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EFFECT OF WELDING FUME SOLUBILITY ON LUNG MACROPHAGE VIABILITY AND FUNCTION *IN VITRO*

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It was shown previously that fumes generated from stainless steel (SS) welding induced more pneumotoxicity and were cleared from the lungs at a slower rate than fumes collected from mild steel (MS) welding. These differences in response may be attributed to the metal composition of SS and MS welding fumes. In this study, fumes with vastly different metal profiles were collected during gas metal arc (GMA) or flux-covered manual metal arc (MMA) welding using two different consumable electrodes, SS or MS. The collected samples were suspended in saline, incubated for 24 h at 37°C, and centrifuged. The supernatant (soluble components) and pellets (insoluble particulates) were separated, and their effects on lung macrophage viability and the release of reactive oxygen species (ROS) by macrophages were examined in vitro. The soluble MMA-SS sample was shown to be the most cytotoxic to macrophages and to have the greatest effect on their function as compared to the GMA-SS and GMA-MS fumes. Neither the soluble nor insoluble forms of the GMA-MS sample had any marked effect on macrophage viability. The flux-covered MMA-SS fume was found to be much more water soluble as compared to either the GMA-SS or the GMA-MS fumes. The soluble fraction of the MMA-SS samples was comprised almost entirely of Cr. The small fraction of the GMA-MS sample that was soluble contained Mn with little Fe, while a more complex

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mixture was observed in the soluble portion of the GMA-SS sample, which contained Mn, Ni, Fe, Cr, and Cu. Data show that differences in the solubility of welding fumes influence the viability and ROS production of macrophages. The presence of soluble metals, such as Fe, Cr, Ni, Cu, and Mn, and the complexes formed by these different metals are likely important in the pulmonary responses observed after welding fume exposure.

An estimated 800,000 workers are employed full-time as welders worldwide (Sundin, 1988). Larger numbers are estimated to perform welding intermittently as part of their work duties. Epidemiology studies have indicated that a large number of welders experience some type of respiratory illness (Sferlazza & Beckett, 1991). Respiratory effects observed have included acute and chronic bronchitis, airway irritation, chemical pneumonitis, and occupational asthma. Acute upper and lower respiratory tract infections are increased in terms of severity, duration, and frequency among welders, and excess mortality from pneumonia has been reported (Howden, 1988).

The high temperatures (~4000°C) of the welding process heat the base metal pieces to be joined and also heat a consumable electrode, which is continuously fed into the weld during the contact (Howden, 1988). Fumes are formed by the evaporation of metals and fluxes at the tip of the electrode. These metal vapors are oxidized on contact with the air and form small particulates composed of a complex mixture of metal oxides. The resulting metal complex of the welding fume and the inhalation exposure of welders to the fume varies according to the materials and process used.

It was shown previously in rats that welding fumes generated from stainless steel (SS) materials induced more pneumotoxicity and were cleared from the lungs at a slower rate than fumes collected from mild steel (MS) welding (Antonini et al., 1996, 1997). These differences in response may be attributed to the different metal composition of SS and MS welding fumes. Compared to MS fumes, SS fumes generally have lower levels of Fe but more Mn as well as Cr and Ni; both are absent from MS fumes. These metals alone or in combination with other materials have been shown to be cytotoxic to pulmonary cells and to be associated with lung disease (Ulrich et al., 1979; Lees, 1991; Camner & Johansson, 1992).

Fluoride-containing fluxes used in consumable electrodes to carry away impurities during the welding processes may also influence the composition of welding fume. The inhalation of gases containing fluorine has been shown to injure lungs (Stavert et al., 1991), and pulmonary exposure to particulate fluorides in the workplace has been implicated as a risk factor for occupational lung disease (O'Donnell, 1995). It has been demonstrated previously by our group and others that welding fumes generated from fluoride-containing flux-covered electrodes cause more lung injury and inflammation in rats than other types of welding fumes (Coate, 1985; Antonini et al., 1997).

Numerous animal studies have suggested that metal components of urban air particulates and residual oil fly ash (ROFA) are major determinants of their potential to induce pulmonary injury and inflammation (Pritchard et al., 1996; Gavett et al., 1997; Carter et al., 1997). Dreher et al. (1997) have provided evidence for the toxic role of soluble metals in acute pulmonary injury induced by ROFA. Goldsmith et al. (1998) demonstrated that the water-soluble components of both concentrated ambient particulates and ROFA significantly increased alveolar macrophage oxidant production as compared to controls. The mechanisms of lung toxicity with welding fumes are less well characterized.

In this study, we attempt to identify the metal constituents that are responsible for the pulmonary effects seen after exposure to welding fumes. Three welding fumes with different metal compositions were collected, characterized, and separated into soluble and insoluble portions, and their effects on lung macrophage viability and the release of reactive oxygen species (ROS) by macrophages were examined. Activation of lung macrophages and the subsequent release of ROS have been shown to be a mechanism by which inhaled substances damage the lungs (Halliwell & Cross, 1994; Farber, 1994; Castranova et al., 1996).

MATERIALS AND METHODS

Collection of Welding Fume

The welding fumes tested were obtained from the American Welding Society and Lincoln Electric Co. (St. Louis, MO) courtesy of Kenneth Brown or collected by the Department of Chemical Engineering at the University of New Hampshire. The fumes were generated in a cubical open-front fume chamber (volume = 1 m^3) by a skilled welder using a manual or semiautomatic technique appropriate to the electrode. The fumes were collected on 0.2-μm Nuclepore filters (Nuclepore Co., Pleasanton, CA) during 2 min of welding. After the 2-min collection period, the filter was removed, the fume sample was recovered (approximately 50–75 mg of fume sample was collected during each 2-min run), and a new filter was inserted to collect additional fume sample. The fume samples were generated in three different ways: (1) gas metal arc welding using a mild steel electrode (GMA-MS); (2) gas metal arc welding using a stainless steel electrode (GMA-SS) with argon and CO_2 shielding gases to protect the weld from oxidation; and (3) manual metal arc welding using a flux-covered stainless steel electrode (MMA-SS). For each of the three samples, approximately 500 mg of fume sample was collected.

Preparation of Welding Fume Samples

The three particle samples (MMA-SS, GMA-SS, and GMA-MS) were suspended in sterile phosphate-buffered saline (PBS), pH 7.4, and soni-

cated for 1 min. The samples were further divided into soluble and insoluble components. The particle suspensions were incubated for 24 h at 37°C containing air and 5% CO_2, and the samples were centrifuged at 12,000 × g for 30 min. The supernatants of the samples (MMA-SS-sol, GMA-SS-sol, and GMA-MS-sol) were recovered and filtered with 0.22-μm filters (Millipore Corp., Bedford, MA). The pellets (MMA-SS-insol, GMA-SS-insol, and GMA-MS-insol) were resuspended in PBS. The pH of each suspension was measured and the effect of the soluble and insoluble components of the fume samples on macrophages was evaluated.

