Molecular and Cellular Biochemistry **255:** 257–265, 2004.
© 2004 *Kluwer Academic Publishers. Printed in the Netherlands.*

Metal composition and solubility determine lung toxicity induced by residual oil fly ash collected from different sites within a power plant

James M. Antonini[1], Michael D. Taylor[1], Stephen S. Leonard[1], Nicholas J. Lawryk[1], Xianglin Shi, Robert W. Clarke[2] and Jenny R. Roberts[1]

[1]*Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, WV;*
[2]*Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA*

Abstract

Residual oil fly ash (ROFA) is a particulate pollutant comprised of soluble and insoluble metals and is produced by the combustion of fossil fuels. The objective was to examine the pulmonary responses to chemically distinct ROFA samples collected from either a precipitator or air heater within the same power plant. The collected ROFA samples were suspended in saline (total sample), incubated for 24 h at 37°C, centrifuged, separated into soluble and insoluble fractions, and the metal composition was determined. In addition, electron spin resonance (ESR) was used to detect short-lived free radical intermediates produced by the ROFA samples and the different fractions. On day 0, Male Sprague–Dawley rats were intratracheally instilled with saline (vehicle control) or the ROFA samples (1 mg/100 g body wt). At day 1, bronchoalveolar lavage was performed, and lung inflammation was assessed. On day 3, additional rats that had been treated with ROFA were intratracheally inoculated with 5×10^5 *Listeria monocytogenes*, and pulmonary bacterial clearance was measured at days 6, 8, and 10. The precipitator ROFA was found to be more soluble and acidic with a significantly greater mass of each metal compared with the air heater ROFA. A prominent hydroxyl radical signal was measured for the total and soluble precipitator ROFA after the addition of H_2O_2, whereas the air heater ROFA and its fractions did not produce a signal. Precipitator ROFA induced a greater inflammatory response than air heater ROFA illustrated by a significant elevation in lung neutrophils. In addition, pulmonary clearance of *L. monocytogenes* was greatly diminished in the rats treated with the soluble and total precipitator ROFA samples. None of the air heater ROFA samples had an effect on lung bacterial clearance. In conclusion, precipitator ROFA, particularly the soluble fraction, generated a metal-dependent hydroxyl radical as measured by ESR and was shown to cause more inflammation and result in reduced lung defense against infection compared with air heater ROFA. These results are most likely due to differences in metal composition and solubility of the ROFA samples. (Mol Cell Biochem **255**: 257–265, 2004)

Key words: residual oil fly ash, electron spin resonance, lung injury, *Listeria monocytogenes*

Introduction

Residual oil fly ash (ROFA) is an occupational and environmental hazard produced by the combustion of fossil fuels. Epidemiology has shown a positive correlation between inhaled ambient air particulate exposure and an increased pulmonary morbidity and infection [1, 2]. ROFA accounts for a significant portion of air pollution, contributing an estimated 2.5×10^5 tons to the ambient particulate burden annually in the United States [3]. Inhalation of ROFA has been associated with adverse respiratory health effects in exposed workers [4, 5]. Animal studies have demonstrated that the bioavailability of

Address for offprints: J.M. Antonini, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, 1095 Willowdale Road, Morgantown, WV 26505, USA (E-mail: jga6@cdc.gov)

soluble transition metals is responsible for the pulmonary injury and inflammation observed after ROFA exposure [6, 7].

Fly ash is defined as the portion of ash produced during fuel combustion that has a sufficiently small particle size to be carried away from the boiler in the flue gas [8]. ROFA is composed of a mixture of metals, sulfates, acids, and other unknown materials complexed to an insoluble, particulate carbon core. The properties of ROFA are dependent on the composition of the fuel source, conditions during combustion, efficiency of emission control devices, and the processing of byproducts [8]. Metal and solubility properties of collected ROFA samples can vary depending on the power plant as well as the position of collection sites within the same plant [7]. By comparing pulmonary responses to chemically distinct ROFA samples with different relative toxicities, it may be possible to determine which components of ROFA may be the most hazardous.

It was our objective to compare the chemical properties of two different ROFA samples and examine their effect on lung responses. ROFA was collected from a precipitator and an air heater from within the same power plant and divided into soluble and insoluble fractions. The metal composition of the ROFA samples and the potential to generate reactive oxygen species as measured by electron spin resonance (ESR) were determined. Male Sprague–Dawley rats were intratracheally instilled with the ROFA samples, and indices of lung injury and inflammation were measured. Additional rats were intratracheally inoculated with *Listeria monocytogenes* after pretreatment with the different ROFA samples, and the effect on lung defense mechanisms was examined.

Materials and methods

Animals

Male Sprague–Dawley [Hla: (SD) CVF] rats (Hilltop Lab Animals, Scottdale, PA, USA) weighing ~250 g were used. The animals were housed in a room with restricted access and HEPA-filtered air, monitored free of pathogens, and allowed to acclimate in an AAALAC-approved animal facility for one week before use. The rats were maintained on ProLab 3500 diet and tap water *ad libitum*. Alpha-Dri virgin cellulose chips and hardwood Beta-chips were used as bedding.

Residual oil fly ash samples

Residual oil fly ash (ROFA) samples were collected from either a precipitator or an air heater at Boston Edison, Mystic Power Plant #4, Everett, MA, USA (see Fig. 1 for collection sites). Particle size of the ROFA samples was characterized by scanning electron microscopy (JSM-#5600 SEM, JEOL, Peabody, MA, USA). The precipitator and air heater ROFA samples were of respirable size with similar count mean diameters of < 3 μm.

