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ScienceDirect
Toxicology and Applied Pharmacology 223 (2007) 234–245
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Effect of short-term stainless steel welding fume inhalation exposure on lung inflammation, injury, and defense responses in rats

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Received 11 May 2007; revised 11 June 2007; accepted 11 June 2007
Available online 6 July 2007

Abstract

Many welders have experienced bronchitis, metal fume fever, lung function changes, and an increase in the incidence of lung infection. Questions remain regarding the possible mechanisms associated with the potential pulmonary effects of welding fume exposure. The objective was to assess the early effects of stainless steel (SS) welding fume inhalation on lung injury, inflammation, and defense responses. Male Sprague–Dawley rats were exposed to gas metal arc-SS welding fume at a concentration of 15 or 40 mg/m^3 × 3 h/day for 1, 3, or 10 days. The control group was exposed to filtered air. To assess lung defense responses, some animals were intratracheally inoculated with 5×10^4 *Listeria monocytogenes* 1 day after the last exposure. Welding particles were collected during exposure, and elemental composition and particle size were determined. At 1, 4, 6, 11, 14, and 30 days after the final exposure, parameters of lung injury (lactate dehydrogenase and albumin) and inflammation (PMN influx) were measured in the bronchoalveolar lavage fluid. In addition, particle-induced effects on pulmonary clearance of bacteria and macrophage function were assessed. SS particles were composed of Fe, Cr, Mn, and Ni. Particle size distribution analysis indicated the mass median aerodynamic diameter of the generated fume to be 0.255 μm. Parameters of lung injury were significantly elevated at all time points post-exposure compared to controls except for 30 days. Interestingly, no significant difference in lung PMNs was observed between the SS and control groups at 1, 4, and 6 days post-exposure. After 6 days post-exposure, a dramatic increase in lung PMNs was observed in the SS group compared to air controls. Lung bacteria clearance and macrophage function were reduced and immune and inflammatory cytokines were altered in the SS group. In summary, short-term exposure of rats to SS welding fume caused significant lung damage and suppressed lung defense responses to bacterial infection, but had a delayed effect on pulmonary inflammation. Additional chronic inhalation studies are needed to further examine the lung effects associated with SS welding fume exposure.
Published by Elsevier Inc.

Keywords: Welding fume; Inhalation; Inflammation; Particulates; Chromium

Introduction

It has been estimated that greater than 5 million workers worldwide are exposed to welding aerosols on a daily basis. Approximately 360,000 workers are classified as full-time welders in the United States (Bureau of Labor Statistics, 2005). Welding is a common industrial process used to join metals. Complex mixtures of aerosol and gaseous by-products are formed during welding. The generated fumes are composed of an array of metals, such as iron (Fe), manganese (Mn), chromium (Cr), and nickel (Ni), volatilized from the welding electrode. Welders also are exposed to gases, such as ozone and carbon monoxide, that may affect their health.

Over the past 40 years, numerous studies have evaluated the health effects of welding (Antonini, 2003). Most studies have focused on the pulmonary effects associated with welding fume exposure. Bronchitis, metal fume fever, lung function changes, siderosis, immunosuppression, and a possible increase in the incidence of lung cancer have all been reported in welders (Antonini et al., 2003). Even less is known about the non-pulmonary effects associated with welding, specifically the potential neurological effects (Josephs et al., 2005; Racette et al., 2005).

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0041-008X/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.taap.2007.06.020

The potential adverse health effects associated with welding fume inhalation can be challenging to study. Welders are not a homogeneous group. Their exposure can vary due to differences in industrial setting, work area ventilation, the types of welding processes and material used, and exposures to other occupational hazards, such as solvents and asbestos. Little information is available about the causes and potential mechanisms by which welding fume inhalation may adversely affect health. The use of animal models and the ability to control the welding fume exposure may be helpful in the elucidation of these mechanisms.

A NIOSH welding program has been developed in the Health Effects Laboratory Division. The goal of the program is to use animal models to assess the effects of welding fume inhalation on possible increases in lung tumorigenicity (Solano-Lopez et al., 2006), susceptibility to lung infection (Antonini et al., 2004), and the development of neurotoxicity (Antonini et al., 2006a). A welding fume generation and inhalation exposure system has been developed by NIOSH that can simulate real workplace exposures and allow for continuous welding for extended periods of time without interruption (Antonini et al., 2006b). The system is completely automated and uses a computer-controlled robotic welder, which welds and replaces materials as they are consumed during the operation. This manuscript describes a series of short-term animal inhalation studies that evaluate dose and time effects of stainless steel (SS) welding fume exposure on lung injury, inflammation, and defense response to infection. The observations and results generated from these acute studies will be the basis for designing a long-term animal inhalation study that will evaluate the potential toxic effects associated with welding fumes.

Materials and methods

Experimental design

Rats were exposed by inhalation to aerosols generated during gas metal arc welding using an SS welding electrode. Four 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.

Welding fume generation system

The welding fume generation system was comprised of a welding power source (Power Wave 455, Lincoln Electric, Cleveland, OH), an automated, programmable six-axis robotic arm (Model 100 Bi, Lincoln Electric, Cleveland, OH), a water-cooled arc welding torch (WC 650 amp, Lincoln Electric, Cleveland, OH), 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 to keep the welding nozzle free of debris and spatter. Gas metal arc welding was performed using 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.

A schematic diagram of the robotic welding fume generation system is shown in Fig. 1. The system can be divided into three different areas: (1) enclosed control room; (2) robotic welding fume generator; and (3) animal exposure chamber with fume and gas characterization equipment as previously described (Antonini et al., 2006b).

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 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. Excessive heat production at the surface may lead to warping of the base metal pieces which in turn could disrupt the arc and result in altered morphology and size of the generated welding particles.

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 min in duplicate during the daily 3-h exposure (see Table 1 for actual fume concentration measurements during exposure). In addition, particle samples were periodically 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.

Two different fume exposure concentrations were used in the study. Animals were exposed to either 15 or 40 mg/m^3 for 3 h/day. The rationale for the selection of the dosing regimen was based on the previously established welding fume threshold exposure limit value–time weighted average (TLV–TWA) of 5 mg/m^3 for 8 h/day (ACGIH, 2001). The 15 mg/m^3 for 3 h/day exposure regimen is

Table 1
Experimental design and exposure regimen

Study	Fume concentration (mg/m^3)[a]	Exposure duration	Sacrifice	Pulmonary endpoints
1. Time course	Target: 15 Actual: 21.8±4.7	3 h/d×1, 3, 10 d	1 d post-exposure	Injury Inflammation
2. Dose response	Target: 15; 40 Actual: 16.6±4.9; 38.5±7.0	3 h/d×10 d	1 d post-exposure	Metal deposition Injury Inflammation
3. Recovery	Target: 40 Actual: 43.5±4.6	3 h/d×3 d	1, 4, 6, 11, 14, 30 d post-exposure	Injury Inflammation Cytokine response
4. Infectivity	Target: 40 Actual: 38.0±7.0	3 h/d×3 d+infection 1 d post-exposure	3 d post-infection	Bacterial clearance Cytokine response