Sample Characterization

Analysis of the metal constituents of the total welding fume as well as its soluble and insoluble fractions was obtained using inductively coupled argon plasma atomic emission spectroscopy (NIOSH, 1994). Seven different metals (Cr, Cu, Fe, Mn, Ni, Ti, and V) commonly found in welding fumes were measured. In order to measure size, particles from the different samples were suspended in sterile PBS, sonicated for 1 min using a Sonicor cell disruptor (Heat Systems–Ultrasonic, Inc., Plainview, NY), dispersed onto glass slides in microwell chambers, and then sized by a Sarastro 2000 (Molecular Dynamics, Inc., Sunnyvale, CA) laser scanning confocal microscope (Optiphot-2, Nikon, Inc., Melville, PA) fitted with an argon-ion laser as described in an earlier study (Antonini et al., 1996). The count mean diameters of the GMA-MS, GMA-SS, and MMA-SS fumes were of respirable size, measuring 1.22 μm, 1.38 μm, and 0.92 μm, respectively.

Other particles to be used in alveolar macrophage cytotoxicity assays were also characterized. Silica and iron oxide were used as positive and negative particle controls, respectively, in initial studies when evaluating the potential of welding fumes to induce macrophage cytotoxicity. Crystalline Min-U-Sil Silica (U.S. Silica Corporation, Berkeley Springs, WV) had a purity of 99.5% alpha-quartz with a mean diameter of 3.5 μm. Iron oxide particles (gamma-Fe_2O_3) were produced by combustion of iron pentacarbonyl [$Fe(CO)_5$] vapors as described by Valberg and Brain (1979). The resulting iron oxide agglomerates had a mean diameter of 0.87 μm.

Animals

Male CD/VAF rats weighing 200–250 g were used for all experiments (Charles River Laboratories, Wilmington, MA). They were given a conventional laboratory diet and tap water ad libitum and were housed in a clean-air, viral- and antigen-free room with restricted access.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed on untreated rats to recover lung macrophages. The rats were deeply anesthetized with an overdose of sodium pentobarbital and then exsanguinated by severing the abdominal

aorta. Their lungs were lavaged with 15 separate 3-ml aliquots of warm, calcium- and magnesium-free PBS, pH 7.4. The samples were centrifuged for 10 min at 500 × g and the cell-free lavage fluid discarded. The cell pellets were washed and resuspended in 1 ml PBS. Total cell numbers were determined using a hemacytometer. Viability of recovered cells was assessed by trypan blue exclusion and found to be >95%.

Experiments were performed to determine the purity of the alveolar macrophage preparations. Using a cytospin centrifuge (Shandon Southern Products, Ltd., Cheshire, England), 1×10^5 cells were spun for 5 min at 800 rpm and pelleted onto a slide. Cells (200/rat) were identified on cytocentrifuge-prepared slides after staining with DiffQuik fixative and stain (Baxter Healthcare Corp., McGaw Park, IL). It was found that >98% of the recovered cells were macrophages.

Experiments With Welding Fume Particles

Particle-Macrophage Association

Confocal microscopy In order to prepare the cells for confocal microscopy, lung macrophages were incubated on glass coverslips in 24-well plates with 1 ml Hanks balanced salt solution (HBSS; pH 7.4) at a concentration of 2×10^5 cells/well for 1 h to allow for attachment. The cells were then washed and treated with a 25 μg/ml concentration of the MMA-SS, GMA-SS, or GMA-MS particles suspended in HBSS and incubated for 30 min, 12 h, and 24 h. Before they were added to the cells, all particles were sonicated for 1 min using a Sonicor cell disruptor (Heat Systems–Ultrasonic, Inc., Plainview, NY). After the incubation period, the cells were washed twice with HBSS, fixed with 2% paraformaldehyde for 30 min, and then stained with the fluorochrome nile red (0.1 μg/ml) for 5 min.

Images were recorded from a Sarastro 2000 laser scanning confocal microscope fitted with an argon-ion laser using 514-nm excitation light. With an emission spectra >535 nm, macrophages were imaged. When using reflected light <535 nm simultaneously passed to a separate optical path, the welding fume could also be imaged and recorded.

Flow cytometry To quantitate particle–macrophage association, right angle scatter of light (RAS) was measured using flow cytometry as described by Stringer et al. (1995). Lung macrophages were suspended in HBSS at a concentration of 5×10^5 cells/ml. The macrophages were treated with MMA-SS, GMA-SS, or GMA-MS samples (25 μg/ml) for 30 min, 12 h, and 24 h. Flow cytometric measurement of samples was performed using an Ortho 2150 cytofluorograph equipped with an argon laser (488 nm, 15 mW output) and a Cyclops data acquisition and analysis hardware/software package (Cytomation, Ft. Collins, CO). RAS (488 nm) was collected unfiltered at 90° incident to the flow cell. The number of cells analyzed was 5000 per sample. Data acquisition and analysis of macrophage uptake of nonfluorescent welding particles were measured using

the increased RAS signal caused by these granular materials. The same photomultiplier gain setting for the RAS parameter was used for all experiments, facilitating interexperiment comparisons. Unbound particles were substantially smaller than macrophages and were removed from the gated window by adjusting the electronic threshold settings when samples incubated in the presence of particulates were analyzed.

Measurement of Macrophage Viability Lung macrophages were suspended in sterile RPMI 1640 culture medium (pH 7.4) enriched with 10% fetal bovine serum and suspended in a 1.5-ml microfuge tubes at a concentration of 3×10^5 cells/ml. The macrophages were then treated with 6.25, 25, or 100 μg/ml of silica, iron oxide, MMA-SS, GMA-SS, or GMA-MS particles suspended in sterile RPMI and incubated for 24 h at 37°C containing air and 5% CO_2 (n = 6 rats/particle group) while continually inverted 360° to increase macrophage–particle interaction. Before cell treatment, all particles were sonicated for 1 min. The control group received an equal volume of PBS. Cell viability was measured on aliquots from each microfuge tube by trypan blue exclusion.