The precipitator and air heater ROFA samples were suspended in sterile phosphate-buffered saline (PBS), pH 7.4, and sonicated for 1 min. The samples were further divided into soluble and insoluble components as follows. The ROFA particle suspension (ROFA-total) was incubated for 24 h at 37°C in a shaking incubator. After incubation, the samples were centrifuged at 12,000 × g for 30 min. The supernatant (ROFA-sol) was recovered and filtered with a 0.22 μm filter, whereas the pellet (ROFA-insol) was resuspended with the initial volume of PBS. The pH of each ROFA sample was measured.

For determination of elemental composition, the samples were wet-ashed with concentrated nitric and perchloric acids, followed by hydrofluoric acid treatment to dissolve metal silicates and remove elemental silicon. Metal analysis of each sample was performed by the Division of Applied Research and Technology at NIOSH, Cincinnati, OH, USA, using inductively coupled argon plasma, atomic emission spectroscopy [9].

Free radical measurement

Electron spin resonance (ESR) was used to detect short-lived free radical intermediates in the ROFA-total, ROFA-sol, and ROFA-insol fractions for both the precipitator and air heater ROFA samples (1 mg/ml) in the presence of H_2O_2 using a cell-free system as previously described [10]. For detection and characterization of the generated free radicals, 0.1 M 5,5-dimethyl-1-pyrroline-oxide (DMPO) was used as a spin trap. In some analyses, the metal chelator deferoxamine (2 mM) and antioxidant enzyme catalase (2000 U/L) were added to the reaction.

All ESR measurements were conducted using a Bruker EMX spectrometer (Bruker Instruments, Billerica, MA, USA) and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reference standards. The relative radical concentration was estimated by multiplying half of the peak height by $(\Delta H_{pp})^2$, where ΔH_{pp} represents peak-to peak width. An Acquisit program (Bruker Instruments, Billerica, MA, USA) was used for data acquisitions and analyses.

Residual oil fly ash treatment

On day 0, rats were lightly anesthetized by an intraperitoneal injection of 0.6 ml of a 1% solution of sodium methohexital

(Brevital; Eli Lilly, Indianapolis, IN, USA) and intratracheally instilled with 1.0 mg/100 g body wt. of the precipitator ROFA, air heater ROFA, or silica (positive control) in 300 μl of saline as previously described [11]. In addition, the soluble and insoluble fractions from each ROFA sample were administered by intratracheal instillation using amounts equivalent to the ROFA-total instillate. Animals in the vehicle control groups were matched to the air heater ROFA and precipitator ROFA groups and dosed intratracheally with 300 μl of sterile saline. Intratracheal treatment of the animals with saline at acidic pH had no significant effect on lung injury and inflammation when compared with using saline at a neutral pH.

Bronchoalveolar lavage

On day 1, bronchoalveolar lavage (BAL) was performed as previously described [12]. Total cell numbers and differentials were determined with a Coulter Multisizer II (Coulter Electronics, Hialeah, FL, USA).

Luminol-dependent chemiluminescence

Luminol-dependent chemiluminescence (CL) measures the light generated as reactive oxygen species are produced by activated cells. CL was performed with an automated Berthold Autolumat LB 953 luminometer (Wallace, Gaithersburg, MD, USA) as previously described [13]. Luminol was used as an amplifier to enhance detection of the light, and 3 μM of phorbol myristate acetate (PMA; Sigma Chemicals, St. Louis, MO, USA) was added immediately prior to the measurement of CL to stimulate the lung phagocytes. Measurement of CL was recorded for 15 min at 37°C using 5×10^5 total BAL cells, and the integral of counts per min (cpm) vs. time was calculated. CL was calculated as the total counts of stimulated cells minus the total counts of the corresponding resting cells.

Biochemical parameters of injury

The albumin content and lactate dehydrogenase (LDH) activity in the acellular first fraction of BAL fluid were measured. These measures reflect increased permeability of the bronchoalveolar-capillary barrier and general cytotoxicity, respectively. Albumin content was determined colorimetrically at 628 nm based on albumin binding to bromcresol green using an albumin BCG diagnostic kit (Sigma, St. Louis, MO, USA). LDH activity was determined by measuring the reduction 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).

Pulmonary clearance of L. monocytogenes

At day 3, additional rats were intratracheally inoculated with 5×10^5 of *L. monocytogenes* in 500 μl of sterile saline, according to the instillation method described above. This bacteria dose was found in a previous study to give a uniform infection and did not kill untreated naive Sprague–Dawley rats [14]. At days 6, 8, and 10, the left lungs were removed from rats in each treatment group. The excised lungs were suspended in 10 ml of sterile water, homogenized using a Polytron 2100 homogenizer (Brinkmann Instruments, Westbury, NY, USA), and cultured quantitatively on brain heart infusion agar plates (Becton Dickinson, Cockeysville, MD, USA). The number of viable colony-forming units (CFUs) was counted after an overnight incubation at 37°C.

Statistical analysis

Results are expressed as means ± standard errors of measurement (S.E.). The significance of the interaction among the different treatment groups for the different parameters was assessed using analysis of variance (ANOVA). The significance of differences between individual groups was analyzed using the Tukey–Kramer post-hoc test. For all analyses, the criterion of significance was set at $p < 0.05$.

Results

ROFA was collected in the precipitator and air heater (Fig. 1). The air heater acts as a heat exchanger that recovers heat from boiler exhaust gases to preheat incoming combustion air [15]. This cools the exhaust gases before they pass to the precipitator and also increases the efficiency of the fuel-burning process. At the end of the process, gases are passed through the precipitator for particulate sampling and collection before reaching the stack and emitted into the environment.