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

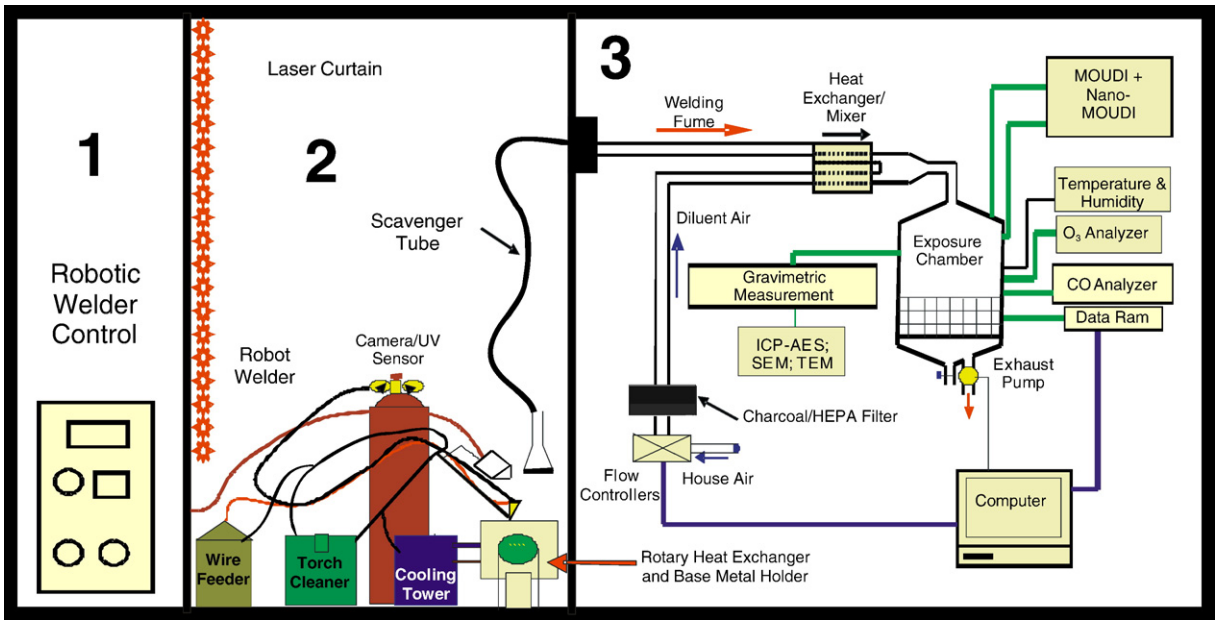

Fig. 1. Diagram of the NIOSH welding fume generation system including: (1) enclosed control room with welding power source and computer controller; (2) robotic welding fume generator with six-axis robotic arm, wire feeder, torch cleaner, coolers, and base metal holder; (3) animal exposure chamber with fume and gas characterization devices. A modification of this figure was previously published (Antonini et al., 2006c). 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.

comparable to the TLV–TWA for welding. Our goal was to expose animals to welding fume at concentrations near or slightly above the recommended TLV–TWA. Risk assessment analyses of common welding exposures and measurements of workplace levels of total welding particulate have indicated that the TLV–TWA of 5 mg/m^3 is commonly exceeded in actual workplace settings (Susi et al., 2000).

In addition, we wanted to use a fume exposure concentration that led to lung particle deposition that was close to a 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). Our calculations were 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:

$$\text{Fume concentration} \times \text{min volume} \times \text{exposure duration} \times \text{deposition efficiency} = \text{Deposited dose}$$

$$15\ \text{mg/m}^3 \times (300\ \text{ml/min} \times 10^{-6}\ \text{m}^3\text{/ml}) \times (30\ \text{hr} \times 60\ \text{min/hr}) \times 0.10 = 0.81\ \text{mg deposited}$$

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

For rats exposed for 3 h/day × 10 days to the higher concentration, the resulting deposited dose was comparable to the 2 mg/rat intratracheal instillation dose.

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 regulated 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, MIE, Inc. DR-2000, Bedford, 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. Low amounts of ozone (0.041 ± 0.019 ppm) were formed in the chamber during the exposure period. Ozone levels generated during welding were not significantly higher than background levels (~0.025 ppm) and were lower than the NIOSH recommended exposure limit (REL) and OSHA permissible exposure limit (PEL) of 0.1 ppm. Carbon monoxide levels also were not significantly higher than background levels and were lower than the NIOSH REL (35 ppm) and OSHA PEL (50 ppm). Temperature and humidity were measured and remained constant in the chamber during the exposure period. Mean temperature and percent relative humidity and standard deviations were 20.9 ± 1.4 °C and 37.7 ± 2.7%, respectively.

Welding particle composition

Stainless 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 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 were analyzed.

Welding particle morphology

Scanning electron microscopy. 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. 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., Tokyo, Japan).

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, it was possible to collect particles in the size range from 0.010 to 18 μm that were separated into 15 fractions.

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 while 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. 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. The animal facilities are specific pathogen-free, environmentally controlled, and accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

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 wt 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×*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×*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.

Pulmonary deposition of metals

Non-lavaged whole lungs from a separate set of exposed animals were excised, weighed, and freeze-dried after an overnight lyophilization. The dried lung tissue were digested in a microwave in the presence of nitric acid. The amount of Fe, Cr, Mn, and Ni deposited in the lung was determined by ICP-AES at NIOSH-DART (Cincinnati, OH) according to NIOSH method 7300 modified for microwave digestion (NIOSH, 1994).

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 as alveolar macrophages (AMs) and neutrophils (PMNs) with Leukostat stain (Fisher Scientific, Pittsburgh, PA, USA).

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, USA). 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, USA).

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 at different time points after exposure. 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, USA). 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, USA).

Chemiluminescence

Luminol-dependent chemiluminescence (CL) measures the light generated as reactive oxygen/nitrogen species are produced by activated AMs. CL was performed with an automated Berthold Autolumat LB 953 luminometer (Wallace, Inc., Gaithersburg, MD, USA) as previously described (Antonini et al., 1994) on macrophages recovered from exposed animals by BAL. Luminol was used as an amplifier to enhance detection of the light, and 2 mg/ml of unopsonized zymosan (Sigma Chemical Co.) was added immediately prior to the measurement of CL to activate the macrophages. Rat lung PMNs have not been observed to respond to unopsonized zymosan in our system; therefore, the zymosan-stimulated CL produced is generated by macrophages. Measurement of CL was recorded for 15 min at 37 °C using 5×10^5 macrophages, and the integral of counts versus time was calculated. Zymosan-stimulated CL was calculated as the total counts of stimulated cells minus the total counts of the corresponding resting cells.

Intratracheal bacterial inoculation

L. monocytogenes (strain 10403S, serotype 1) was cultured overnight in brain heart infusion broth (Difco Laboratories, Detroit, MI, USA) at 37 °C in a shaking incubator. Following incubation, the bacterial concentration was determined at an optical density of 600 nm using a spectrometric 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 exposed to SS welding fume (40 mg/m^3 × 3 h/day × 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 cytokine levels in the recovered acellular lavage fluid.

Statistical analysis

Results are expressed as means ± standard error of measurement. Statistical analysis was performed using JMP statistical software (SAS, Inc., 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 $p<0.05$.

