

Emission-Particle-Induced Ventilatory Abnormalities in a Rat Model of Pulmonary Hypertension

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Preexistent cardiopulmonary disease in humans appears to enhance susceptibility to the adverse effects of ambient particulate matter. Previous studies in this laboratory have demonstrated enhanced inflammation and mortality after intratracheal instillation (IT) and inhalation (INH) of residual oil fly ash (ROFA) in a rat model of pulmonary hypertension induced by monocrotaline (MCT). The present study was conducted to examine the effects of ROFA in this model on ventilatory function in unanesthetized, unrestrained animals. Sixty-day-old male CD rats were injected with MCT (60 mg/kg) or vehicle (VEH) intraperitoneally 10 days before IT of ROFA (8.3 mg/kg) or saline (SAL) (control) or nose-only INH of ROFA [15 mg/m³ for 6 hr on 3 consecutive days or air (control)]. At 24 and 72 hr after exposure, rats were studied individually in a simultaneous gas uptake/whole-body plethysmograph. Lungs were removed at 72 hr for histology. Pulmonary test results showed that tidal volume (V_T) decreased 24 hr after IT of ROFA in MCT-treated rats. Breathing frequency, minute volume (V_E), and the ventilatory equivalent for oxygen increased in MCT- and VEH-treated rats 24 hr after IT or INH of ROFA and remained elevated 72 hr post-IT. O₂ uptake (V_{O_2}) decreased after IT of ROFA in MCT-treated rats. Carbon monoxide uptake decreased 24 hr after IT of ROFA, returning to control values in VEH-treated rats but remaining low in MCT-treated rats 72 hr post-IT. ROFA exposure induced histologic changes and abnormalities in several ventilatory parameters, many of which were enhanced by MCT treatment. **Key words:** air pollution, cardiopulmonary, monocrotaline, particle, residual oil fly ash, ventilation. *Environ Health Perspect* 112:872–878 (2004). doi:10.1289/ehp.6583 available via <http://dx.doi.org/> [Online 17 February 2004]

Epidemiologic associations between levels of ambient particulate matter (PM) and morbidity (Burnett et al. 1995; Committee of Departmental Officers and Expert Advisers 1954; Poloniecki et al. 1997; Schwartz and Morris 1995; Wichmann et al. 1989) and mortality (Pope et al. 1992; Schwartz 1994; Schwartz and Dockery 1992; Wordley et al. 1997) from cardiovascular and pulmonary diseases in humans have been reported. These studies have also indicated that groups with preexisting cardiopulmonary disease appear to be at highest risk of the acute impacts of PM. Our laboratory is currently using animal models of cardiovascular and pulmonary disease to investigate mechanisms that may be involved in these findings. One such model is that induced by monocrotaline (MCT) in rats, which imparts significant cardiopulmonary disease (White and Roth 1989). MCT is a pyrrolizidine alkaloid found in leaves and seeds of the plant *Crotalaria spectabilis*. In rats, administration of MCT causes pulmonary vascular injury and inflammation, pulmonary hypertension, and right ventricular hypertrophy (Meyrick et al. 1980). Many of the MCT-induced changes in rats are similar to those seen in certain types of chronic pulmonary hypertension in humans (Meyrick and Reid 1979).

Previous studies in our laboratory have demonstrated acute lung injury with alveolar inflammation in rats, after exposure to residual oil fly ash (ROFA), an emission source PM of which the primary toxic component is the bioavailable metal fraction (Dreher et al. 1997; Kodavanti et al. 1997a, 1997b). In addition, studies in our laboratory have demonstrated enhanced inflammation, increased frequency and severity of cardiac arrhythmias, and increased mortality after intratracheal instillation (IT) and inhalation (INH) of ROFA in rats pretreated with MCT (Kodavanti et al. 1999; Watkinson et al. 1998, 2000). Mortality and enhanced injury with ROFA INH in this model have also been reported by Killingsworth et al. (1997), but the etiology of death was unknown. Many of the cardiac arrhythmias in the Watkinson studies appeared consistent with myocardial hypoxia. We hypothesized that the cardiac effects might be secondary to ventilatory and pulmonary gas exchange impairments. To test this hypothesis, we measured breathing parameters and diffusing capacity as indirect measures of gas exchange competence in unanesthetized, unrestrained animals as opposed to directly measuring gas exchange, which requires handling and arterial cannulation, potentially altering the validity of results in these compromised rats.

Severe impairments would be self-evident from vastly abnormal values, but less dramatic changes might suggest the transient yet significant impairments as seen in individuals with chronic obstructive pulmonary disease. If coupled with underlying cardiac dysfunction, these less dramatic changes might be sufficient to trigger such cardiac events.

Materials and Methods

Animals. Sixty-day-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Raleigh, NC) were housed in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care. Food (Purina Rodent Lab Chow, St. Louis, MO) and water were available *ad libitum*.