Table 1 depicts the measured pH and solubility properties of the different ROFA samples. The total, insoluble, and soluble fractions of the air heater ROFA sample were found to be mostly neutral, whereas the total and soluble fractions of the precipitator ROFA were both highly acidic with a pH of 5.00 and 4.10, respectively. In addition, the precipitator ROFA sample had a soluble-to-insoluble ratio of 0.605 and was substantially more water soluble than the air heater ROFA sample which had a ratio of 0.198.

The elemental analysis of the different ROFA samples is shown in Table 2. The predominant components of the air heater sample were Fe, V, Ni, and Al in a mostly insoluble form. Conversely, a substantially greater mass of each ele-

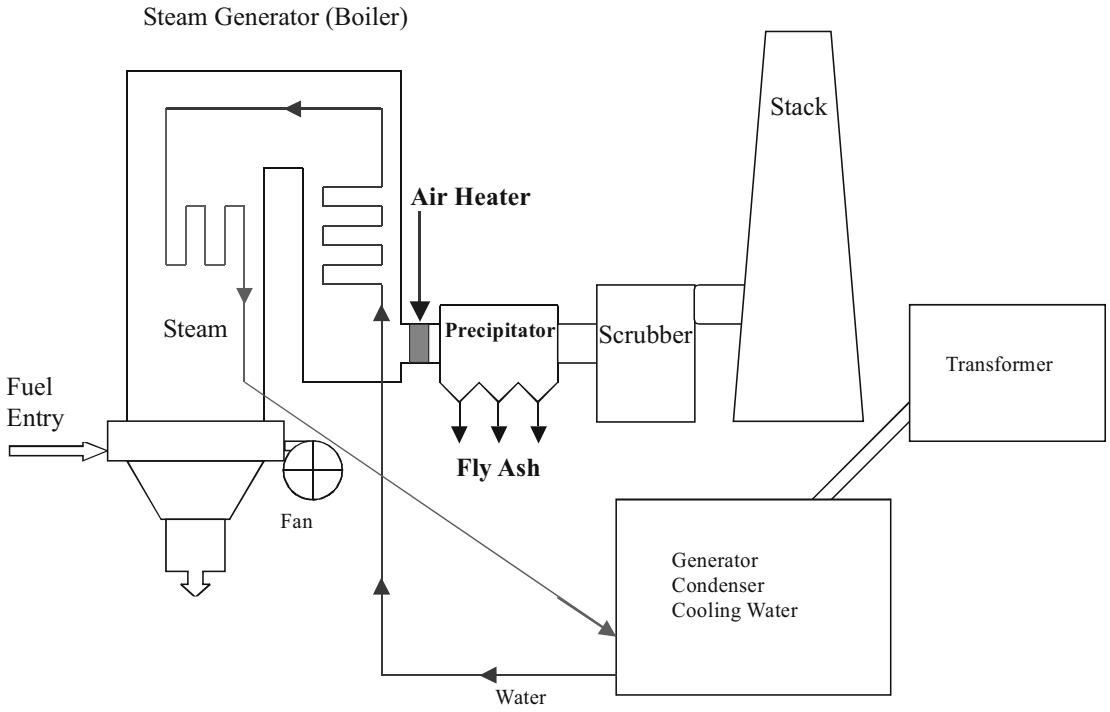

Fig. 1. Schematic diagram of a fossil fuel-burning power plant. ROFA was collected from both the precipitator and air heater areas within the plant.

ment was observed for the precipitator ROFA sample as compared to the air heater sample. The predominant elements of the precipitator sample were Fe, Al, V, Ni, Ca, Mg, and Zn. Measurable amounts of soluble Fe, Al, Ni, Ca, Mg, and Zn were present in the precipitator sample, whereas V was found to be mostly insoluble.

Using ESR to measure free radical formation in an acellular system, the precipitator ROFA generated a 1:2:2:1 quartet signal in the presence of DMPO and H_2O_2 which is representative of the highly biologically reactive hydroxyl radical (Fig. 2A). The total and soluble forms of the precipitator ROFA produced the greatest signal. This signal was dependent upon metals because it was reduced after the addition of the metal chelator deferoxamine to the reaction (data not shown). The addition of the antioxidant catalase significantly reduced the hydroxyl radical formation generated by the precipitator ROFA (data not shown). Air heater ROFA produced no hydroxyl radical in the presence of DMPO and H_2O_2 as measured by ESR (Fig. 2B).

In the assessment of pulmonary cellular responses one day after treatment, no significant difference in the number of alveolar macrophages recovered from the lungs was observed among the different groups (Fig. 3A). Lung neutrophil numbers were significantly elevated after intratracheal instillation of silica (positive control) when compared with all the other groups (Fig. 3B). Treatment with the different precipitator

Table 1. pH and solubility properties of ROFA samples

Sample	pH	Soluble/insoluble ratio
Air heater – ROFA	Total 7.27 Insoluble 7.76 Soluble 7.27	0.198
Precipitator – ROFA	Total 5.00 Insoluble 7.06 Soluble 4.10	0.605

Table 2. Elemental composition of ROFA samples

	Element Mass (μg/mg)					
	Air Heater – ROFA			Precipitator – ROFA		
	Total	Insoluble	Soluble	Total	Insoluble	Soluble
Fe	45.7	62.9	2.71	157	91.4	15.7
Al	11.1	11.9	1.86	72.9	37.1	22.9
V	20.0	18.6	0.05	48.6	42.9	0.63
Ni	12.3	7.00	3.43	35.7	7.71	21.4
Ca	11.4	0.00	10.0	21.4	0.00	20.0
Mg	3.71	0.59	1.30	41.4	5.29	28.6
Zn	1.29	0.86	0.59	5.29	0.89	3.43
Pb	0.16	0.24	0.00	2.71	1.29	0.05
Mn	0.29	0.12	0.23	1.41	0.36	0.74
Co	0.29	0.14	0.11	1.03	0.23	0.64

Note: Trace elements measured in both ROFA samples included Ba, Cd, Cr, and Cu.