Results

The generated welding aerosols from the robotic welder were characterized to determine if they were similar to fume generated in the workplace. To quantify the amount of each metal present, bulk particle samples were collected onto filters during 30 min of welding (Table 2). The welding particles were composed of (in descending order of amount present) Fe, Cr, Mn, Ni, and Cu. Trace amounts of Si, Al, and V also were present.

Because of the potential to generate nanometer-sized particles during welding, particle size distribution was determined using a combination of MOUDI and Nano-MOUDI samplers that determines particle sizes from 0.010 to 18 μm. The most significant mass of particles was in the fine size range with cut-off diameters of 0.10–1.0 μm (Fig. 2). Additional ultrafine particles in the range of 0.010–0.10 μm as well as larger, coarse particles with diameters from 1.0 to 10 μm in size also were observed. The mass median aerodynamic diameter was calculated to be approximately 0.255 μm with a geometric standard deviation (σ_g) of 1.352. Scanning and transmission electron microscopic analysis demonstrated that most of the aerosols generated were arranged in homogeneous, chain-like agglomerates of primary nanoparticles (Figs. 3A and B).

To ensure that the animals in the welding fume group were receiving a consistent exposure of welding particles, the pulmonary deposition of the primary metals associated with SS welding fume was measured. There was a dose-dependent increase in the lung concentrations of Fe, Cr, Mn, and Ni after SS welding fume exposure for 10 days (Fig. 4). Fe, the most abundant metal in the fume, deposited at the highest concentration in the lungs followed by Cr, Mn, and Ni.

To assess the effect of exposure time on lung injury and inflammation, animals were exposed for 3 h/day for 1, 3, or 10 days to 15 mg/m^3 of SS welding fume. Exposure to SS welding fume for 1 and 3 days had no effect on lung inflammation and

Table 2
Metal composition of generated stainless steel welding fume

Metals analyzed	μg/sample	Weight % of metal[a]
Fe	1207.0±161.7	57.0±2.6
Cr	427.5±69.1	20.2±3.0
Mn	295.0±48.4	13.8±0.9
Ni	185.0±24.0	8.8±0.4
Cu	3.3±0.49	0.2±0.0

Note. Values are means ± standard error; $n=4$ welding collection periods of 30 min.

[a] Relative to all metals analyzed. Trace amounts of Si, Al, and V also were present. These data were previously presented in Antonini et al. (2006b) and were included to illustrate the metal profile of the particles that were inhaled by the animals in this study.

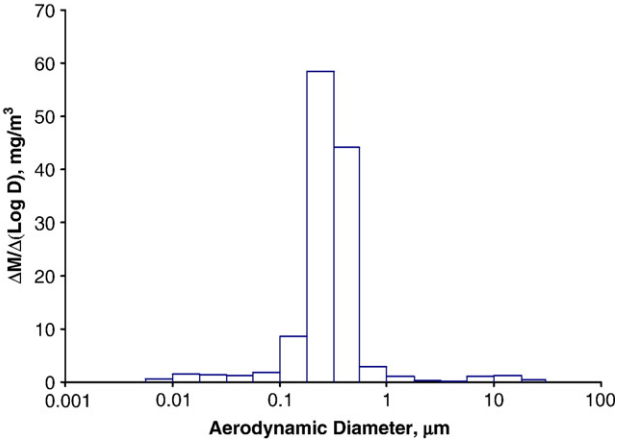

Fig. 2. Representative particle size distribution graph of generated SS welding particles comparing mass concentration versus particle size. Random daily measurements of particle size distribution were made during exposures throughout the course of the study.

injury compared to air controls (Fig. 5). A 10-day exposure to SS welding fume caused a significant increase in lung injury (Figs. 5A and B). In addition, the number of PMNs recovered from the welding fume group was significantly elevated at 10 days. However, despite the elevation in inflammation, only 10% of the cells recovered from the welding fume rats were PMNs (Fig. 5C).

To assess the effect of dose on lung injury and inflammation, animals were exposed for 3 h/day for 10 days to 15 or 40 mg/m^3

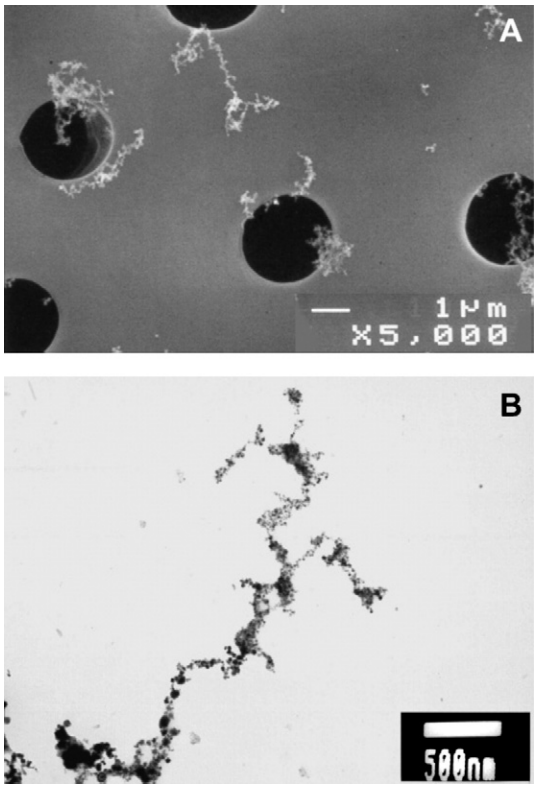

Fig. 3. Representative (A) scanning and (B) transmission electron micrograph of an SS welding particles. Note the many primary nanoparticles that form the chain-like agglomerates.