Particles. ROFA had been previously collected using a Teflon-coated fiber filter at a temperature of 204°C by Southern Research Institute (Birmingham, AL) downstream from the cyclone of a power plant in Florida burning low-sulfur no. 6 residual oil. The mass mean aerodynamic diameter of ROFA was 1.95 μm , and the geometric mean was 2.19 μm (Dreher et al. 1997). For IT exposures, ROFA was suspended in sterile 0.9% sodium chloride solution (saline, SAL; Fujisawa USA Inc., Deerfield, IL) at a concentration of 8.3 mg/mL. ROFA has been tested and found to be free of endotoxin (Cape Cod Associates, South Yarmouth, MA).

Treatment. MCT (Sigma Chemical Co., St. Louis, MO) was dissolved in 1.0 M

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hydrochloric acid, diluted with sterile distilled water, and adjusted to a pH of 7.0 with sterile 1.0 M NaOH; the final concentration was adjusted to 60 mg/mL. Twelve rats were injected intraperitoneally with this MCT suspension (60 mg/kg) 10 days before IT, and another 12 rats were injected intraperitoneally with this MCT suspension (60 mg/kg) 10 days before INH. Twelve control rats were injected intraperitoneally with sterile water adjusted to a pH of 7.0 (vehicle, VEH; 1 mL/kg) 10 days before IT, and another 12 control rats were injected intraperitoneally with sterile water adjusted to a pH of 7.0 (VEH; 1 mL/kg) 10 days before INH exposure.

IT exposure. IT was performed as previously described (Dreher et al. 1997). ROFA suspension (8.3 mg/kg) or SAL (control) was instilled intratracheally (1 mL/kg) in six rats that had been treated with MCT 10 days before and six rats that had been treated with VEH 10 days before they were exposed to ROFA. Six rats that had been treated with MCT 10 days before, and six rats that had been treated with VEH 10 days before, were exposed to SAL.

INH exposure. Nose-only INH exposure was performed as previously described (Kodavanti et al. 1999; Ledbetter et al. 1998) for 6 hr on 3 consecutive days at a concentration of 15 mg/m³ ROFA or air (control). Briefly, the generator, designed on the principle of a carpenter's chalk line, used a continuously moving string to carry adherent particles upward from a reservoir containing ROFA. Aerosolization was accomplished using compressed air to pulse-dislodge particles from the string into the dilution air stream. Six rats that had been treated with MCT 10 days before, and six rats that had been treated with VEH 10 days before, were exposed to ROFA. Six rats that had been treated with MCT 10 days before, and six rats that had been treated with VEH 10 days before were exposed to air. The anticipated cumulative dose of ROFA was calculated based on the mean weight of rats (300 g) at the time of exposure.

Ventilation measurements. A gas uptake/whole body plethysmograph system (Figure 1) was used for unrestrained simultaneous measurement of all ventilation parameters. The gas uptake chamber (McGee et al. 1995) is a closed, recirculated system that provides oxygen uptake rate (V_{O_2} ; milliliters per minute per kilogram) and carbon monoxide uptake rate. Attachment of a differential pressure transducer (model DP45; Validyne Engineering Corp., Northridge, CA) allows the chamber to serve as a whole-body plethysmograph (Wong and Alarie 1982) for measurement of breathing frequency (breaths/min), tidal volume (V_T ; milliliters per breath), and minute volume (V_E ; milliliters per minute). The barometric measurement algorithm and syringe pump calibrator

used for V_T were as described by Perkins et al. (1996). Rats were studied individually at 24 and 72 hr postexposure. After the two exposure scenarios, six rats from each of the four groups were studied: *a*) VEH-treated/SAL IT or air INH, *b*) VEH-treated/ROFA IT or ROFA INH, *c*) MCT-treated/SAL IT or air INH, *d*) MCT-treated/ROFA IT or ROFA INH. After a 20-min acclimation period, V_{O_2} , breathing frequency, V_T , and V_E were measured for 40 min and averaged. Two milliliters of pure CO was then injected into the exposure chamber, and the CO concentration (parts per million) was measured continuously using a nondispersive single-beam infrared technique, with alternate modulation of the sample and reference cells (model 8501-5CA; Bendix Corp., Lewisburg, WV) and recorded on a strip chart (model BD 111; Kipp and Zonen, Delft, Holland). The CO analyzer was calibrated by a static dilution technique (Nelson 1972) of a certified gas standard (1,200 ppm CO in nitrogen; National Specialty Gases, Durham, NC). The initial concentration of CO within the chamber after injection was 1,100 ppm. A ratio of the CO concentration in the plethysmograph at 10 min after injection (C_{10}) to the concentration of CO in the plethysmograph immediately after injection (C_0), $C_{10}:C_0$ (unitless), was used as an index of the respiratory CO uptake (Yokoyama 1984). The ventilatory equivalent for O_2 was calculated as $V_E:V_{O_2}$ (unitless; Mauderly 1995).