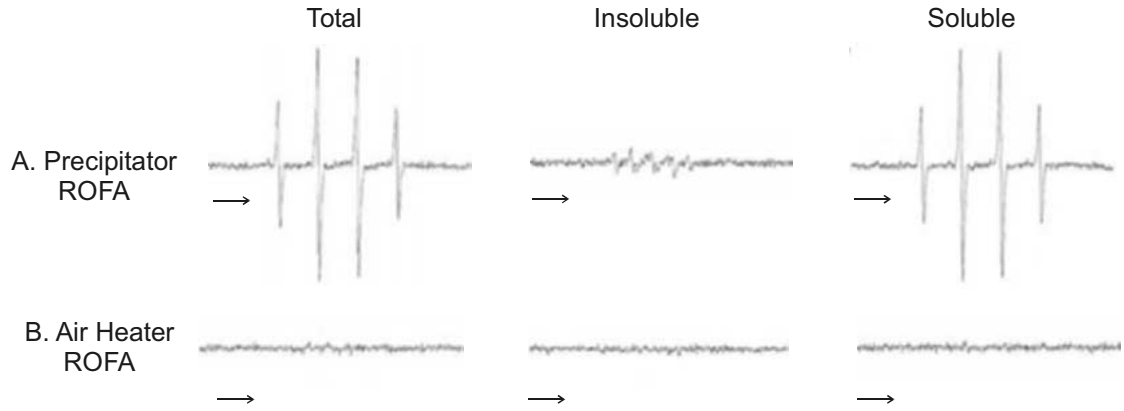

Fig. 2. Electron spin resonance spectra of the different fractions from (A) precipitator ROFA and (B) air heater ROFA using spin trapping with DMPO in addition to H_2O_2. The ESR spectrometer settings were: receiver gain, 2.5×10^4; time constant, 0.04 sec; modulation amplitude, 1.0 G; scan time, 1 min; magnetic field, 3440 μ 100 G. Arrows represent 15 G and direction of scan.

ROFA samples induced an inflammatory response as evidenced by a significant elevation in neutrophil number when compared with the air heater ROFA sample and the saline control groups. The numbers of neutrophils recovered from the groups treated with the soluble and insoluble fractions of the precipitator ROFA were significantly less than the number recovered from the precipitator ROFA-total group. No significant increase in lung neutrophil number was observed for the different air heater ROFA samples when compared with control.

PMA-stimulated CL was measured as an index of cellular activation. Lung phagocytes recovered from animals treated with silica generated the greatest CL signal when compared with the other groups (Fig. 4). CL produced by each of the precipitator and air heater ROFA groups was not significantly different from saline control.

LDH activity and albumin content were measured in the acellular first fraction of BAL fluid to assess lung injury after different treatments. LDH activity was significantly higher for the silica group when compared with the other groups (Fig. 5A). No significant difference was observed in LDH activity when comparing the precipitator ROFA groups with the saline control. LDH activity was significantly higher for the total and insoluble fractions of the air heater ROFA sample when compared with the corresponding saline control. Albumin values were significantly higher for the groups receiving the total and insoluble groups for both the precipitator and air heater ROFA samples when compared with the groups receiving the corresponding soluble ROFA fractions or saline (Fig. 5B).

In the assessment of lung defense function, pulmonary bacterial clearance of *L. monocytogenes* was measured in rats

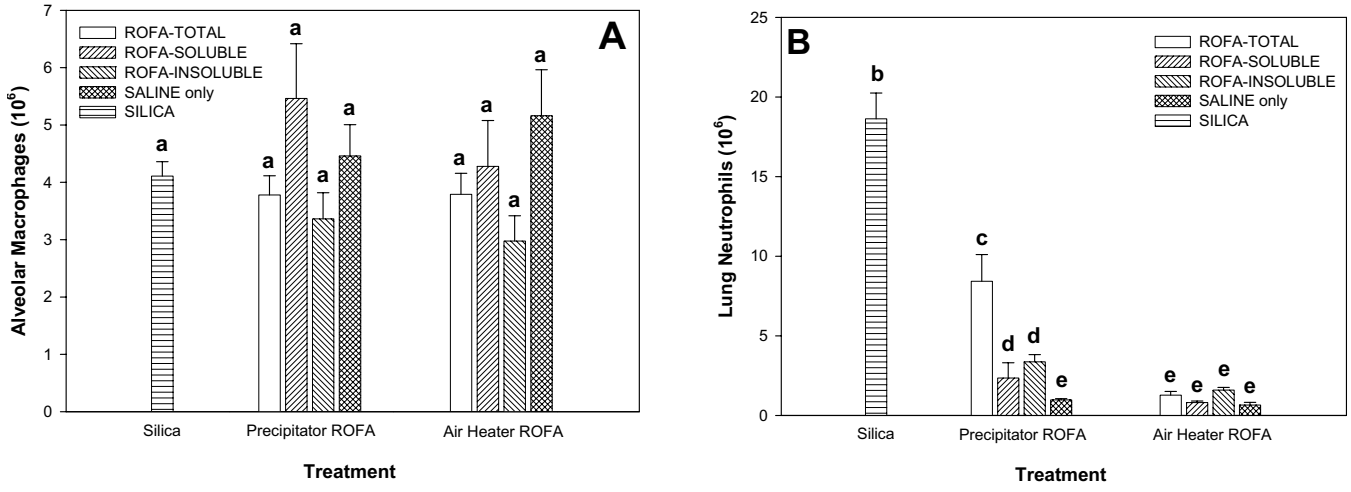

Fig. 3. Number of (A) alveolar macrophages and (B) neutrophils recovered 1 day after treatment with silica (positive control), the different ROFA samples, and the corresponding saline control groups. Values are means ± S.E. (n = 5–10 rats/group); for each cell type, groups with different symbols are statistically different from each other; $p < 0.05$.