Experiments With Soluble and Insoluble Fractions of Welding Fumes

Incubation With Macrophages Lung macrophages were suspended in sterile RPMI 1640 culture medium as described previously in this report and suspended in a 1.5 ml microfuge tubes at a concentration of 3×10^5 cells/ml. The cells were then incubated for 1 and 24 h at 37°C containing air and 5% CO_2 and treated with a 25-μg/ml concentration of the entire particle suspension of the 3 fumes, as well as equal quantities of the soluble and insoluble portions of the samples (n = 3–6 rats/treatment group). This concentration of 25 μg/ml was chosen after the findings from the initial concentration response experiment performed (see Figure 3). For all analyses, the vehicle control group received an equal volume of sterile PBS. The concentration of 25 μg/ml was chosen after performing the initial concentration-response experiment (see Figure 3). This concentration was found to be high enough to generate responses from macrophages so that the different particle groups could be compared.

Assessment of Cell Viability After a 24-h incubation with the samples, macrophage viability was assessed by measuring lactate dehydrogenase activity of recovered cell-free supernatants spectrophotometrically (Pesce et al., 1964) or trypan blue exclusion on aliquots of recovered cells as described previously in this report.

Macrophage Function

Release of β-N-*acetyl-glucosaminidase (*β*-NAG)* After a 1-h incubation with the different treatment samples (while cell viability was >90%), the cells were spun for 1 min in a microfuge at the minimum speed setting. β-NAG activity was quantified spectrophotometrically in the recovered cell-free supernatants by the method of Sellinger et al. (1960).

Release of reactive oxygen species (ROS) Kobzik et al. (1990) described a method to measure the release of ROS using dichlorofluo-

rescin (DCFH). Lung macrophages were suspended in sterile PBS and incubated for 15 min with the nonfluorescent precursor, DCFH diacetate (15 μ*M*) at 37°C containing air and 5% CO_2. The cells were then added to a 96-well Ultra Low Attachment plate (Corning Costar Corp., Cambridge, MA) at a concentration of 2×10^5 cells/well and incubated with the different treatment groups for 1 h (while cell viability was >90%) at 37°C containing air and 5% CO_2. The oxidation of DCFH to the fluorescent form dichlorofluorescein (DCF) was measured in a Cytofluor 2300 96-well microplate reader (Millipore Corp., Bedford, MA).

Desferrioxamine (DES) Pretreatment To investigate whether the chelation of different metals in welding fumes inhibits macrophage viability and function, the fume samples were incubated for 24 h at 37°C containing air and 5% CO_2 with 2.5, 5.0, or 10 m*M* concentrations of DES before treatment with the cells.

Statistical Analysis

Results are expressed as means ± standard error of measurement (SE). Statistical analyses were carried out with the Statview statistical program (Abacus Concepts, Inc., Berkeley, CA). The significance of the interaction among the different treatment groups for the different parameters was assessed using an analysis of variance (ANOVA). The significance of difference between individual groups was analyzed using Fisher's least significant difference post hoc test. For the analysis of the effect of DES pretreatment of welding fumes on macrophage viability and function (see Table 3), statistical comparisons were made between the individual fume sample pretreated with DES and its corresponding control that was not treated with DES. For all analyses, the criterion of significance was $p < .05$.

RESULTS

Characterization of Welding Fumes and Welding Fume Fractions

The welding fume samples were suspended in PBS and the relative amounts of seven different metals were measured as percent by weight (Table 1). The GMA-MS sample was comprised almost entirely of Fe (85.9%) and Mn (14.6%). The two SS samples (MMA-SS and GMA-SS) were composed of similar Mn levels but much less Fe as compared to the

TABLE 1. Total Metal Composition of Welding Fumes (Weight %)[a]

Sample	Fe	Mn	Cr	Ni	Cu	Ti	V
MMA-SS	41.1	16.7	28.5	2.53	0.40	10.7	0.11
GMA-SS	53.1	23.2	18.6	4.85	0.31	n.d.	n.d.
GMA-MS	85.9	14.6	0.07	0.01	0.41	0.02	n.d.

Note. n.d., Metal concentration in sample is below the method's limit of detection.
[a]Relative to all metals analyzed.

GMA-MS fume. Cr and Ni were also present in the two SS samples, but they were absent in the GMA-MS sample. An appreciable amount of Ti was measured in the MMA-SS fume.

Some of the properties of the soluble fractions of the welding fumes are shown in Table 2. Little change was observed in the pH of the three particle suspensions. The GMA-SS and GMA-MS samples were relatively insoluble, with soluble-to-insoluble ratios of 0.006 and 0.020, respectively. The flux-covered MMA-SS sample was much more soluble than the other two samples, with a soluble-to-insoluble ratio of 0.345. In the GMA-SS and GMA-MS samples, the predominant metal in the soluble fraction was Mn. Little Fe was present in the soluble fraction of the GMA-MS samples, while some Fe, Cr, Ni, and Cu were measured in the soluble portion of the GMA-SS fume. For the MMA-SS sample, the majority of the soluble fraction was comprised of Cr with some Mn.

Particle-Macrophage Association

Lung macrophages were exposed to the different welding fumes for 30 min, 12 h, and 24 h. By 30 min, most of the particles from each of the fume samples were bound to the surface of the macrophages or internalized (Figure 1). As observed by flow cytometry, no differences were observed in

TABLE 2. pH and Solubility Properties of Welding Fumes

Sample	pH	Soluble/insoluble ratio	Weight % of metals in soluble fraction[a]
MMA-SS	Total 6.92 Soluble 7.05 Insoluble 7.09	0.345	Fe 0.39 Mn 11.7 Cr 87.0 Ni 0.65 Cu 0.08 Ti 0.13 V n.d.
GMA-SS	Total 6.94 Soluble 6.97 Insoluble 7.01	0.006	Fe 9.26 Mn 68.2 Ni 11.8 Cu 4.50 Ti 0.79 V n.d.
GMA-MS	Total 7.02 Soluble 7.44 Insoluble 7.03	0.020	Fe 4.60 Mn 93.0 Cr 0.20 Ni n.d. Cu 0.70 Ti 1.38 V n.d.

Note. n.d., Metal concentration in sample is below the method's limit of detection.
[a]Relative to all metals analyzed in soluble fraction of welding sample.