Histopathology. At the completion of the 72 hr postexposure ventilation measurements, rats were anesthetized by an intraperitoneal

injection of sodium pentobarbital (50 mg/kg) and exsanguinated via the abdominal aorta. The lungs were fixed intratracheally with 4% paraformaldehyde in phosphate-buffered SAL (28 mL/kg) and placed in 4% paraformaldehyde. After fixation, the tissues were embedded in paraffin and 4- μ m-thick midsagittal sections were mounted and stained with hematoxylin and eosin (Experimental Pathology Laboratory, Research Triangle Park, NC). Slides were read by a pathologist in a blind and random manner. The slides were evaluated for the degree of *a*) alveolitis, defined as inflammatory changes manifested principally within the intra-alveolar space and alveolar zones not contiguous with alveolar duct regions; *b*) bronchiolar hyperplasia, defined as increased size and numbers of epithelial cells within the alveolar duct and terminal bronchiolar structures; *c*) bronchiolocentric inflammation, defined as thickening of airway wall with inflammatory cells and inflammatory changes in the centriacinar zone; *d*) angiocentric inflammation, defined as thickening of vascular structures and perivascular tissue with inflammatory cells; *e*) alveolar hyperplasia, defined as increased numbers of alveolar type II cells lining alveolar septal regions; and *f*) alveolar septal thickening, defined as increased numbers of inflammatory cells and expansion of interstitial tissue within alveolar septal walls. The following code was used for scoring: 0, no lesions present; 1, minimal lesions present; 2, mild lesions present; 3, moderate lesions present; 4, severe lesions present.

Statistical analysis. Results are expressed as means \pm SE. The data were analyzed using

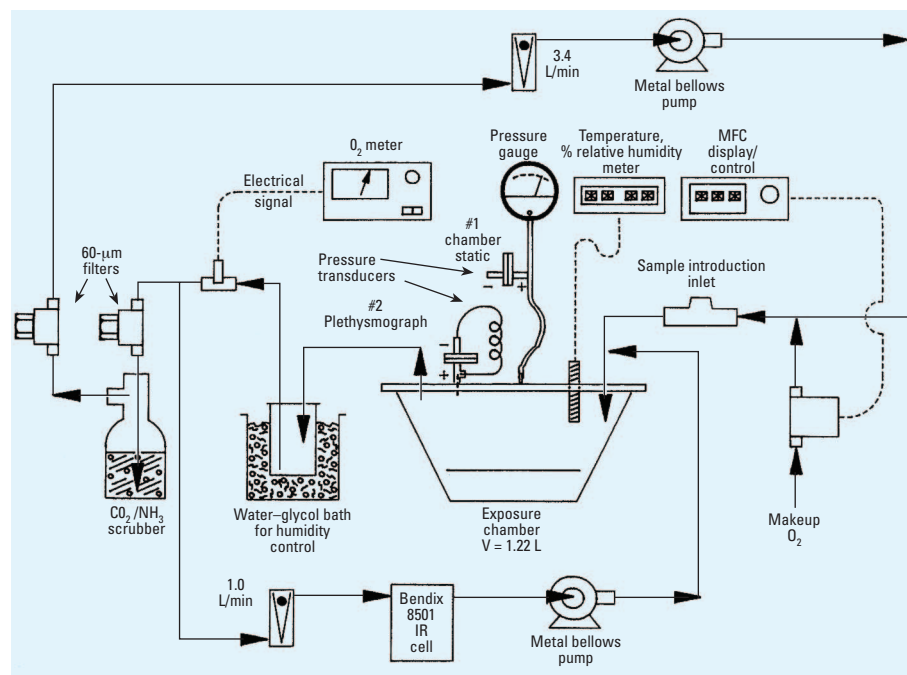
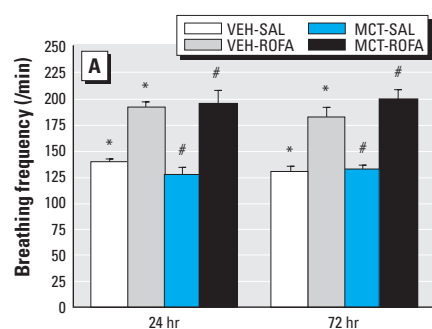


Figure 1. Schematic of simultaneous gas uptake chamber/whole body plethysmograph. Modified from McGee et al. 1995.

a general linear model. A multivariate analysis of variance was performed simultaneously evaluating the effect of MCT and ROFA on the response variables. For significant findings, a univariate analysis of variance was performed on each of the dependent variables. Pairwise comparisons were performed as subsets of the overall analysis. A Bonferroni correction was applied to the multiple pairwise comparisons. The level of significance was set at 0.05.

Results

IT exposure. Two of the six MCT-treated rats that were instilled with ROFA intratracheally died between the 24 hr and 72 hr post-IT ventilatory measurements. Breathing frequency increased by approximately one-half at 24 hr and remained elevated by the same amount at 72 hr after IT of ROFA in both VEH- and MCT-treated rats (Figure 2A). V_T decreased slightly 24 hr after IT of ROFA in MCT-treated rats (Figure 2B) but otherwise showed little change. V_E increased 24 and 72 hr after IT of ROFA in both VEH- and MCT-treated rats (Figure 2C), largely due to the increases in breathing frequency. The impact of ROFA on V_E at either time point for the healthy (VEH-treated) and MCT-treated rats was similar.