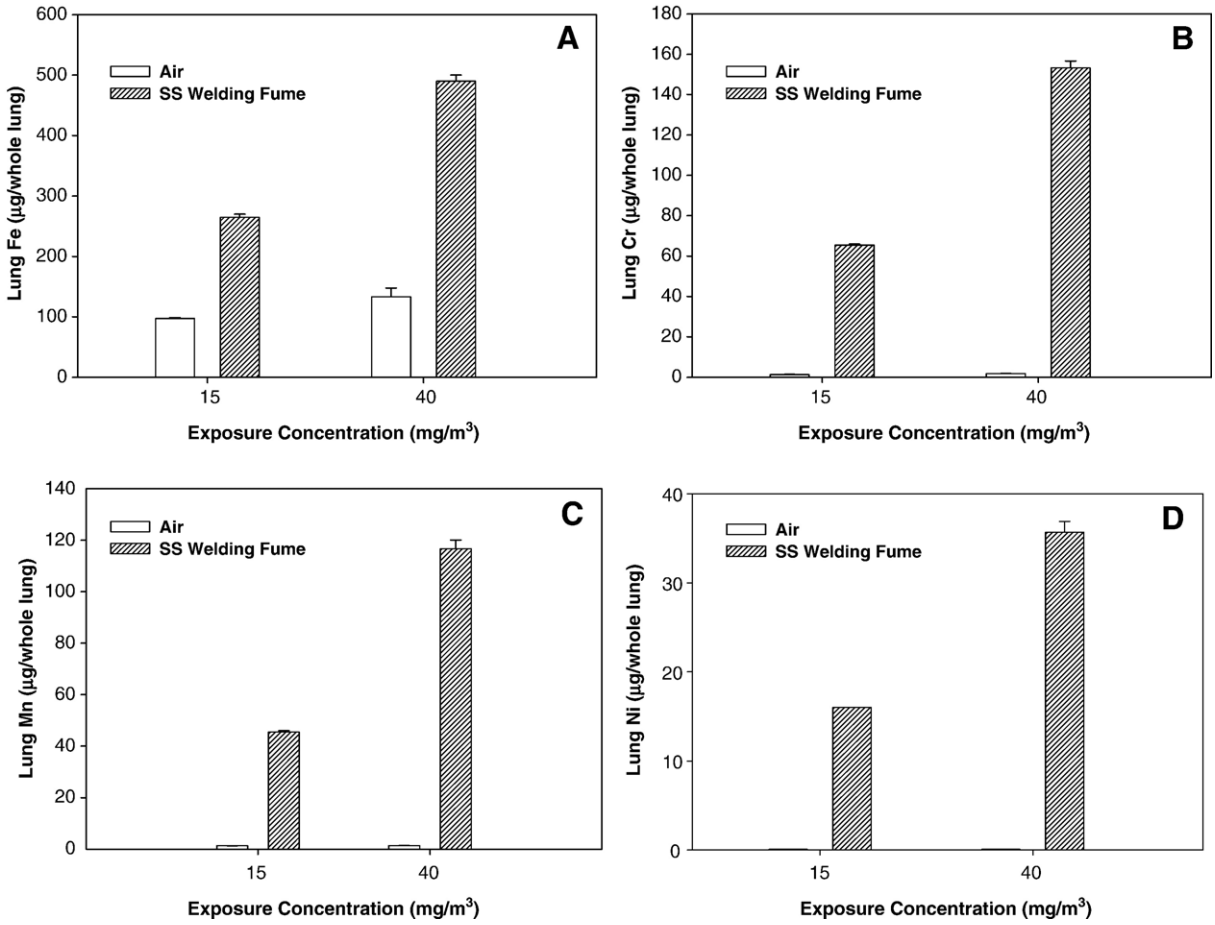

Fig. 4. Lung deposition of (A) Fe; (B) Cr; (C) Mn; and (D) Ni at 1 day after inhalation of 15 or 40 mg/m^3 of stainless steel (SS) welding fume for 3 h/day for 10 days. Control animals were exposed to filtered air.

of SS welding fume. Both doses caused a significant increase in lung injury and inflammation (Figs. 6A and B). Interestingly, only 10% of the cells recovered from the welding fume-treated rats at both doses were PMNs (Fig. 6C). The inflammatory lung response appears to peak after exposure to the 15 mg/m^3 dose, and 40 mg/m^3 caused no further elevation in PMN number.

To examine lung recovery after inhalation exposure to SS welding fume, injury and inflammation were measured at 1, 4, 6, 11, 14, and 30 days after exposure for 3 h/day for 3 days to 40 mg/m^3. Both parameters of lung injury remained significantly elevated at all time points post-exposure except for 30 days compared to controls (Figs. 7A and B). However, no significant difference in the number of lung PMNs recovered was observed between the SS and control groups at 1, 4, and 6 day post-exposure (Fig. 7C). After 6 days post-exposure, a dramatic increase in lung PMNs, indicative of a delayed inflammatory response, was observed in the SS group compared to air controls. By 30 days, the number of PMNs recovered from the SS welding fume group returned to control level values. Slight, but significant, elevations were observed in the number of AMs recovered from the SS welding fume group at 14 and 30 days after exposure compared to air controls (Fig. 7D).

Images were taken of the lung cells recovered from the animals exposed to the SS welding fume at different time points after the final exposure (Fig. 8). Numerous AMs were observed to have phagocytized the inhaled welding particles (Fig. 8, arrows). Even up to 30 days after exposure, a significant number of AMs contained SS welding particles (Fig. 8D). In confirmation of the cell counting measurements, relatively few PMNs were recovered from the welding fume-exposed animals up to 6 days after exposure (Figs. 8A and B). However, at 11 days after exposure, a significant number of PMNs were recovered from the welding fume-exposed animals (Fig. 8C). It also is interesting to note the progression in the increase in macrophage cell size over time after exposure (Figs. 8A–D).

In assessment of the response of an inflammatory cytokine and chemokine after exposure to SS welding fume, the concentrations of TNF-α and MIP-2 were measured in the acellular BALF, respectively (Fig. 9). No difference was observed in TNF-α levels between the SS welding fume and air control groups at any time point after exposure (Fig. 9A). MIP-2, a chemoattractant for PMNs, was significantly elevated in the welding fume group at 4, 6, 11, and 14 days after exposure compared to air control (Fig. 9B). By 30 days, the MIP-2 value had returned to control levels. When plotted together for the SS welding fume group, the increase in MIP-2 levels at 4 days after exposure preceded the increase in PMN influx that was observed at 6 days after exposure (Fig. 9C).

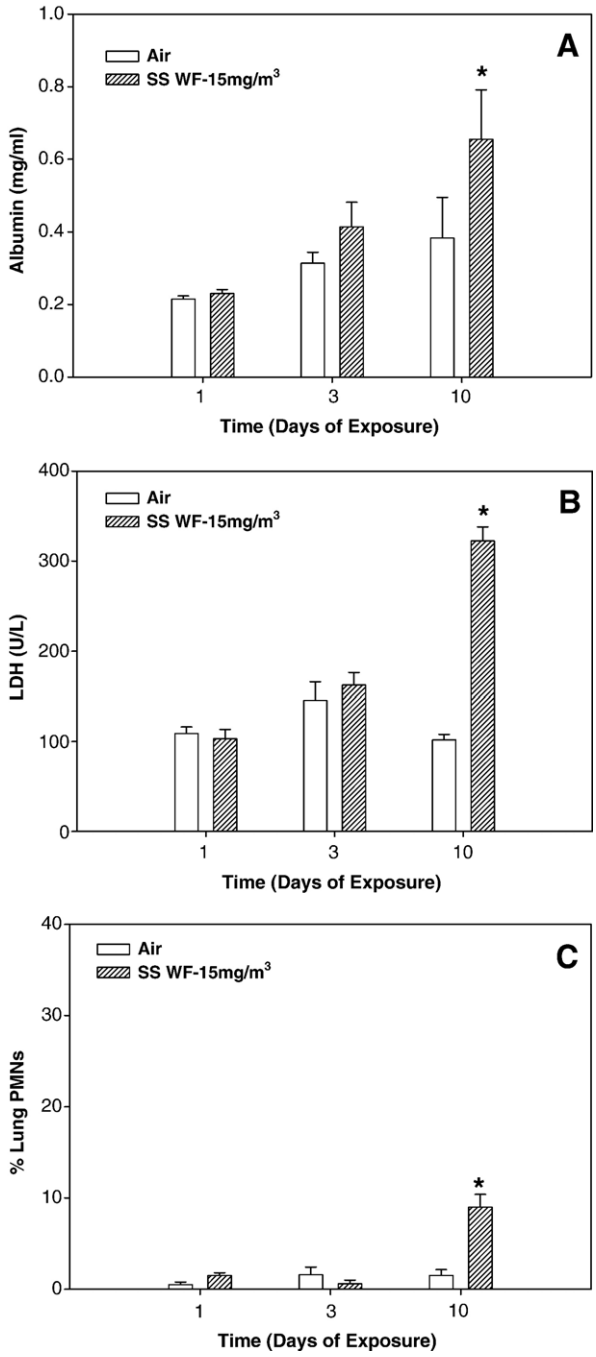

Fig. 5. Time course study: (A) albumin; (B) lactate dehydrogenase (LDH); and (C) % lung neutrophils (PMNs) at 1 day after inhalation of 15 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 1, 3, and 10 days. Control animals were exposed to filtered air. *Significantly greater than air controls, $p<0.05$ ($n=4–5$).

