron and manganese oxides, and possessed the typical morphological features of welding fume in that the particles were arranged as chain-like agglomerates of aggregated nanometer-sized primary particles. The majority of the mass of the welding agglomerates was in the size range between 0.2–0.6 μm in aerodynamic diameter, giving the particles a high probability of depositing in the alveolar regions of the lungs.

In regard to pulmonary toxicity, the current study indicates that inhalation of a relatively high concentration of MS welding fume (40 mg/m^3) had no effect on lung injury and inflammation after exposure for 3 h/day for 3 or 10 days. No changes were observed in lung PMN influx and *in vivo* pulmonary cell cytotoxicity and breakdown of air-capillary barrier after short-term exposure to MS welding fume. In agreement with most worker studies (as reviewed by Antonini, 2003), the current animal study has demonstrated that continual exposure to MS welding fume produces no inflammation and injury in the lungs of exposed rats. The pneumotoxic and fibrogenic potential of MS welding fume appears to be quite low. Indeed, a consistent observation in the lungs of long-time welders is the presence of multiple iron oxide deposits without evidence of inflammation or fibrosis. This condition is known as siderosis and is classified as a benign form of pneumoconiosis (Kleinfeld et al., 1969; Morgan, 1989).

The same cannot be said about the potential pneumotoxicity of fume generated from welding of stainless steel or other specialized steels, indicating that certain groups of welders may be at a greater risk for adverse health effects. Stainless steel welding fume contains significant amounts of chromium and measurable amounts of nickel that are not present in MS welding fume. We have clearly demonstrated that the inhalation of a lower concentration (15 mg/m^3) of stainless steel welding fume induced persistent lung injury and a delayed inflammatory response using the same generation system and a similar exposure regimen described in the current study (Antonini et al., 2007). In addition, pulmonary treatment with stainless steel welding fumes produced atypical hyperplastic changes in the lung tumor-susceptible A/J mouse strain after 48 weeks that has not been shown to occur after MS exposure (Solano-Lopez et al., 2006).

A common ailment of many welders, not specific to any type of welding (e.g., mild steel versus stainless steel), is a possible increased susceptibility to lung infection. Severity, frequency, and duration of upper and lower respiratory tract infections have been reported to be increased among welders (Howden et al., 1988), and excess mortality from pneumonia has been observed in welders and metal fume workers (Doig and Challen, 1964; Coggon et al., 1994; Hoffmaster et al., 2006; Avashia et al., 2007). A previous intratracheal instillation animal study that compared the effects of three chemically distinct welding fumes on lung defense responses after bacterial challenge indicated that