Impairment in the lung's overall ability to transfer gas into the blood was indicated by a significant and similar increase in $C_{10}:C_0$ of approximately 10–15% by both MCT treatment and IT of ROFA at 24 hr post-IT (Figure 3A). At 72 hr post-IT, $C_{10}:C_0$ in the VEH-treated/ROFA-instilled rats had begun to subside toward control and was no longer significantly different. At 72 hr post-IT, $C_{10}:C_0$ was similarly increased by MCT treatment in the SAL-instilled rats as at 24 hr. However, in the MCT-treated/ROFA-instilled rats at 72 hr, $C_{10}:C_0$ was increased more than additively, but to a similar degree as at 24 hr (Figure 3A). This pattern of gas transfer impairment was reflected in V_{O_2} , which was decreased by MCT treatment (~10%) alone but decreased further (> 20%) with the addition of IT of ROFA at 24 hr post-IT (Figure 3B). At 72 hr post-IT, V_{O_2} remained decreased by ROFA in MCT-treated rats only (Figure 3B); effects were similar at 24 and 72 hr. The ventilatory equivalent for O_2 increased substantially 24 and 72 hr after IT of ROFA, synergistically so in MCT-treated rats (Figure 4).

MCT treatment resulted in significant alveolitis, angiocentric inflammation, and alveolar septal thickening (Table 1, Figure 5). IT of ROFA induced alveolitis, bronchiolar

hyperplasia, bronchiolocentric inflammation, angiocentric inflammation, alveolar hyperplasia, and alveolar septal thickening in both VEH- and MCT-treated rats (Table 1, Figure 5).

INH exposure. INH of ROFA produced several changes in breathing parameters at 24 hr after exposure. Breathing frequency increased by approximately 50% at 24 hr in both VEH- and MCT-treated rats (Figure 6A). MCT alone depressed V_T slightly but not significantly at 24 hr; however, this difference reached significance at the 72 hr time point (Figure 6B) but in general showed little change. V_E increased 24 hr after INH of ROFA in both VEH- and MCT-treated rats (Figure 6C), consistent with the rise in breathing frequency and small change in V_T . The frequency, V_T , and V_E results were very similar for both the IT and INH studies. The index of respiratory CO uptake ($C_{10}:C_0$) increased in MCT-treated rats exposed to air 24 and 72 hr after air exposure (Figure 7A); however, the magnitudes of $C_{10}:C_0$ increases for the INH study were only half those of the IT study. V_{O_2} decreased in MCT-treated rats exposed to air compared with VEH-treated rats exposed to air 72 hr after air exposure (Figure 7B). The V_{O_2} effects for MCT-treated rats were much smaller for the INH study relative to the

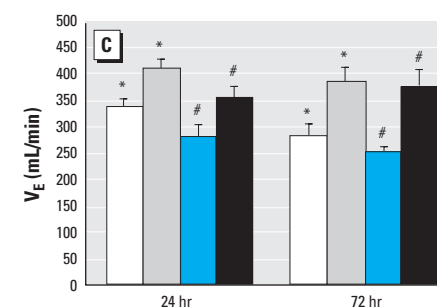
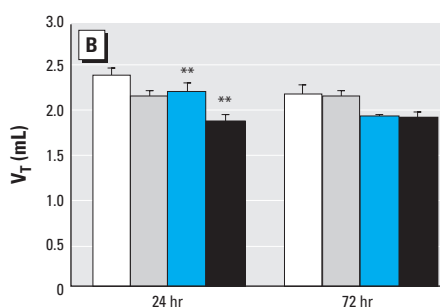


Figure 2. (A) Breathing frequency (f) increased after IT of ROFA. Results are expressed as mean frequency \pm SE. Groups that are significantly different are denoted by the same symbol (i.e., *, #). (B) V_T decreased 24 hr after IT of ROFA in MCT-treated rats. Results are expressed as mean $V_T \pm$ SE. Groups that are significantly different are denoted by the same symbol (i.e., **). (C) V_E increased after IT of ROFA. Results are expressed as mean $V_E \pm$ SE. Groups that are significantly different are denoted by the same symbol (i.e., *, #).

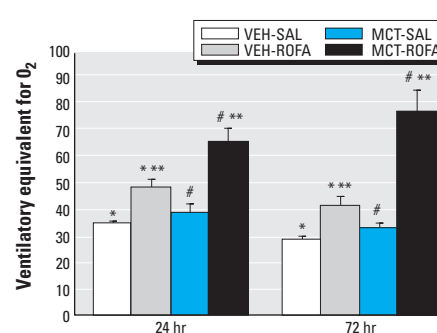
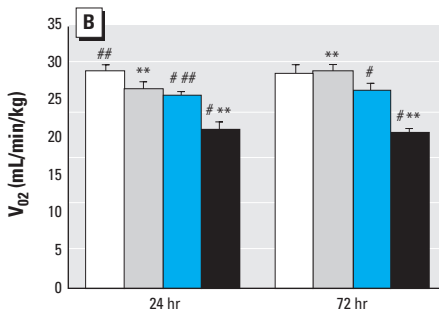
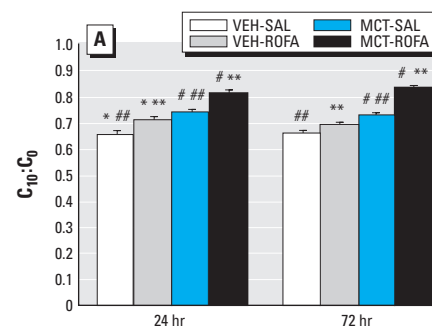