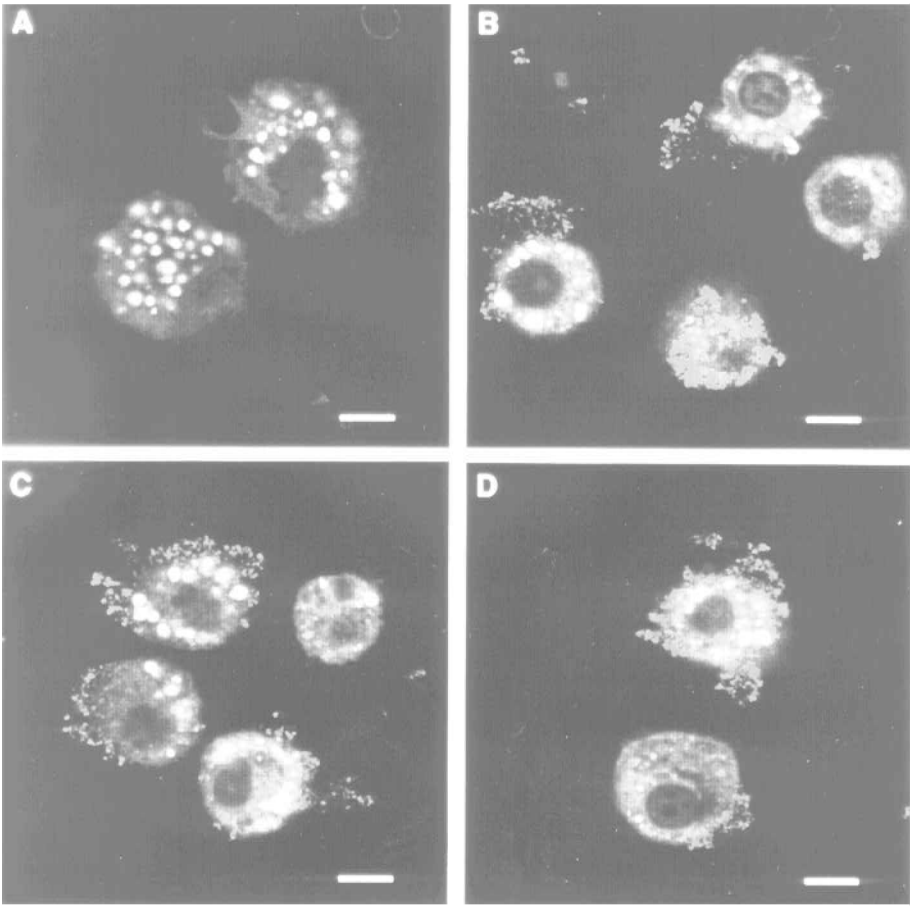

FIGURE 1. Confocal micrograph of lung macrophages treated with (A) saline (vehicle control), (B) GMA-SS, (C) MMA-SS, or (D) GMA-MS for 30 min. The final concentration of the particle samples was 25 μg/ml. The particles are red. Bar = 5 μm.

the phagocytosis of the different fume samples (Figure 2). The amounts of particles associated with the macrophages at the later time points were not different from those observed at the 30-min time point (data not shown).

Effect of Welding Fumes Particles on Macrophage Viability

The viability of lung macrophages was determined by trypan blue exclusion after treatment for 24 h with the different particle samples at concentrations of 6.25, 25, and 100 μg/ml (Figure 3). Silica and iron oxide were used as positive and negative particle controls, respectively. At the 6.25 μg/ml concentration, very little macrophage death was observed for the different particle sample groups. There were no significant differences among the different treatment groups.

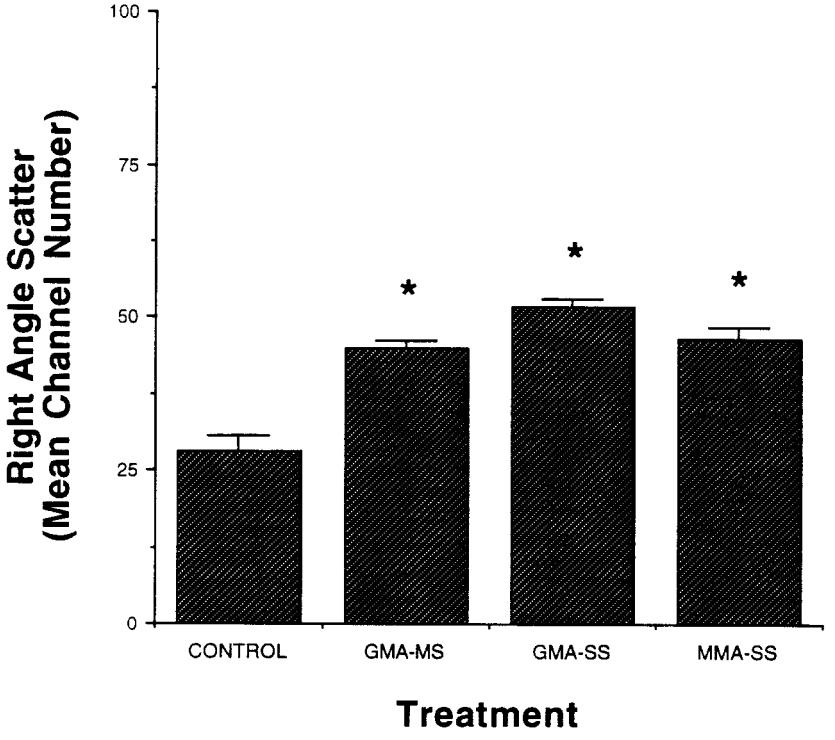

FIGURE 2. Right-angle scatter measurements of lung macrophages treated with the welding fumes for 30 min. The final concentration of the particle samples was 25 μg/ml. Values are means ± SE (n = 6); asterisk indicates significantly greater than saline control ($p < .05$).

At the 25 μg/ml concentration, the MMA-SS and silica samples caused a 56% and a 62% loss in cell viability, respectively. These increases in macrophage death were significantly greater than the loss in viability caused by the other groups. A significantly greater number of macrophages died when treated with GMA-SS (30%) as compared to the GMA-MS group (16%) and the iron oxide group (12%). A loss of viability of only 6% was observed for the control group, which was significantly less than all the other groups except the GMA-MS group.

When the particle concentration was increased to 100 μg/ml, silica caused an 81% loss in macrophage viability, which was significantly greater than all other groups. Sixty-one percent of the cells died when exposed to the MMA-SS fume. A loss in macrophage viability was observed for the iron oxide, GMA-MS, and GMA-SS groups that was significantly greater than the cell death seen with the saline control group.

Solubility Effects on Macrophage Viability

The effects of the soluble and insoluble components of the three different welding fumes on macrophage viability were determined (Figures 4

and 5). As assessed by trypan blue exclusion and LDH activity, the total MMA-SS sample caused a significant elevation in macrophage death as compared to the total GMA-SS and GMA-MS fumes after 24 h. There was no difference in loss of macrophage viability between the total GMA-MS and control groups. The soluble portions of the MMA-SS and GMA-SS samples demonstrated significant elevations in macrophage death as compared to the insoluble components. There was no marked difference in effect on macrophage viability between the soluble and insoluble components of the GMA-MS fume.