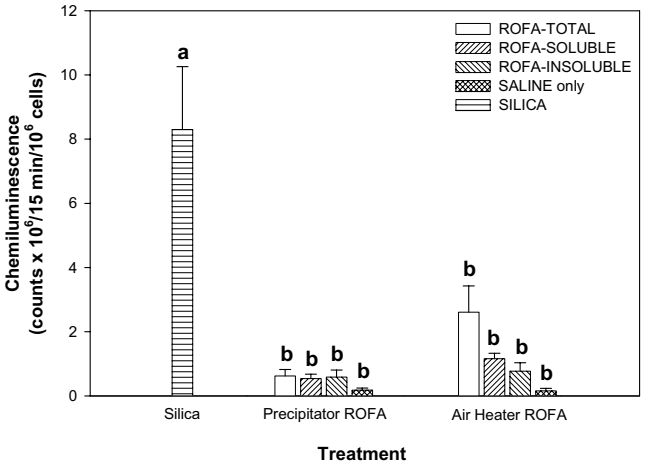

Fig. 4. PMA-stimulated chemiluminescence (CL) of lung BAL cells recovered one day after treatment with silica (positive control), the different ROFA samples, and the corresponding saline control groups. Values are means ± S.E. (n = 5–10 rats/group); groups with different symbols are statistically different from each other; $p < 0.05$.

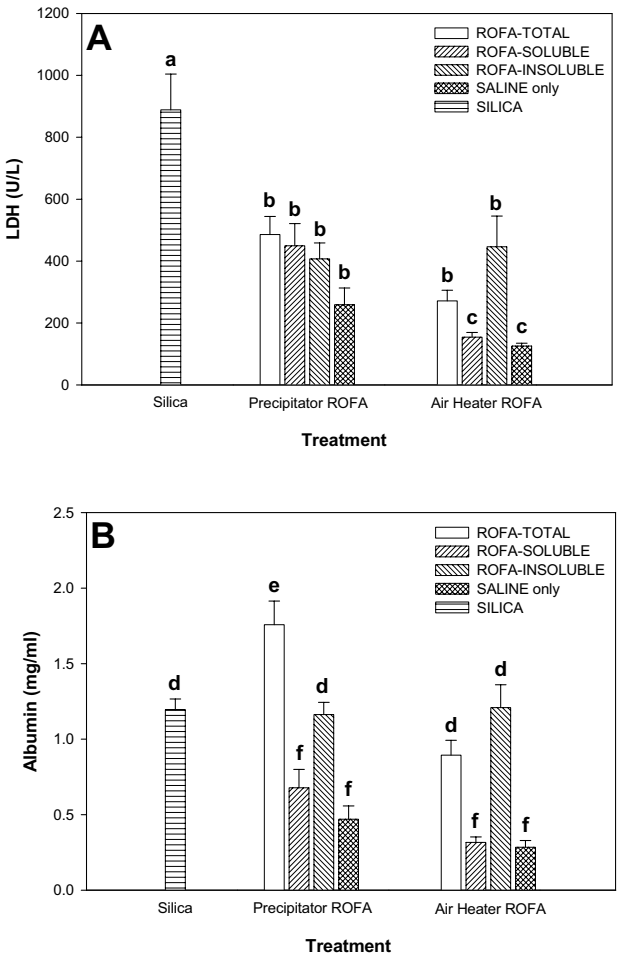

Fig. 5. (A) LDH activity and (B) albumin content of the acellular first fraction BAL fluid one day after treatment with silica (positive control), the different ROFA samples, and the corresponding saline control groups. Values are means ± S.E. (n = 5–10 rats/group); for each parameter, groups with different symbols are statistically different from each other; $p < 0.05$.

treated with the different ROFA samples. Pre-exposure with the soluble and total fractions of the precipitator ROFA sample dramatically slowed the clearance of bacteria from the lungs of these groups as compared to the insoluble precipitator and saline control groups (Fig. 6A). At day 10, there were 661 and 204 times more bacteria in the lungs of the soluble and total precipitator ROFA groups, respectively, as compared to the saline control group. No significant difference in bacterial clearance was observed when comparing the air heater ROFA and saline control groups (Fig. 6B).

Discussion

In the present study, the pulmonary responses of two ROFA particulate samples collected from different sites within a power plant were evaluated. It is known that different operations in a power plant can have a significant effect on the chemical composition of generated particulates. Changes in particulate properties as a function of location in the processing equipment of a power plant can be caused by many factors, including temperature and pressure changes, gas velocities, continuing reactions, duct work configuration, residence times, and control equipment [16]. Time–temperature relationships at different plant locations affect carbon burnout and the fusion of mineral components, and thus influence particulate density, color, and chemical composition.