Figure 3. (A) $C_{10}:C_0$ increased by MCT treatment and after IT of ROFA in MCT-treated rats only. Results are expressed as mean $C_{10}:C_0 \pm$ SE. Groups that are significantly different are denoted by the same symbol (i.e., *, **, #, ###). (B) V_{O_2} decreased by MCT treatment and after IT of ROFA in MCT-treated rats only at 24 hr. At 72 hr V_{O_2} decreased after IT of ROFA in MCT-treated rats only. Results are expressed as mean $V_{O_2} \pm$ SE. Groups that are significantly different are denoted by the same symbol (i.e., *, **, #, ###).

Figure 4. The ventilatory equivalent for O_2 ($V_E:V_{O_2}$) increased after IT of ROFA. Results are expressed as mean ventilatory equivalent for $O_2 \pm$ SE. Groups that are significantly different are denoted by the same symbol (i.e., *, **, #).

IT study. The ventilatory equivalent for O₂ increased substantially 24 hr after INH of ROFA in both VEH- and MCT-treated rats but was much less at 72 hr (Figure 8).

MCT treatment produced significant alveolitis and angiocentric inflammation (Table 2,

Figure 9). INH exposure of ROFA induced alveolitis, bronchiolar hyperplasia, bronchiolo-centric inflammation, and alveolar septal thickening in both VEH- and MCT-treated rats (Table 2, Figure 9). The combination of MCT treatment and ROFA exposure appeared to

have a greater than additive effect on bronchiolar and alveolar hyperplasia (Table 2, Figure 9).

Discussion

The present study was conducted to compare ventilatory function and histology in a rat model of MCT-induced pulmonary vasculitis and hypertension after exposure to the model emission particle, ROFA, by IT or nose-only INH. ROFA is emitted from oil-fired power plants, and although it represents a very small fraction of ambient PM, its metal sulfate composition has been associated with the toxic effects of various model and ambient PM administered to experimental animals in relatively high doses (Costa and Dreher 1997; Dreher et al. 1997). The model has proved useful in the study of PM-associated health effects and mechanisms. Although the issue of dose imparts uncertainty in the interpretation of the effects seen in experimental animals, the coherence in the effects seen in animals with model and ambient PM lends credence to its use to explore mechanistic questions surrounding rare effects seen in the exposed human population (Costa and Dreher 1997; Dye et al. 2001). The inflammatory responses to the IT dose of ROFA used in this study have been well characterized in healthy and compromised animal models and have been compared with INH of comparable doses of the metal sulfates themselves (Campen et al. 2002). The estimated cumulative dose of ROFA resulting from the INH exposure based on 15% deposition was 3.03 mg/kg. This INH dose also compares with other INH exposures that have shown altered cardiac function in cardiac-compromised rats (Kodavanti et al. 2000). Although no mortality has been observed in our laboratory with these high INH exposures, mortality has been reported with a similar ROFA exposure in MCT rats at only approximately 500 µg/m³ (Killingsworth et al. 1997). Thus, we tested the hypothesis of altered ventilation and gas exchange (represented by C₁₀:C₀, a steady-state diffusion measure), using

Table 1. Mean scores of pathologic lesions.^a

Lesion	VEH-SAL	VEH-ROFA	MCT-SAL	MCT-ROFA
Alveolitis	0.5 ^{*,**}	2.8 [*]	1.4 ^{**,##}	3.5 ^{##}
Bronchiolar hyperplasia	0 [*]	2.3 [*]	0 ^{##}	3.2 ^{##}
Bronchiolocentric inflammation	0 [*]	2.2 [*]	0.4 ^{##}	2.5 ^{##}
Angiocentric inflammation	0 ^{*,**}	1.3 ^{*,#}	2.0 ^{**,##}	2.8 ^{##}
Alveolar hyperplasia	0 [*]	3.2 ^{*,#}	0.6 ^{##}	1.8 ^{##}
Alveolar septal thickening	0 ^{*,**}	3.0 [*]	1.0 ^{**,##}	2.5 ^{##}

^aLung sections were taken from rats (*n* = 4 for MCT-ROFA; *n* = 6 for each of the other three groups) 13 days after injection of VEH or MCT and 72 hr after IT of SAL or ROFA. Slides were read by a pathologist in a blind and random manner and the following code was used for scoring: 0, not present, 1, minimal, 2, mild, 3, moderate, 4, severe. Results are expressed as mean scores. ^{*}, ^{**}, ^{##} Groups that are significantly different are denoted by the same symbol.