It has been observed that welders are more susceptible to upper and lower respiratory infections as compared to the general population. Lung macrophage function as assessed by chemiluminescence was reduced by 74.1% and 38.1% after exposure to 15 or 40 mg/m^3 of SS welding fume, respectively, compared to corresponding air controls (Fig. 10). To assess the effect of SS welding exposure on defense responses to bacterial challenge, the lungs of animals were inoculated with *L. monocytogenes* 1 day after inhalation of SS welding fume for 3 days. Animals pre-exposed to SS welding fume before infection could not clear the bacteria from the lungs as readily (Fig. 11A) and lost significantly more weight (Fig. 11B) than air control animals.

In the assessment of cytokines important in immune lung responses, a significant difference was observed for only MIP-2 in non-infected animals pre-exposed to SS welding fume

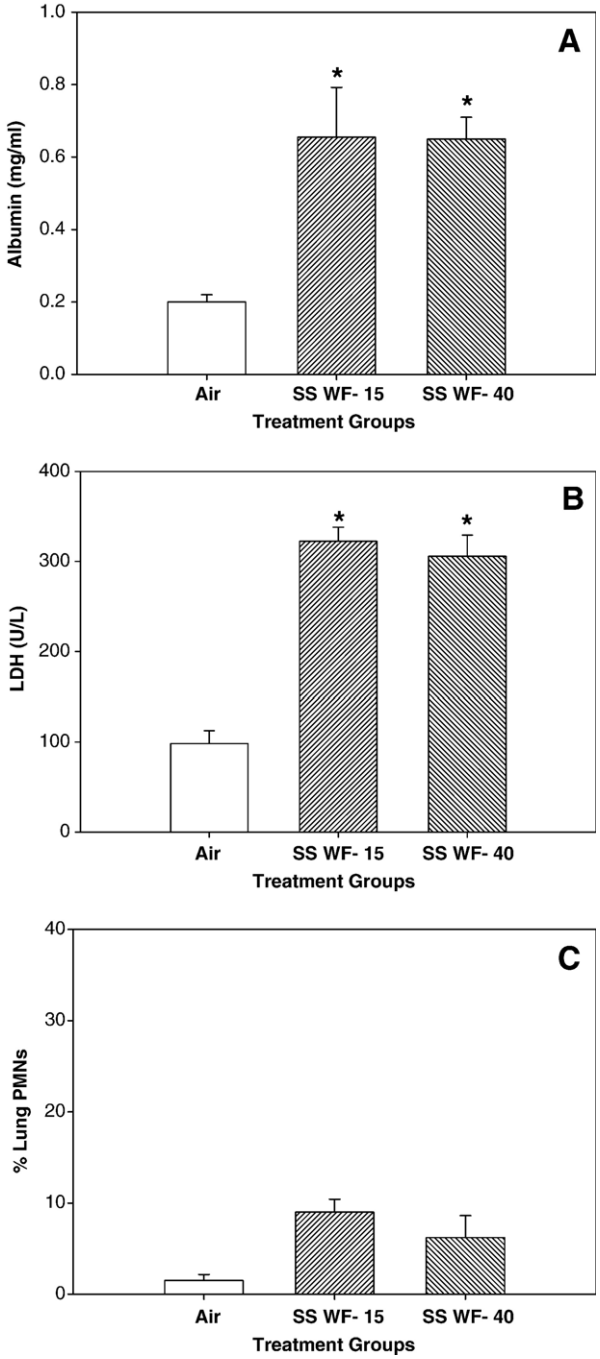

Fig. 6. Dose–response study: (A) albumin; (B) lactate dehydrogenase (LDH); and (C) % lung neutrophils (PMNs) at 1 day after inhalation of 15 or 40 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 10 days. Control animals were exposed to filtered air. *Significantly greater than air controls, $p<0.05$ ($n=4–5$).