Solubility Effects on Macrophage Function

The effects of the soluble and insoluble components of the three different welding fumes on macrophage function were determined (Figures 6 and 7). The total MMA-SS fume caused a significant elevation in macrophage release of β-NAG as compared to the total GMA-SS and GMA-MS samples after 1 h (Figure 6). The soluble portion of all three fume samples significantly increased B-NAG release as compared to the

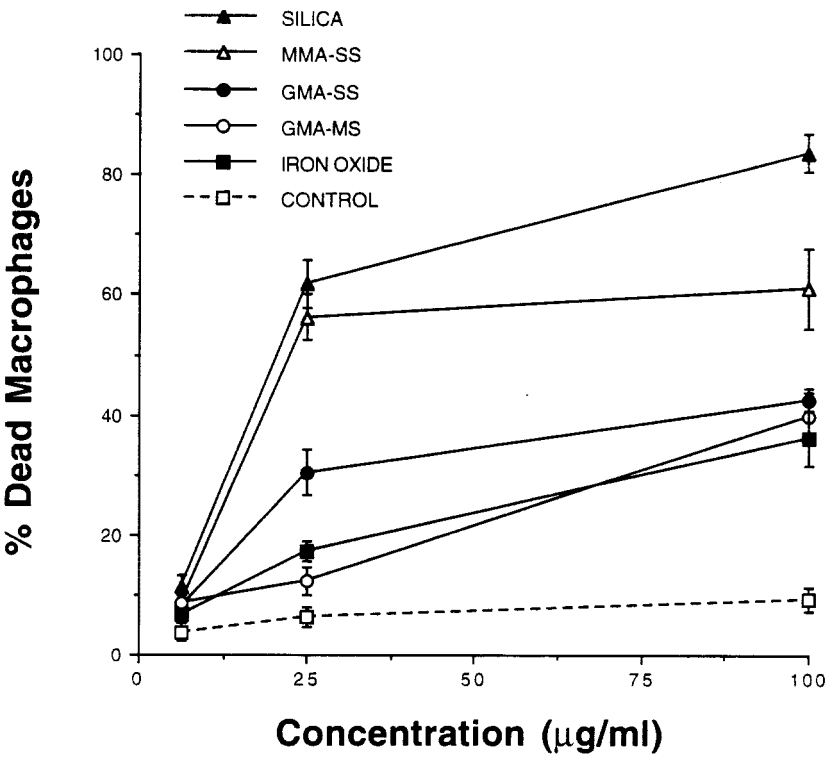

FIGURE 3. Percentage of dead macrophages as measured by trypan blue exclusion after a 24-h incubation with silica, iron oxide, MMA-SS, GMA-SS, or GMA-MS welding fumes. Saline was used as the vehicle control. The final concentrations of the particle samples were 6.25, 25, and 100 μg/ml. Values are means ± SE (n = 6).

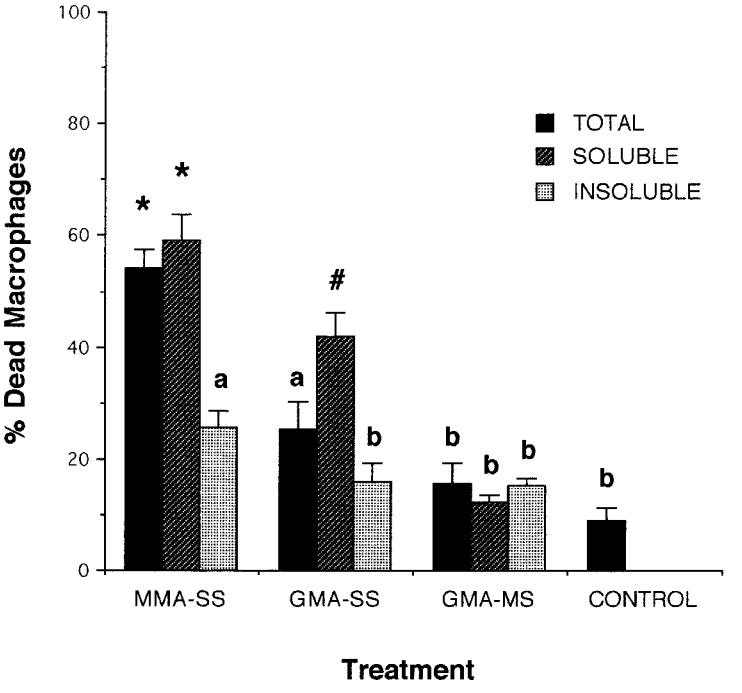

FIGURE 4. Percentage of dead macrophages as measured by trypan blue exclusion after a 24-h incubation with the soluble and insoluble components of MMA-SS, GMA-SS, or GMA-MS welding fumes. The final concentration of the particle samples was 25 μg/ml. Equal quantities of the soluble and insoluble portions of the samples were used. Values are means ± SE (n = 3–4). Groups with the same symbols are not statistically different ($p < .05$).

insoluble components of the samples and control values. The insoluble portion of the MMA-SS fume significantly elevated β-NAG release from macrophages as compared to control values, while the insoluble components of the GMA-SS and GMA-MS samples caused significant decreases in β-NAG release.

The total MMA-SS fume caused a significant increase in ROS production (70% increase over control levels) as compared to the total GMA-SS and GMA-MS fumes (Figure 7). The ROS production induced by the soluble portion of the MMA-SS fume was significantly decreased as compared to the total MMA-SS sample. There was no significant difference in the ROS production when comparing the GMA-SS and GMA-MS fumes. There were no marked differences in ROS production when comparing the total samples and their corresponding insoluble fractions for all three fumes.

Effect of Desferrioxamine Pretreatment

Pretreatment of the welding fumes with 2.5 m*M* DES had no significant effect on macrophage viability and release of LDH and β-NAG

except in one case (Table 3). A significant decrease in the loss of viability was observed when macrophages were exposed to DES-treated GMA-SS-sol fume. Pretreatment of welding fumes with DES concentrations of 5 and 10 m*M* also showed no effect on macrophage function (data not shown). These higher DES concentrations were found to be slightly cytotoxic to macrophages. DES pretreatment significantly inhibited ROS production induced by the insoluble portions of the three fumes and the total samples of the GMA-SS and GMA-MS fumes (Table 4). DES pretreatment had no effect on ROS production of any of the soluble portions of the three fumes.