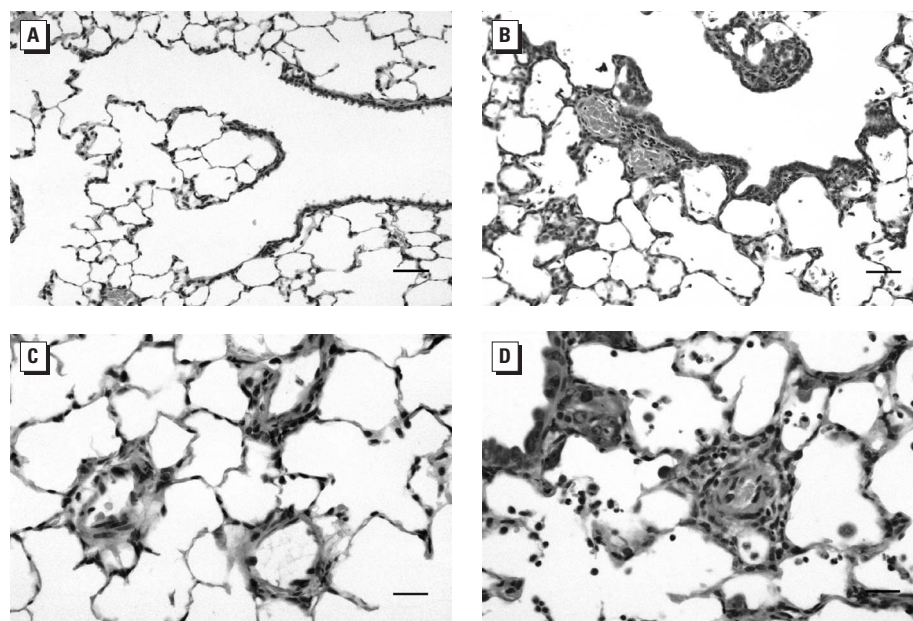


Figure 5. Light micrographs of lung tissue from rats 13 days after injection of VEH or MCT and 72 hr after IT of SAL or ROFA. (A) VEH-SAL. Scale bar, 70 µm. (B) VEH-ROFA. Scale bar, 55 µm. (C) MCT-SAL. Scale bar, 35 µm. (D) MCT-ROFA. Scale bar, 35 µm. (B) is an example of bronchiolocentric inflammation, whereas (C) is an example of angiocentric inflammation. The tissue in photomicrograph (D) demonstrates angiocentric inflammation, alveolar hyperplasia, and alveolitis.

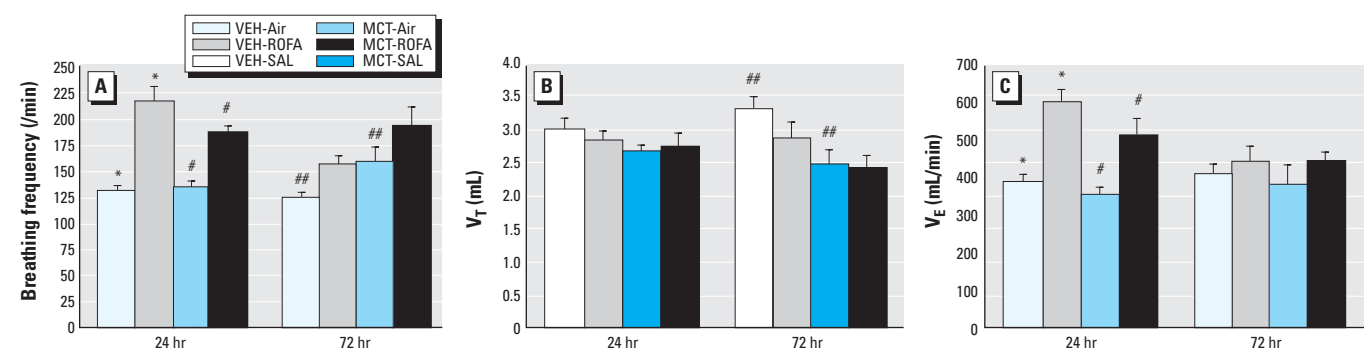


Figure 6. (A) Breathing frequency (*f*) increased after INH of ROFA at 24 hr. At 72 hr, frequency was increased in MCT-air-treated rats compared with those treated with VEH-air. Results are expressed as mean frequency ± SE. Groups that are significantly different are denoted by the same symbol (i.e., *, #, ##). (B) *V_T* decreased in MCT-air-treated rats compared with those treated with VEH-air at 72 hr. Results are expressed as mean *V_T* ± SE. Groups that are significantly different are denoted by the same symbol (i.e., ##). (C) *V_E* increased after INH of ROFA at 24 hr. Results are expressed as mean *V_E* ± SE. Groups that are significantly different are denoted by the same symbol (i.e., *, #).

what in our hands would be a worst-case scenario, to assess the likely involvement of altered physiology in the systemic outcomes observed.