The two ROFA samples which were collected and used in the study had distinctively different chemical and physical characteristics. The air heater ROFA was found to be neutral when suspended in PBS and was relatively water-insoluble, whereas the precipitator ROFA was acidic in solution and contained elevated quantities of water-soluble Fe, Ni, Al, Ca, and Mg. The difference in composition of the two samples may be due to the extremely high temperatures (usually between 150–300°C) present in the air heater which can prevent acid condensation and promote metal vaporization of the particulates. Due to the differences in the physical and chemical properties of the ROFA samples, it was our goal to attempt to make determinations of what properties or components of ROFA may be the most hazardous after inhalation.

ESR spin trapping was used to measure the reactivity of the different ROFA samples in a cell-free system by detecting the formation of short-lived free radical intermediates. It is well-documented that free radical generation is an important contributor to the pathogenesis of lung disease caused by particulate inhalation [17]. Vallyathan *et al.* [18] correlated

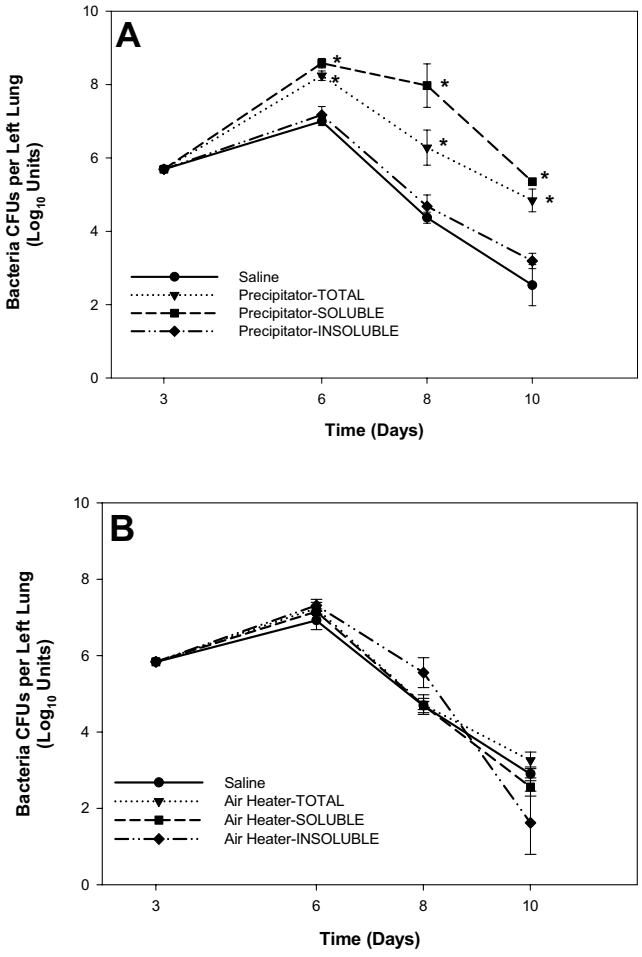

Fig. 6. Pulmonary clearance of 5×10^5 *Listeria monocytogenes* at 6, 8, and 10 days after treatment with (A) precipitator or (B) air heater ROFA. Values are means ± S.E. (n = 4–12 rats/group); *, significantly different than saline and precipitator-insoluble groups; $p < 0.05$.

the lung injury associated with freshly-fractured silica with the generation of reactive oxygen species using ESR spin trapping. In addition, ESR analysis has been used to evaluate the oxidant-induced cell injury associated with different metals, such as chromium [10, 19], vanadium [20], nickel [21], and arsenic [22].

In a previous ESR study, van Maanen *et al*. [23] measured hydroxyl radical generation by different fly ash samples in the presence of H_2O_2 and correlated the acellular production of oxidants with DNA damage in rat lung epithelial cells. In addition, it was observed that pulmonary treatment with the soluble fraction of ROFA induced the *in vivo* generation of carbon-centered free radical adducts [24]. In the current study, using ESR spin trapping, the total and soluble precipitator ROFA samples both produced the highly reactive hydroxyl radical in the presence of H_2O_2, whereas the insoluble precipitator fraction did not. Neither the soluble nor the insoluble fractions of the air heater ROFA generated a hydroxyl radical signal in the presence of H_2O_2 and the spin trap. The response was significantly reduced after treatment with the metal chelator deferoxamine suggesting that the soluble metals associated with the precipitator ROFA were likely responsible for the ROS generated.

Based on the ESR findings, it was hypothesized that the lung responses associated with the precipitator ROFA sample would be more dramatic, possibly due to the presence of soluble metals. Dreher *et al*. [6] have demonstrated that water-soluble transition metals are likely the causative agents of ROFA-induced lung injury. Transition metal content, bioavailability, and the metal-to-metal interactions were shown to influence lung responses after ROFA exposure. Not surprisingly, the precipitator ROFA samples used in the current study were significantly more inflammatory than the air heater ROFA samples as indexed by pulmonary neutrophil influx. However, few differences were observed in the lung cytotoxicity as measured by LDH activity when comparing the precipitator and air heater ROFA samples. In assessing the integrity of the alveolar-capillary barrier, significantly more albumin was measured in the BAL fluid of the total precipitator ROFA group as compared to the total air heater group. Interestingly, significantly greater levels of BAL fluid albumin were observed in the insoluble fraction for both the precipitator and air heater samples when compared with the soluble fractions.