DISCUSSION

It has been demonstrated that differences exist in the extent of pulmonary inflammation and injury in rats after exposure to fumes generated using different welding processes and materials. Fumes formed from SS materials during GMA welding induced more pneumotoxicity than fumes collected from MS welding (Antonini et al., 1996, 1997). In an acute toxi-

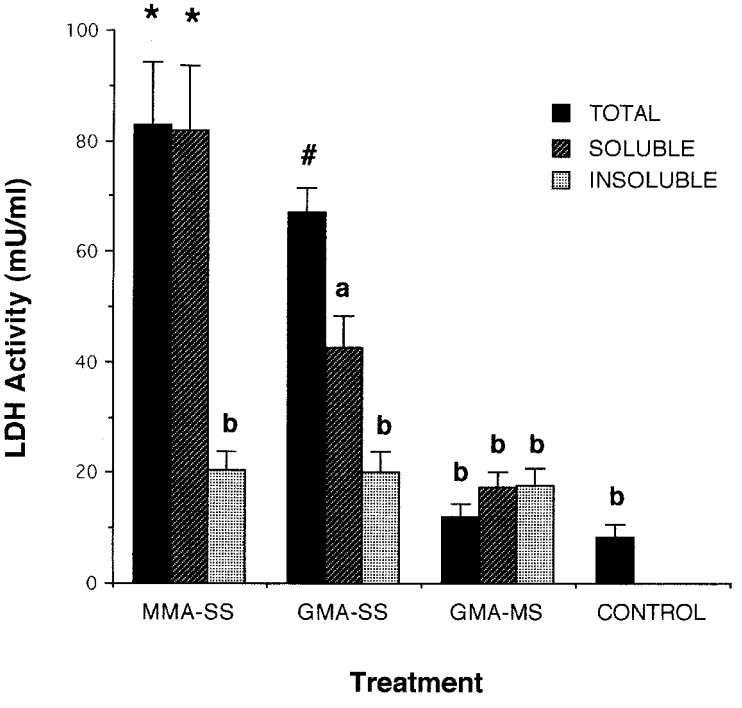

FIGURE 5. Lactate dehydrogenase (LDH) release from lung macrophages incubated for 24 h with the soluble and insoluble components of MMA-SS, GMA-SS, or GMA-MS welding fumes. The final concentration of the particle samples was 25 μg/ml. Equal quantities of the soluble and insoluble portions of the samples were used. Values are means ± SE ($n = 6$). Groups with the same symbols are not statistically different ($p < .05$).

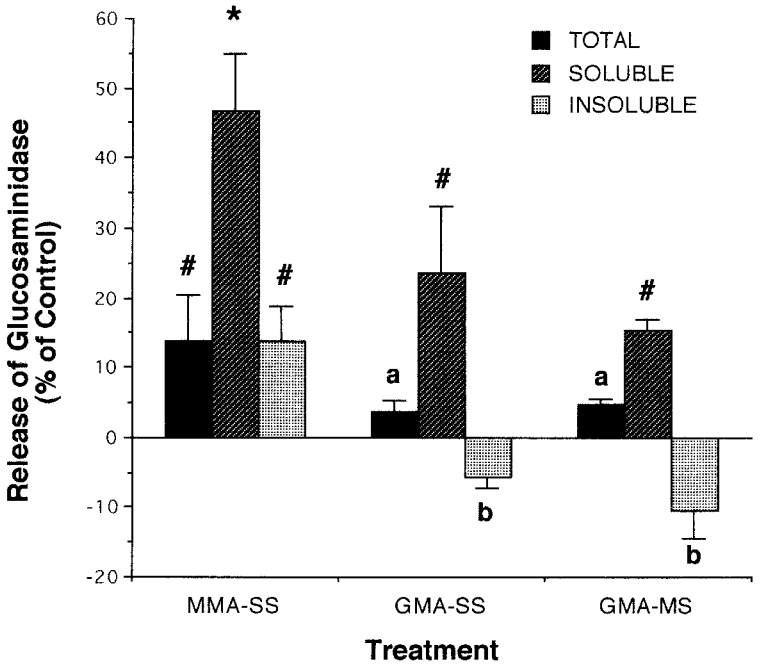

FIGURE 6. β-*N*-Acetyl-glucosaminidase release from lung macrophages incubated for 1 h with the soluble and insoluble components of MMA-SS, GMA-SS, or GMA-MS welding fumes. The final concentration of the particle samples was 25 μg/ml. Equal quantities of the soluble and insoluble portions of the samples were used. Values are means ± SE (n = 3–4). Groups with the same symbols are not statistically different ($p < .05$).

cology study by Coate (1985), SS fumes from flux-covered MMA welding (where fluorides were present) induced a significant pneumonitis after a single 6-h inhalation exposure, while MS fumes generated from GMA welding were relatively nontoxic to the lungs.

In previous in vitro studies, Stern and Pigott (1983) and Pasanen et al. (1986) demonstrated that flux-covered MMA-SS fumes were much more cytotoxic to rat peritoneal macrophages than fumes from a variety of other welding processes. Hooftman et al. (1988) have shown that bovine lung macrophage viability and phagocytosis were greatly reduced by particles generated in flux-covered MMA welding using SS electrodes as compared to welding using MS materials. It was also shown that flux-covered MMA-SS fumes were more cytotoxic and induced a greater release of ROS when compared to GMA-MS fumes (Antonini et al., 1997).

The differences in the lung and macrophage responses seen in these previous studies may be attributed to the particular metals present in the different welding fumes. It has been shown that soluble transition metals, such as Fe, V, and Ni, adsorbed to ROFA particulates participate in reduction/oxidation cycling and are likely responsible for the pulmonary toxicity by

producing ROS and activating proinflammatory mediators (Dreher et al., 1997). Transition metal pH, content, bioavailability, and their interactions with each other were found to influence ROFA-induced pulmonary toxicity. The content of soluble metals and sulfates leached from ROFA particles was found to be critical in the development of airway hyperreactivity and lung injury (Gavett et al., 1997). Goldsmith et al. (1998) demonstrated that transition metals associated with ROFA and concentrated ambient air particulates mediated ROS production in lung macrophages and increased TNF-α and macrophage inflammatory protein-2 message in a monocyte-macrophage cell line, RAW 264.7. However, the information examining the role individual metals play in the pulmonary responses after exposure to welding fumes is incomplete.