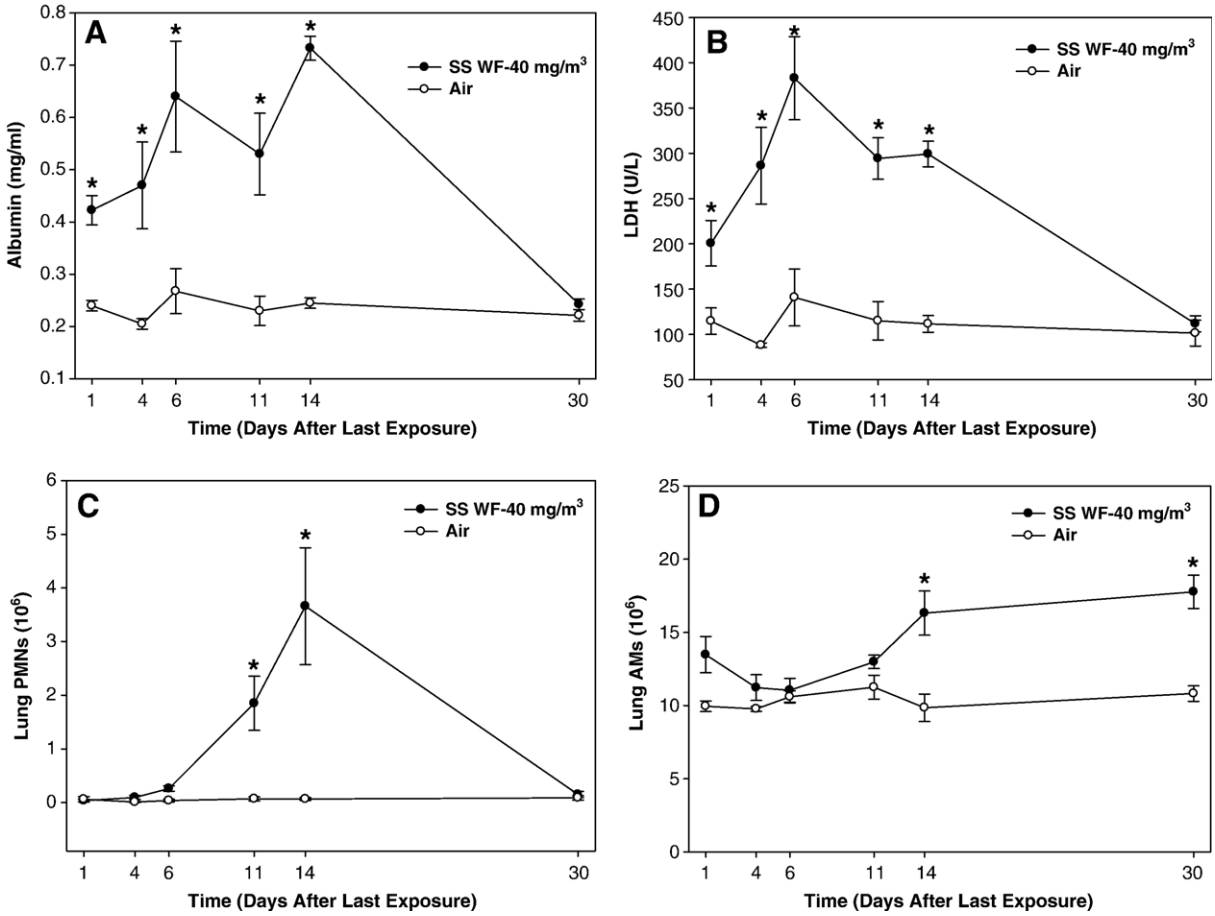

Fig. 7. Recovery study: (A) albumin; (B) lactate dehydrogenase (LDH); (C) number of lung neutrophils (PMNs); and (D) number of alveolar macrophages (AMs) at 1, 4, 6, 11, 14, and 30 days after the final exposure to 40 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 3 days. Control animals were exposed to filtered air. *Significantly greater than air control at corresponding time point, $p<0.05$ ($n=4$–8).

compared to air control (Table 3). However after infection, significant alterations were observed in all the cytokines examined between the infected welding fume and all other groups. Inflammatory cytokines (TNF-α, IL-6, and IL-10) and chemokines (MCP-1 and MIP-2) were significantly elevated in the infected SS welding fume group (Table 3) compared to infected control. In addition, cytokines important in T cell responses (IL-2 and IL-12) were altered in the animals pre-exposed to SS welding fume after infection compared to infected air controls. It appears that SS welding fume inhalation alters lung immune responses after bacterial infection.

Discussion

The NIOSH welding exposure team has developed an automated, computer-controlled robotic welding fume generation and inhalation exposure system that can be programmed for welding for up to 8 h/day without interruption (Antonini et al., 2006b). Extensive characterization of the SS welding fume generated in our system has been performed (Antonini et al., 2006b). Particle size, morphology, and composition were observed to be comparable to SS fume studied by other investigators (Hewett, 1995; Zimmer and Biswas, 2001; Jenkins and Eagar, 2005; Jenkins et al., 2005). In the breathing zone of a welder, the particles are in the form of agglomerates. Therefore, it is the aerodynamic size of the agglomerates, not the size of the individual primary particles which form the agglomerates, that determine the sites of pulmonary deposition after welding fume inhalation. The mass median aerodynamic diameter of the agglomerates generated by the robotic welder was 0.255 μm, giving them a high probability of being deposited in the alveolar regions of the lungs. Electron microscopy clearly showed the typical chain-like agglomerates formed during this type of welding. The metal profile of the generated SS particles mimicked that of the SS welding wire that was consumed in the process. Significant amounts of Fe, Cr, Mn, and Ni were present. Cr and Ni are of interest toxicologically because different chemical speciations of each have been classified as human carcinogens. In addition, exposure to high levels of pure Mn has been observed to cause a neurological condition referred to as manganism (Olanow, 2004).

In terms of evaluating pulmonary responses after welding fume treatment in animals, the primary route of particle administration has been via a single intratracheal instillation (White et al., 1981; Naslund et al., 1990; Antonini et al., 1996; Taylor et al., 2003). There are obvious limitations when using the intratracheal instillation method in welding fume studies compared to inhalation. Exposure to potentially toxic gases that

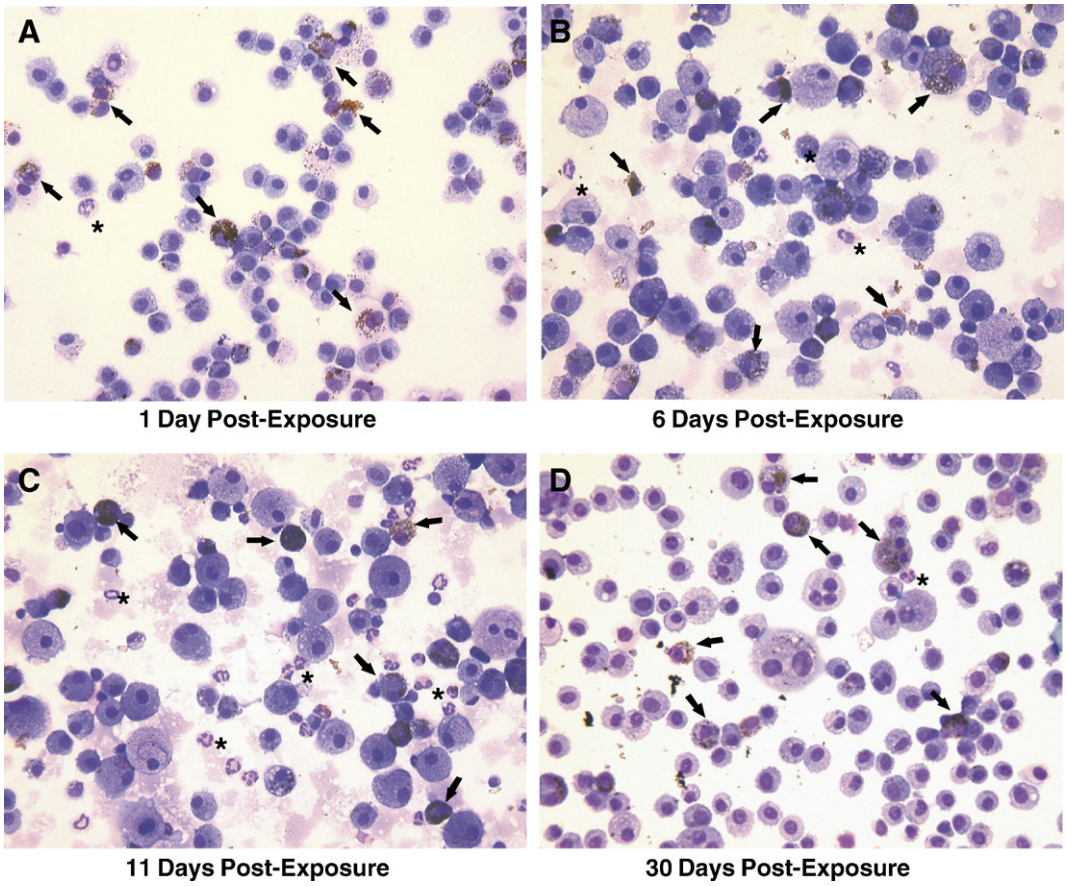

Fig. 8. Recovery study: cytospin images of lung cells recovered by BAL from animals at (A) 1 day; (B) 6 days; (C) 11 days; and (D) 30 days after exposure to 40 mg/m^3 of stainless steel welding fume for 3 h/day for 3 days. Arrows point to alveolar macrophages that contain welding particles; asterisks highlight PMNs that have infiltrated the airspaces of the exposed animals.

are commonly formed during welding fume generation is absent. In addition, particle surface characteristics and reactivity may be altered due to fume collection, storage procedures, or suspension in aqueous solutions for intratracheal delivery. Previously, we have observed that freshly generated 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). Lastly, and most importantly, a large, non-physiological bolus of particles at a much higher concentration is delivered to the lungs after intratracheal instillation compared with particle concentrations that are inhaled over time with inhalation exposures. Lung clearance mechanisms may become overloaded and the associated pulmonary responses after intratracheal delivery may result from particle overload and not the inherent chemical and physical properties of the welding fume.