IT ROFA exposure of MCT-treated rats has been shown to produce apparent hypoxia-associated cardiac dysfunction (Watkinson et al. 1998), leading to arrhythmias and death (~50%). Concurrent inflammation and edematous injury are observed under these study conditions. Analogous findings regarding mortality and histology reported by Killingsworth et al. (1997) at much lower exposure concentrations appear at first at odds with the doses used by Watkinson and coworkers but may represent differences in degree of disease. However, mortality was observed by Watkinson and colleagues at doses as low as 250 μg . The IT exposure was conducted initially as a definitive test of the feasibility of the impaired diffusion hypothesis and the merit of further analysis in the INH study. The INH exposure, although clearly high based on ambient levels, more closely represents occupational exposures to boilermakers where airway and lung dysfunction have been reported (Hauser et al. 1995).

The MCT model of pulmonary hypertension presents with features of acute respiratory inflammatory disease with altered respiratory function and circulatory effects (Meyrick et al. 1980). Many of the MCT-induced changes in rats are similar to those seen in primary and certain other types of chronic pulmonary hypertension in humans, such as occurs with chronic bronchitis and emphysema, cystic fibrosis, and, when advanced, acute respiratory distress syndrome (Meyrick and Reid 1979). This model was induced to represent a susceptible population with systemic cardiopulmonary stress and impairment to address a potential mechanistic explanation for the epidemiologic associations reported between exposure to ambient PM and hospital admissions (Burnett et al. 1995; Wichmann et al. 1989) and mortality (Schwartz 1994; Schwartz and Dockery 1992; Wordley et al.

1997) in humans suffering from preexisting cardiopulmonary disease.

As predicted from previous studies, two of the six rats treated with MCT died between 24 and 72 hr after IT of ROFA (Kodavanti et al. 1999; Watkinson et al. 1998). ROFA itself imparted some degree of ventilatory dysfunction in healthy controls, but these effects were most notable in rats pretreated with MCT and therefore cardiopulmonaryly compromised. Breathing frequency was dramatically increased after IT of ROFA in both control and MCT-treated rats, but V_T decreased significantly only in MCT-treated rats 24 hr postexposure. The alterations in V_E (breathing frequency $\times V_T$) paralleled those of breathing frequency. The etiology of these alterations is uncertain and could be secondary to the inflammatory injury or perhaps related to direct and/or indirect (via the degree of lung injury) stimulation of irritant C-fibers in the tracheobronchial tree and/or vagal non-myelinated fibers in the pulmonary acinus (Costa and Schelegle 1999).

The $C_{10}:C_0$ ratio (index of the respiratory CO uptake), inversely related to CO-diffusing capacity, a measure of the lung's overall ability to transfer gas into the blood, was increased by MCT treatment, indicating impaired gas transport. An analogous decrease in CO-diffusing capacity has been previously reported in MCT-treated rats (Lai et al. 1991). Twenty-four hours after ROFA exposure, $C_{10}:C_0$ increased in both control and MCT-treated rats. However, at 72 hr postexposure, a significant and more than additive increase persisted in the MCT-treated but not control rats. V_{O_2} was likewise decreased by MCT treatment and decreased further in a synergistic manner after ROFA exposure. The ventilatory equivalent for O_2 ($V_E:V_{O_2}$) was only slightly (not significantly) increased by MCT treatment. ROFA IT alone significantly increased this parameter of ventilatory insufficiency but, when overlaid upon the MCT pretreatment and associated lung disease, resulted in a substantial and apparent

synergistic increase. This ratio of volume of air breathed per unit of O_2 consumed is a somewhat crude but useful index of the overall efficiency of ventilation indicating a reduced uptake of O_2 per volume of air breathed (Mauderly 1995). The alterations in $C_{10}:C_0$, V_{O_2} , and the ventilatory equivalent for O_2 indicate that ROFA instillation hinders the lung's overall ability to transfer gas into the blood, and MCT pretreatment further impaired its ability.

It is difficult to relate these changes directly to cardiac hypoxia, because blood gas measures were not taken and likely would not have been very elucidating because of probable sympathetic stress interplay. However, as with patients with chronic obstructive pulmonary disease who have seemingly mild or moderate diffusion impairments, there may be significant ventilation/perfusion mismatching and episodic hypoxia with other cardiopulmonary stressors or demands. Thus these changes may be responsible, at least in part, for some of the arrhythmias and electrocardiographic changes that were noted after ROFA exposure in rats similarly studied in our laboratory (Watkinson et al. 1998). Electrocardiographic abnormalities in these studies included bradycardia, depressed S-T segment area, and high peaked T-waves, changes associated with hypoxia. Arrhythmias occurred in both control and MCT-treated rats after ROFA exposure; however, the frequency and severity were greatly exacerbated by MCT treatment, with half of the MCT-treated rats progressing to death.