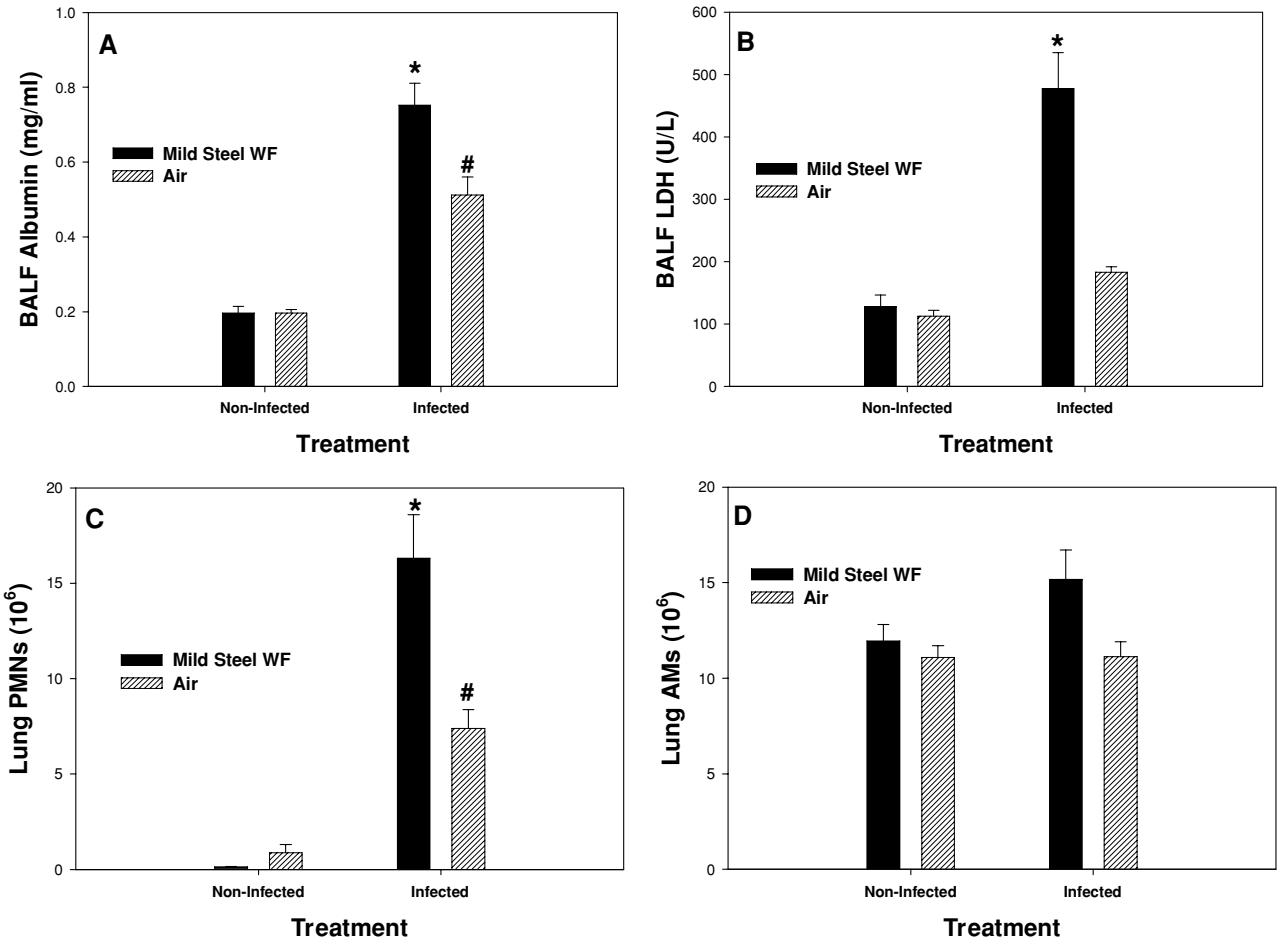

FIG. 6. Infectivity study: (A) albumin; (B) lactate dehydrogenase (LDH); (C) neutrophil (PMN) number; and (D) alveolar macrophage (AM) number at 3 days after pulmonary infection with 5×10^4 *L. monocytogenes* in rats pre-exposed to 40 mg/m^3 of mild steel welding fume (WF) for 3 h/day for 3 days. Control animals were exposed to filtered air; n = 4–5 rats/treatment group. *significantly different from all other groups; #significantly different from non-infected groups, $p < 0.05$.

the increase in susceptibility to infection may be isolated to processes that generate highly water-soluble stainless steel welding fume (Antonini et al., 2004). Lung defenses remained normal after treatment with the MS fume used in the intratracheal instillation study. In attempting to determine which individual metals in welding fume may alter lung immunity, we observed soluble chromium, a primary component of stainless steel fume, to increase susceptibility to lung infection (Antonini and Roberts, 2007). Importantly, lung defense responses to bacterial challenge were normal after intratracheal treatment with iron oxide, the primary component of the MS fume.

In disagreement with the previous intratracheal instillation study (Antonini et al., 2004), inhalation of MS welding fume in the current study appears to suppress lung defense responses. Exposure to the MS fume before bacterial infection delayed lung clearance of the bacteria, caused weight loss, and induced a greater inflammatory response compared to infected air control animals. The differences in response to infection comparing the intratracheal instillation and the current inhalation studies are likely due to the differences in the rate and pattern of lung deposition of the MS particles as well as recruitment and activation of lung phagocytes. Intratracheal instillation studies have been shown to result in heavy, more centralized deposits of particles, whereas the inhalation pattern of particle deposition is lighter, and more evenly and widely distributed (Brain et al., 1976). For the intratracheal instillation study, a significant increase in PMNs, possibly in response to the bolus dose of particles, was observed in the lungs one day after treatment with the MS fume. This initial increase in the number of PMNs recruited to the lungs, which is not observed in the inhalation studies, may aid the AMs in the killing and clearance of the bacteria in the lungs.

In addition, alterations in signaling and activation of recruited inflammatory cells may explain the differences in bacterial clearance that were observed between exposure by intratracheal instillation and inhalation of MS fume. A battery of different

cytokines and chemokines were measured in the lavage fluid of infected and non-infected air and MS exposed animals to address this question and the possible mechanisms of immunosuppression. Previous studies have indicated that pulmonary treatment with soluble stainless steel welding fume and soluble chromium reduced IL-2 (involved in T cell proliferation) before and after infection (Antonini et al., 2004; Antonini et al., 2004), and stainless steel fume enhanced IL-10 (involved in inhibiting macrophage function) after infection (Antonini et al., 2004). These alterations in cytokine production were not observed in the current study. MS fume inhalation had no effect on lung cytokine or chemokine levels before infection, and increased only TNF-α and MIP-2, two proteins important in inflammation, after infection. Interestingly, IL-6 was not significantly elevated when comparing the infected MS fume group with the non-infected groups, but was significantly elevated in the infected air control group compared to the non-infected groups. This finding indicates that there may have been a delay in the acute phase response after infection in the MS fume group, which would correlate with the observed delay in pulmonary bacterial clearance. Additional infectivity studies are needed that use multiple MS fume inhalation concentrations and different times of exposure as well as multiple bacteria doses to further elucidate the possible mechanisms of the suppressed lung defense responses observed in the current study.

In summary, a novel robotic welding fume generation and inhalation exposure system has been developed that exposes animals to well-controlled and characterized aerosols that are physically and chemically identical to fume generated in the workplace. It appears from the current inhalation study and previous intratracheal instillation studies (Antonini et al., 1996; Taylor et al., 2003) that exposure to MS welding fumes, even at relatively high concentrations, has little potential to produce debilitating lung damage and persistent inflammation. These negative findings are corroborated after review of the pulmonary health effects of mild steel welders (Antonini, 2003). However, it does appear that MS welding fumes can suppress lung immune responses and increase the susceptibility to infection. Not surprisingly, Wergeland and Iversen (2001) warned about the possible lethal risk of an association of pneumonia with the inhalation of metal fumes. It was suggested that pneumonia after exposure to fumes from welding, cutting, or grinding may require hospitalization and that the inhalation of metal fumes may seriously aggravate the prognosis of pneumonia. Moreover, Palmer et al. (2003) have reported that pulmonary exposure to ferrous metal fume workers may increase the susceptibility to infection. They suggest that the excess of free body iron after exposure may serve as a nutrient for microorganisms, promoting their growth and possibly enhancing their pathogenic potential. It has been demonstrated that innate immune responses to bacteria may be dependent on iron regulation and sequestration by the host during infection (Flo et al., 2004). Studies currently are ongoing to investigate this hypothesis.

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