Preliminary short-term animal exposures were performed to test the welding fume generator that has been developed by NIOSH. SS welding fume was observed to increase lung damage after 10 days of exposure at both concentrations used in the study. The responses appeared to peak after exposure to the lower concentration of 15 mg/m^3 for 10 days. An interesting finding, considering the exposure concentrations used, was the slight increase in PMN influx in the lungs of the SS welding fume group at 1 day after exposure. Based on results of intratracheal instillation studies using the same type of welding fume (Antonini et al., 1996), as well as a similar concentration of particles (~2 mg) delivered to the lungs observed in this current inhalation study, we expected to see a more substantial PMN infiltration. This finding highlights an important difference in response when exposing animals by inhalation versus intratracheal instillation, further questioning the relevance of the instillation procedures in welding fume toxicology studies. A substantial inflammatory response was observed in the lungs of animals at 1 day after intratracheal instillation of SS welding fume and persisted for up to 35 days after treatment (Antonini et al., 1996).

To investigate the inflammatory response in more detail, animals were exposed to the SS welding fume for 3 days and allowed to recover after exposure. It was not until after 6 days of SS welding fume exposure that an increase in PMN influx was observed. It appears that the SS particles were inhibiting chemotactic signaling and function of AMs during exposure. Indeed, SS welding fume exposure delayed the overproduction of the PMN chemokine, MIP-2, and had no effect on production of the inflammatory cytokine, TNF-α. A significant elevation in MIP-2 was not observed until 4 days after exposure. This elevation in MIP-2 immediately preceded the increase in lung PMNs (Fig. 9C). In addition, AM function, as measured by chemiluminescence, was suppressed.

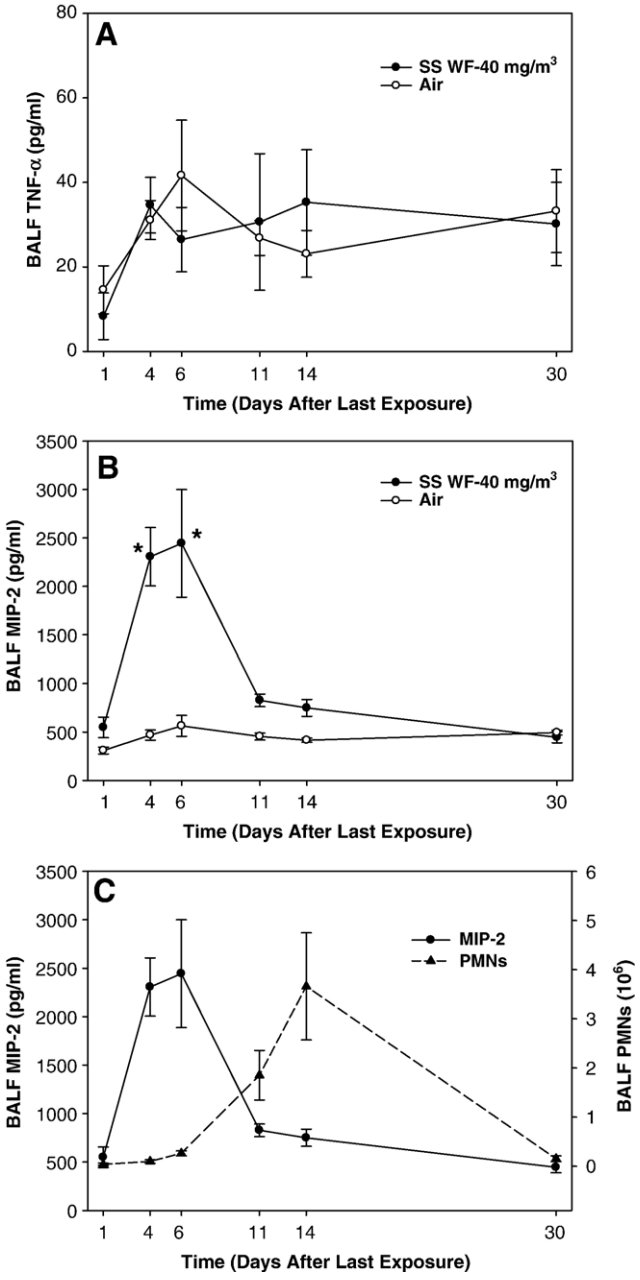

Fig. 9. Recovery study: (A) TNF-α; (B) MIP-2; and (C) a comparison of MIP-2 and number of PMNs recovered in BALF at 1, 4, 6, 11, 14, and 30 days after the final exposure to 40 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 3 days. Control animals in (A and B) were exposed to filtered air. *Significantly greater than air control at corresponding time point, $p<0.05$ ($n=4$–8).

The observation of the delayed response in the inhalation studies compared to the immediate response of previously published intratracheal instillation studies (Antonini et al., 1996; Taylor et al., 2003) using a stainless steel welding fume of the same composition is most likely due to the differences in particle lung deposition associated with the treatment methods. In comparing intratracheal instillation versus aerosol inhalation, the rate and distribution pattern of particle deposition in the lungs are clearly different between the two methods. Morphological measurements indicate that instillation results in heavy,

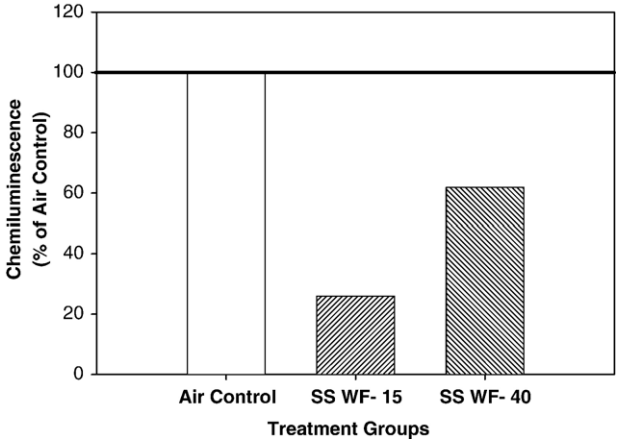

Fig. 10. Macrophage Function: Chemiluminescence of alveolar macrophages recovered from animals at 1 day after inhalation of 15 or 40 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 10 days as expressed as % of air control value ($n=4$–5). Control animals were exposed to filtered air.

more centralized deposits of particles, whereas the inhalation pattern is lighter, and both more evenly and widely distributed (Brain et al., 1976). Because of these findings, comparative