In studying the toxicity profile of different ROFA samples, Kodavanti *et al*. [7] indicated that the precipitator samples, which had the greatest amount of soluble metals, induced the most lung injury as measured by BAL fluid protein and LDH activity. Significant amounts of water-soluble V, Fe, and Ni were present in the precipitator samples used in their study. The precipitator ROFA used in our current study contained little V in the soluble fraction, which may be one reason why the soluble fraction of the precipitator ROFA induced little lung injury as compared to the insoluble fractions (which did contain significant amounts of non-water soluble V). The complex interaction among the different metals may offer another explanation for our observations. Kodavanti *et al*. [25] demonstrated that when Ni, V, and Fe were combined, lung pathology and cytokine production caused by the three metals together were less severe than by Ni alone. In a related study, it was observed that the lung damage caused by a mixture of V and Ni was less than the injury caused by Ni alone [6].

Previous *in vitro* CL studies have indicated that some ROFA samples may be strong activators of lung macrophages [7, 26]. However, neither of the ROFA samples (or the soluble and insoluble fractions) which were used in our current study had an effect on the *ex vivo* production of CL from recovered lung phagocytes. Kodavanti *et al*. [7] showed that a specific ROFA sample without water-soluble V (as was the case with the ROFA samples used in the current study) did not cause an

elevation in macrophage CL. In addition, it appeared that soluble Ni had a suppressive effect on *in vitro* macrophage activation. Because the precipitator ROFA sample used in this current study contained a considerable amount of soluble Ni, the potential toxic lung responses and cell activation associated with the other soluble metals present in the ROFA sample may have been suppressed by the presence of Ni.

To further evaluate the possible suppressive effect of soluble metals on macrophage function, bacterial clearance was measured in ROFA-treated rats inoculated with *L. monocytogenes*. The gram-positive, facultative intracellular bacterial pathogen, *L. monocytogenes*, has been used by our group and others to assess non-specific lung defense mechanisms [12, 27–31]. None of the air heater ROFA groups (which contained minimal amounts soluble metals) had an effect on the lung clearance of *L. monocytogenes*. However, bacterial clearance was greatly diminished in the rats treated with the total and soluble precipitator ROFA groups, further implicating the suppression of macrophage function by soluble metals.

It is important to indicate that lung responses associated with certain particulate samples are not entirely dependent on the bioavailability of soluble metals. Imrich *et al*. [32] demonstrated that the effects of ambient air particles on lung macrophage function, cytokine production, and phagocytosis, were caused mostly by the insoluble components. In addition, it has been observed that the ability of a highly water-soluble welding fume to generate free radicals, activate macrophages, and produce lung inflammation was dependent on the soluble as well as the insoluble metals of the fume [33, 34]. Different lung responses to specific particulates are likely due to the heterogeneity in the characteristics of the samples. Ambient air particle samples collected on different days [32] and ROFA collected from different locations within the same power plant have been shown to demonstrate a wide range of biologic activity. Thus, the pneumotoxic profile of collected workplace and environmental particulate samples is strongly influenced by the elemental and physical properties of the sample.

Acknowledgment

The authors thank Anthony Lewis and Joel Yednock for their invaluable help with all aspects of the study. The authors also acknowledge Mark Millson at NIOSH in Cincinnati, OH for performing the metal analysis of the ROFA samples.

References

1. Schwartz J: Air pollution and daily mortality: A review and meta analysis. Environ Res 64: 36–52, 1994
2. Pope CA, Dockery DW, Schwartz J: Review of epidemiological evidence of health effects of particulate air pollution. Inhal Toxicol 7: 1–18, 1995
3. Costa DL, Dreher KL: Bioavailable transition metals in particulate matter mediate cardiopulmonary injury in healthy and compromised animal models. Environ Health Perspect 105 (suppl 5): 1053–1060, 1997
4. Levy BS, Hoffman L, Gottsegen S: Boilermakers' bronchitis: Respiratory tract irritation associated with vanadium pentoxide exposure during oil-to-coal conversion of a power plant. J Occup Med 26: 567–570, 1984
5. Hauser R, Elreedy S, Hoppin JA, Christiani DC: Airway obstruction in boilermakers exposed to fuel oil ash: A prospective study. Am J Respir Crit Care Med 152: 1478–1484, 1995
6. Dreher KL, Jaskot RH, Lehmann JR, Richards JH, McGee JK, Ghio AJ, Costa DL: Soluble transition metals mediate residual oil fly ash induced acute lung injury. J Toxicol Environ Health 50: 285–305, 1997
7. Kodavanti UP, Hauser R, Christiani DC, Meng ZH, McGee J, Ledbetter A, Richards J, Costa DL: Pulmonary responses to oil fly ash particles in the rat differ by virtue of their specific soluble metals. Toxicol Sci 43: 204–212, 1998
8. El-Mogazi D, Lisk DJ, Weinstein LH: A review of physical, chemical, and biological properties of fly ash and effects on agricultural ecosystems. Sci Total Environ 74: 1–37, 1988
9. NIOSH: Elements (ICP): Method 7300. In: U.S. Department of Health and Human Services (eds). NIOSH Manual of Analytical Methods, 4th edition, Publication No. 98–119. NIOSH, Washington DC, 1994, pp 1–10
10. Leonard S, Wang S, Zang L, Castranova V, Vallyathan V, Shi X: Role of molecular oxygen in the generation of hydroxyl and superoxide anion radicals during enzymatic Cr (VI) reduction and its implication to Cr (VI)-induced carcinogenesis. J Environ Pathol Toxicol Oncol 19: 49–60, 2000
11. Reasor MJ, Antonini JM: Pulmonary responses to single versus multiple intratracheal instillations of silica in rats. J Toxicol Environ Health 62: 9–21, 2001
12. Antonini JM, Yang H-M, Ma JYC, Roberts JR, Barger MW, Butterworth L, Charron TG, Castranova V: Subchronic silica exposure enhances respiratory defense mechanisms and the pulmonary clearance of *Listeria monocytogenes* in rats. Inhal Toxicol 12: 1017–1036, 2000
13. Antonini JM, Van Dyke K, Ye Z, DiMatteo M, Reasor MJ: Introduction of luminol-dependent chemiluminescence as a method to study silica inflammation in the tissue and phagocytic cells of rat lung. Environ Health Perspect 102 (suppl 10): 37–42, 1994
14. Antonini JM, Roberts JR, Clarke RW: Strain-related differences of non-specific respiratory defense mechanisms in rats using a pulmonary infectivity model. Inhal Toxicol 13: 85–102, 2001
15. Edison Electric Institute: Electric utilities must maintain their generating units to keep power plants operating, July 2001. Available at: http://www.eei.org/issues/environ/nsr/coal2.pdf. Accessed 9-15-02.
16. Burckle JO: Particulate emission measurement models. In: D. Considine (ed). Energy Technology Handbook. McGraw-Hill, New York, 1977, pp 9.391–9.406
17. Vallyathan V, Shi X: The role of oxygen free radicals in occupational and environmental lung disease. Environ Health Perspect 105 (suppl 1): 165–177, 1997
18. Vallyathan V, Shi X, Dalal NS, Irr W, Castranova V: Generation of free radicals from freshly fractured silica dust: Potential role in acute silica-induced lung injury. Am Rev Respir Dis 138: 1213–1219, 1988
19. Ye J, Wang S, Leonard SS, Sun Y, Butterworth L, Antonini JM, Ding M, Rojanasakul Y, Vallyathan V, Castranova V, Shi X: Role of reactive oxygen species and p53 in chromium (VI)-induced apoptosis. J Biol Chem 274: 34974–34980, 1999