In this current study, three welding fumes were collected, suspended in PBS, and divided into soluble and insoluble fractions. The metal content and the pH of the samples were measured. Because there was little change in pH after the different welding samples were prepared, it is unlikely that pH played a role in the welding fume-induced changes observed in the macrophage response. However, the metal profiles for the

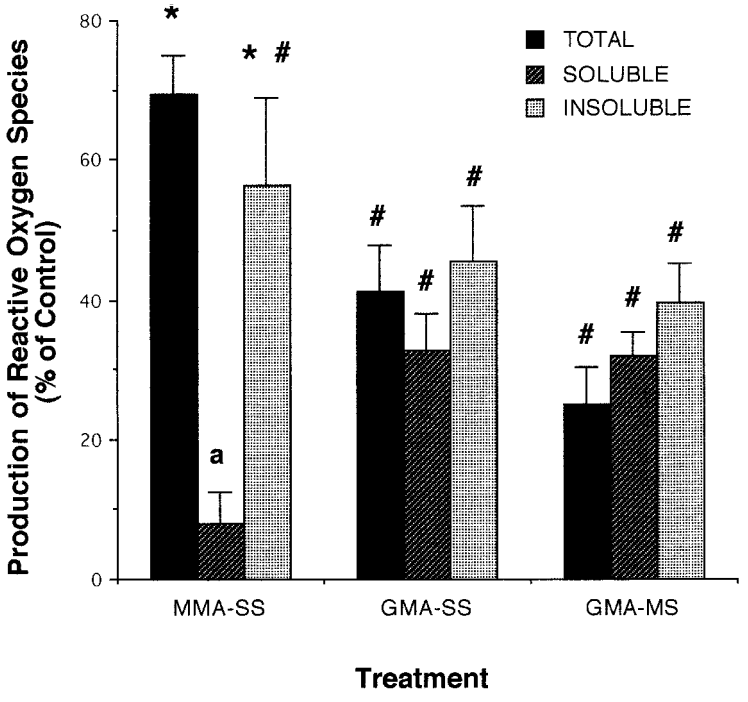

FIGURE 7. Production of reactive oxygen species by lung macrophages incubated for 1 h with the soluble and insoluble components of MMA-SS, GMA-SS, or GMA-MS welding fumes. The final concentration of the particle samples was 25 μg/ml. Equal quantities of the soluble and insoluble portions of the samples were used. Values are means ± SE ($n = 6$). Groups with the same symbols are not statistically different ($p < .05$).

TABLE 3. Effect of Pretreating Welding Fumes With Desferrioxamine on Macrophage Viability and Function

Treatment	Percent dead LMS	LDH (mU/ml)	β-NAG (mU/ml)
MMA-SS-Total	45.3 ± 2.91	88.2 ± 12.6	71.9 ± 10.5
with DES 2.5 m*M*	38.3 ± 4.98	91.2 ± 13.1	72.8 ± 8.64
MMA-SS-sol	45.7 ± 5.61	77.8 ± 0.76	73.9 ± 6.51
with DES 2.5 m*M*	44.0 ± 3.06	82.4 ± 2.36	73.6 ± 6.29
MMA-SS-insol	11.7 ± 2.85	28.3 ± 1.89	51.2 ± 12.3
with DES 2.5 m*M*	15.7 ± 3.53	27.8 ± 3.13	51.3 ± 10.9
GMA-SS-Total	25.7 ± 1.77	80.1 ± 3.20	53.4 ± 3.60
with DES 2.5 m*M*	22.3 ± 4.38	93.2 ± 6.73	56.1 ± 3.13
GMA-SS-sol	19.7 ± 1.45[a]	56.5 ± 10.0	67.1 ± 4.08
with DES 2.5 m*M*	9.33 ± 1.76	48.5 ± 11.2	63.6 ± 3.89
GMA-SS-insol	6.67 ± 2.19	33.5 ± 2.14	51.2 ± 4.09
with DES 2.5 m*M*	8.67 ± 0.88	36.5 ± 2.44	51.1 ± 4.41
GMA-MS-Total	9.67 ± 1.86	36.5 ± 1.55	55.7 ± 3.56
with DES 2.5 m*M*	9.33 ± 0.67	37.5 ± 1.64	55.1 ± 3.10
GMA-MS-sol	10.3 ± 2.03	38.5 ± 1.62	61.5 ± 4.67
with DES 2.5 m*M*	12.3 ± 1.20	38.7 ± 1.02	65.4 ± 3.77
GMA-MS-insol	11.0 ± 2.08	36.8 ± 1.92	48.9 ± 6.23
with DES 2.5 m*M*	9.00 ± 0.58	36.9 ± 1.55	48.8 ± 5.89
Control	3.00 ± 0.58	37.8 ± 2.34	57.2 ± 2.09
with DES 2.5 m*M*	2.31 ± 1.33	42.2 ± 2.21	55.7 ± 2.20

Note. Values are means ± SE; $n = 3–6$.
[a]Significantly different from DES group ($p < .05$).

TABLE 4. Effect of Pretreating Welding Fumes With Desferrioxamine on the Production of Reactive Oxygen Species

Treatment	Percent inhibition of ROS production by DES[a]
MMA-SS-Total	n.d.
MMA-SS-sol	n.d.
MMA-SS-insol	23.6 ± 5.95[a]
GMA-SS-Total	21.2 ± 4.66[a]
GMA-SS-sol	n.d.
GMA-SS-insol	8.90 ± 3.27[a]
GMA-MS-Total	19.4 ± 6.78[a]
GMA-MS-sol	n.d.
GMA-MS-insol	7.73 ± 1.58[a]
Control	2.98 ± 1.19

Note. Values are means ± SE; $n = 4$. n.d., No detectable inhibition.
[a]Significantly greater than control ($p < .05$).

three different welding fumes were vastly different. One fume sample examined was collected during MMA welding of SS electrodes in which fluxes were used. The MMA-SS sample was much more soluble and had a different metal composition than the other two fumes. Fe (41.1%), Cr (28.5%), Mn (16.7%), Ti (10.7%), and Ni (2.53%) were present.