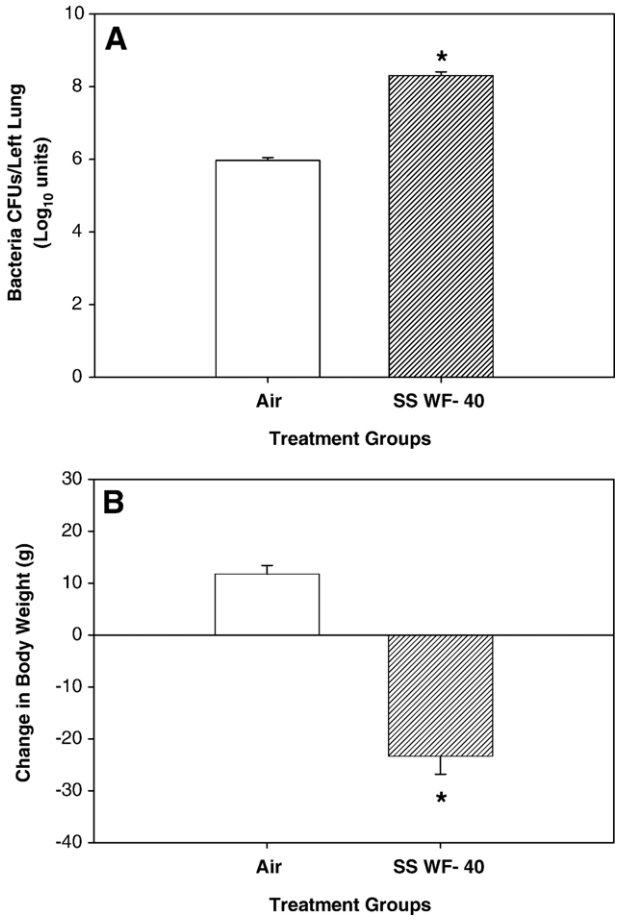

Fig. 11. Infectivity study: (A) pulmonary bacterial load and (B) change in body weight at 3 days after pulmonary infection with 5×10^4 *Listeria monocytogenes* in rats pre-exposed to 40 mg/m^3 of stainless steel welding fume (SS WF) for 3 h/day for 3 days. Control animals were exposed to filtered air. *Significantly different from air control, $p<0.05$ ($n=8$).

Table 3
Cytokine profile after infection in lungs pre-exposed to SS 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	31.1±4.60	108±9.72	45.9±11.4	172±4.93	0.67±0.67	11.5±1.84	468±53.2
SS WF	No	34.6±6.60	103±13.1	12.6±6.57	188±22.2	3.33±1.55	12.9±1.63	2306±301*
Air	Yes	243±35.9	116±17.9	192±46.5	101±14.8	132±22.2	445±44.8	574±42.5
SS WF	Yes	1191±116*	56.9±4.48*	1161±83.7*	537±149*	227±53.2*	1965±164*	1632±269*

Note. Values are means ± standard error ($n=4$–8); *significantly different from air controls for a specific cytokine ($p<0.05$). Cytokines were measured by ELISA in lavage fluid recovered at 3 days after pulmonary infection with 5×10^4 *Listeria monocytogenes* or without (non-infected controls) in rats pre-exposed to 40 mg/m^3 of stainless steel (SS) welding fume (WF) for 3 h/day for 3 days. Control animals were exposed to filtered air.

studies are ongoing using other welding fumes (e.g., mild steel) with different metal profiles (data not shown) to further evaluate this observed effect on inflammation as it relates to method of pulmonary exposure.

As further confirmation for a suppressive effect of SS welding fume exposure on AM function and lung defense responses, it has been reported that the severity, frequency, and duration of upper and lower respiratory tract infections are elevated in welders (Howden et al., 1988). Moreover, an excess in mortality from pneumonia in welders has been associated with the inhalation of metal fumes (Doig and Challen, 1964; Coggon et al., 1994; Wergeland and Iversen, 2001). In a previous animal study, intratracheal pretreatment with a highly water-soluble SS fume, collected from a welding process different from the one used in the current study, before infection significantly slowed the pulmonary clearance of a bacterial pathogen and increased animal morbidity (Antonini et al., 2004). Interestingly, intratracheal instillation of an insoluble SS welding fume, like the one used in this study, before pulmonary infection was observed to have no effect on the clearance of bacteria from the lungs of treated animals. However, a 3-day inhalation exposure using the same insoluble SS welding fume before pulmonary inoculation with *L. monocytogenes* led to animal morbidity, significant body weight loss, and a slowing of bacteria clearance from the lungs, again illustrating the difference in responses due to the method of treatment. Using a similar animal infectivity model, we have observed the immunosuppressive effects associated with SS welding fume to be associated with Cr (Antonini and Roberts, 2007). The lung immunosuppressive effects of Cr have been well documented (Cohen, 2004).

Cytokines and chemokines that are important in inflammatory responses were all significantly increased in the SS welding fume group after infection compared to the infected air control. This elevation in inflammatory signaling is likely due to an enhanced innate immune response to the elevated bacterial burden in the lungs of the SS welding fume group. Activation of the T cell immunity also is needed for the elimination of *L. monocytogenes*. IL-12 induces the expression of IFN-γ, and in the presence of IFN-γ, drives the differentiation of naïve CD4+ T cells toward the CD4+ T_H1 subset, leading to an increase in T cell activity (Hsieh et al., 1993; Mosmann and Sad, 1996). IL-2 is produced by CD4+ T_H1 cells and some CD8+ T cells and is involved in T cell growth and differentiation (Mosmann, 1992). Despite the increase in IL-12, T cell production of IL-2 was decreased in the SS welding fume group after infection compared to control. This reduction in IL-2 suggests that the SS fume may be altering T cell activity directly, resulting in a slowing in the clearance of the bacteria. Thus, the necessary T_H1 cell-stimulation of innate immune cells to further kill and clear *L. monocytogenes* from the lungs, as well as the killing of infected cells by CD8+ T cells may have been impaired. A similar response was observed in a previous infectivity study when animals were intratracheally treated with a water-soluble SS fume collected from a welding process different from the one used in the current study (Antonini et al., 2004).

In summary, an automatic robotic welding fume generation and inhalation exposure system has been developed. Short-term exposure of rats to SS welding fume caused significant lung damage and suppressed lung defense responses to bacterial infection, but had a delayed effect on pulmonary inflammation. In addition, the magnitude and temporal pattern of the inflammatory response after inhalation differed from a previously published study (Antonini et al., 1996) of inflammatory responses after intratracheal instillation of an identical sample of stainless steel welding fume. With the completion of the short-term animal exposure studies described in this paper, we have demonstrated the feasibility of longer term toxicology studies along with the added advantage of inhalation exposure over intratracheal treatment. The goal is to develop animal models by using subchronic and chronic exposures to low daily concentrations of welding fume. Additional inhalation infectivity studies are ongoing, evaluating different types of welding fume, using multiple fume concentrations, and assessing immune defense responses to infection at different times after welding exposure. New studies have begun to evaluate the tumorigenicity after the inhalation of specific welding fumes using lung tumor-susceptible and tumor-resistant mouse strains. Also, studies are taking place to follow the fate of metals after deposition in the respiratory tract of animals that have been exposed to welding fume and to evaluate the potential biochemical, histopathological, and physiological effects these metals may have on other organs systems, such as the central nervous and cardiovascular systems.

Acknowledgments

The authors wish to acknowledge the expert technical assistance on the project of Michelle Donlin, Amy Frazer, and Jared Cumpston. The authors also thank Mark Millson of the Division of Applied Research and Technology of NIOSH for the metal analysis of the welding particles and exposed lung tissue.

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