20. Huang C, Ding M, Li J, Leonard SS, Rojanasakul Y, Castranova V, Vallyathan V, Ju G, Shi X: Vanadium-induced nuclear factor of activated T cells activation through hydrogen peroxide. J Biol Chem 276: 22397–22403, 2001
21. Huang C, Li J, Costa M, Zhang Z, Leonard SS, Castranova V, Vallyathan V, Ju G, Shi X: Hydrogen peroxide mediates activation of NFAT by nickel subsulfide. Cancer Res 61: 8051–8057, 2001
22. Huang C, Li J, Ding M, Wang L, Shi X, Castranova V, Vallyathan V, Ju G, Costa M: Arsenic-induced NFkB transactivation through ERKs- and JNKs-dependent pathways in mouse epidermal JB6 cells. Mol Cell Biochem 222: 29–34, 2001
23. Van Maanen JMS, Borm PJA, Knaapen A, Van Herwijnen M, Schilderman P, Smith KR, Aust AE, Tomatis M, Fubini B: *In vitro* effects of coal fly ashes: Hydroxyl radical generation, iron release, and DNA damage and toxicity in rat lung epithelial cells. Inhal Toxicol 11: 1123–1141, 1999
24. Kadiiska M, Mason R, Dreher K, Costa D, Ghio A: *In vivo* evidence of free radical formation in the rat lung after exposure to an emission source air pollution particle. Chem Res Toxicol 10: 1104–1108, 1997
25. Kodavanti UP, Jaskot RH, Costa DL, Dreher KL: Pulmonary pro-inflammatory gene induction following acute exposure to residual oil fly ash: Roles of particle-associated metals. Inhal Toxicol 9: 679–701, 1997
26. Ghio AJ, Meng ZH, Hatch GE, Costa DL: Luminol-enhanced chemiluminescence after *in vitro* exposures of rat alveolar macrophages to oil fly ash is metal dependent. Inhal Toxicol 9: 255–271, 1997
27. Van Loveren H, Rombout PJA, Wagenaar SS, Walvoot HC, Vos JG: Effects of ozone on the defense to a respiratory *Listeria monocytogenes* infection in the rat. Toxicol Appl Pharmacol 94: 374–393, 1988
28. Jakab GJ: The toxicologic interactions resulting from inhalation of carbon black and acrolein on pulmonary antibacterial and antiviral defenses. Toxicol Appl Pharmacol 121: 167–175, 1993
29. Antonini JM, Roberts JR, Clarke RW, Yang H-M, Barger MW, Ma JYC, Weissman DN: Effect of age on respiratory defense mechanisms: Pulmonary bacterial clearance in Fischer 344 rats after intratracheal instillation of *Listeria monocytogenes*. Chest 120: 240–249, 2001
30. Cohen MD, Sisco M, Li Y, Zelikoff JT, Schlesinger RB: Ozone-induced modulation of cell-mediated immune responses in the lungs. Toxicol Appl Pharmacol 171: 71–84, 2001
31. Antonini JM, Roberts JR, Jernigan MR, Yang H-M, Ma JYC, Clarke RW: Residual oil fly ash increases the susceptibility to infection and severely damages the lungs after pulmonary challenge with a bacterial pathogen. Toxicol Sci 70: 110–119, 2002
32. Imrich A, Ning Y, Kobzik L: Insoluble components of concentrated air particles mediate alveolar macrophage responses *in vitro*. Toxicol Appl Pharmacol 167: 140–150, 2000
33. Antonini JM, Lawryk NJ, Krishna Murthy GG, Brain JD: Effect of welding fume solubility on lung macrophage viability and function *in vitro*. J Toxicol Environ Health 58: 343–363, 1999
34. Taylor MD, Roberts JR, Leonard SS, Shi X, Antonini JM: Effects of welding fumes of differing composition and solubility on free radical production and acute lung injury and inflammation in rats. Toxicol Sci 75: 181–191, 2003