The enhanced macrophage response induced by the MMA-SS sample fractions was likely due to an increase in water solubility as compared to the other fume samples. The soluble fraction was comprised almost entirely of Cr (87%). It has been well established that hexavalent chromium, Cr(VI), is a carcinogen (Lees, 1991) and is often present in the water-soluble, CrO_3 form in fumes generated during welding of SS materials (Griffith & Stevenson, 1989; Eagar et al., 1998). Glaser et al. (1985) found an increase in the phagocytic activity of recovered alveolar macrophages after rat inhalation exposure to low concentrations of a highly soluble Cr(VI) form, sodium dichromate. At higher concentrations, the phagocytic activity decreased, which was likely due to an increase in macrophage death. Johansson et al. (1986) observed morphological changes and a reduction in the metabolic and phagocytic activities of rabbit alveolar macrophages after inhalation exposure to Cr(VI). Sodium chromate, another water-soluble form of Cr, was found to be highly cytotoxic in a concentration-dependent manner to murine peritoneal macrophage (Christensen et al., 1992). Elias et al. (1991) demonstrated that the solubilization of Cr(VI) compounds is a critical step for their cytotoxic and transforming activities in hamster embryo cells.

The contribution of fluoride on the macrophage response also can't be ignored. The fluoride originates from the flux consumed during the MMA welding process. In a previous analysis using x-ray photoelectron spectroscopy, the surface of the MMA-SS particles consisted almost entirely (>97%) of complex fluoride compounds (Antonini et al., 1997). Fluorides have been shown to affect macrophages, by increasing cell death at low concentrations (Meryon & Stephens, 1983) and stimulating eicosanoid release (Dodam & Olson, 1995).

However, water solubility is not a complete guide to the bioavailability of metal constituents in welding fumes. After phagocytosis by macrophages, fume particles are found in phagosomes and phagolysosomes. These intracellular structures become acidified and thus solubility within macrophages is greater than water solubility. Moreover, Kreyling et al. (1990) have shown that metal oxide particle dissolution is related to particle size and surface area as well as the inherent solubility of the particles.

The other fumes tested were collected during GMA welding using either a SS or MS electrode. The GMA-MS fume was comprised of mostly Fe (85.9%) with some Mn (14.6%) present, while the GMA-SS fume contained Fe (53.1%), Mn (23.2%), Cr (18.6%), and Ni (4.85%). Both the GMA-SS and GMA-MS fumes were found to be relatively water insoluble. The differences observed between the GMA-MS and GMA-SS samples

may be due to the differences in the metal composition of both the insoluble and soluble fractions of the two samples. The small fraction of the GMA-MS sample that was soluble was Mn (93%) with little Fe (4.61%), while the portion of the GMA-SS sample that was soluble was a more complex mixture with additional metals present. Mn (68.2%), Ni (11.8%), Fe (9.26%), Cr (5.53%), and Cu (4.4%) were measured in the soluble fraction of the GMA-SS sample. The presence of a mixture of both insoluble and soluble Ni, Cr, and Cu (present only in the GMA-SS sample) in combination with Mn and Fe may then be responsible for the increased loss of macrophage viability observed in the GMA-SS sample as compared to the GMA-MS fume.

It is well documented that the presence of multiple metals may influence the effects of each other. A number of different trace elements were shown to be antagonistic or synergistic to the toxic effects on alveolar macrophages in vitro, depending on the combination of the metals examined (Fisher et al., 1986). Intracellular soluble Cr affected the transforming activity in hamster embryo cells of Ca, Sr, and Zn chromates, while Pb acted synergistically with Cr in the induction of transformation by Ph chromates (Elias et al., 1991). Inhalation studies have shown that Ni potentiates the effect of Co-induced pneumotoxicity (Johansson et al., 1991). Metal content was found to have an impact on ROFA-induced lung injury due to an antagonistic effect of Fe and V on the pulmonary toxicity of Ni (Dreher et al., 1997). Gavett et al. (1997) observed a lack of antagonism by Fe of Ni effects in the lungs.

It is likely that some of the metals found in welding fumes increase macrophage ROS production via such processes as the Fenton reaction (Halliwell, 1989). Although Cu, Ni, and V are capable of reduction/oxidation recycling, which may initiate production of ROS, such as hydrogen peroxide, superoxide anion, and hydroxy radical, it is generally accepted that Fe is the principal metal that mediates these reactions (Halliwell & Gutteridge, 1986). DES is predominately known as a potent chelator of Fe, but it also binds other metals, effectively inhibits iron-dependent lipid peroxidation, and prevents the generation of highly reactive oxidizing species from superoxide anion and hydrogen peroxide in the presence of Fe ions (Halliwell, 1989). The production of ROS induced by ROFA and concentrated ambient air particulates was significantly inhibited by DES, implicating Fe as the metal most likely responsible for the response (Goldsmith et al., 1998). Dreher et al. (1997) reported that DES could inhibit the pulmonary effects of Fe and V, but not Ni, demonstrating the complexity of the metal to metal interaction associated with ROFA-induced lung injury.

In the present study, pretreatment of the soluble fractions of all three welding fumes with DES had no effect in inhibiting the ROS production by macrophages. This can be explained by the fact the GMA-SS and GMA-MS samples were relatively insoluble, and little soluble Fe was

available from the MMA-SS fume. Obviously, other metals were responsible for the increase in ROS production induced by the soluble fractions of each of the fume samples. Since the MMA-SS-sol sample had the least effect on ROS formation, it appears that soluble Cr plays a lesser role in the ROS production as compared to different metals seen to have an effect in other studies, such as Fe and V (Gavett et al., 1997; Dreher et al., 1997). Interestingly, we observed a discordance between the cytotoxicity of the soluble fraction of MMA-SS and its low oxidant potential for macrophages. This resembled a similar discordance in oxidant potential and biological effect produced after exposure to ROFA and TiO_2, depending on the fluorescent probe used to assess ROS production (Imrich et al., 1999). Due to the significant amounts of Fe present in the GMA-SS-Total and GMA-MS-Total and all three insoluble samples, a significant inhibition in ROS production by DES was observed. The increased ROS responses seen were likely attributable to Fe. Except for a slight protective effect in GMA-SS-sol sample, DES had little effect in improving macrophage viability after treatment with any of the other samples.

In conclusion, the effects on macrophage viability observed were likely due to differences in the bioavailability and the combination of both insoluble and soluble metals present in the different welding fumes and not sample pH. The MMA-SS fume was the most soluble and exerted the greatest changes in macrophage viability and function. Unlike the studies examining the pulmonary effects of ROFA and concentrated ambient air particulates (Dreher et al., 1997; Gavett et al., 1997; Goldsmith et al., 1998), very little Fe was present in the soluble portion of the welding samples. The presence of other soluble metals, such Cr, Ni, Cu, and Mn, and the complexes formed by these different metals, as well as fluoride compounds from the fluxes used in certain welding fumes, are likely important in the pulmonary responses observed after welding fume exposure. Still, additional in vivo studies are needed to assess what role the soluble components of welding fumes play in the potential development of lung injury in welders.

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