Histologically in our IT study, ROFA treatment alone induced minimal to mild angiocentric inflammation, mild to moderate bronchiolocentric inflammation and bronchiolar and alveolar hyperplasia, alveolitis, and alveolar septal thickening in VEH-treated rats. The MCT treatment alone induced minimal to mild angiocentric inflammation, alveolitis, and alveolar septal thickening. In MCT-treated rats, ROFA induced minimal to mild alveolar hyperplasia, mild to moderate

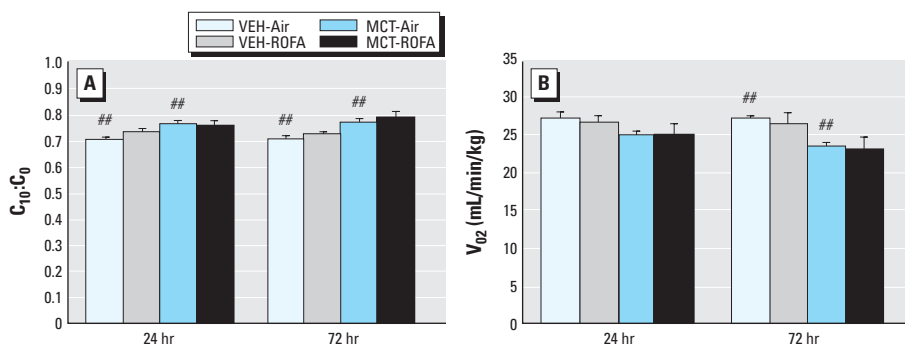


Figure 7. (A) $C_{10}:C_0$ increased in MCT-air-treated rats compared with those treated with VEH-air at 24 and 72 hr. Results are expressed as mean $C_{10}:C_0 \pm SE$. Groups that are significantly different are denoted by the same symbol (i.e., ##). (B) V_{O_2} decreased in MCT-air-treated rats compared with those treated with VEH-air at 72 hr. Results are expressed as mean $V_{O_2} \pm SE$. Groups that are significantly different are denoted by the same symbol (i.e., ##).

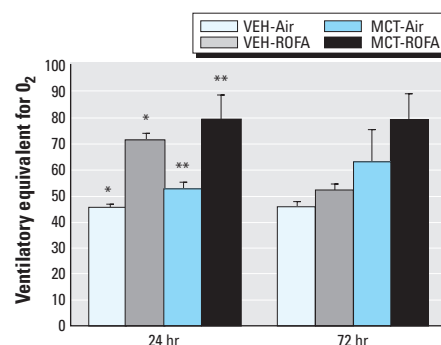


Figure 8. The ventilatory equivalent for O_2 ($V_E:V_{O_2}$) increased after INH of ROFA at 24 hr. Results are expressed as mean ventilatory equivalent for $O_2 \pm SE$. Groups that are significantly different are denoted by the same symbol (i.e., *, **).

bronchiolocentric inflammation, angiocentric inflammation and alveolar septal thickening, and moderate to severe alveolitis and bronchiolar hyperplasia. These histologic changes may also have contributed to the abnormalities in gas exchange.

Regarding the comparison of the modes of ROFA exposure, ROFA INH affected many of the same ventilatory parameters observed after the IT route, but the changes generally were less dramatic, most likely an effect of dose. In contrast to IT, however, there was no clear augmentation of effects associated with MCT pretreatment. Breathing frequency was dramatically increased 24 hr after ROFA INH in both control and MCT-treated rats, surprisingly similar to those observed in the IT study, but there were no significant changes in V_T after INH. Thus, changes in V_E paralleled closely those of breathing frequency. As in the IT exposure, pretreatment with MCT resulted in impaired gas exchange as indicated by a baseline increase in $C_{10}:C_0$ and a decrease in V_{O_2} ; however, there were no additional changes associated with ROFA INH exposure. The ventilatory equivalent for O_2 increased significantly 24 hr

after ROFA exposure in both control and MCT-treated rats, to a similar extent to that observed in the IT study. Histologically, ROFA alone induced minimal to mild bronchiolar hyperplasia and bronchiolocentric inflammation, alveolitis, and alveolar septal thickening, whereas MCT pretreatment alone induced minimal to mild angiocentric inflammation and alveolitis. The combination of MCT treatment and ROFA exposure, however, resulted in apparent synergistic severity of bronchiolar and alveolar hyperplasia. The nature of the histologic changes after INH of ROFA were similar to those observed after IT of ROFA, but the degree of severity was less with the exception of alveolar hyperplasia, which was more severe in MCT-treated rats.

In this study, ROFA induced histologic changes and abnormalities in several ventilatory parameters, many of which were enhanced by MCT treatment. Although the overall results of the INH study were not quite as marked as those observed in the IT study based on dose, the 24-hr results were comparable for breathing frequency, V_E , and the ventilatory equivalent for O_2 , and in MCT-treated rats for the histologic

change of alveolar hyperplasia, demonstrating that the INH route of a smaller dose (2.7 times less than the IT exposure) can cause significant effects. The observed ventilatory abnormalities indicative of impaired gas transfer capacity of the lung may be responsible, at least in part, for the apparent hypoxia-associated electrocardiographic changes and arrhythmias previously reported in our laboratory after ROFA exposure in normal and MCT-pretreated rats. These ventilatory and gas exchange abnormalities represent one potential mechanism involved in the cardiac outcomes revealed by epidemiologic associations linking PM with morbidity and mortality from cardiopulmonary disease.

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Table 2. Mean scores of pathologic lesions.^a

Lesion	VEH-Air	VEH-ROFA	MCT-Air	MCT-ROFA
Alveolitis	0.0*.*	1.8*	1.2*.*#	2.2##
Bronchiolar hyperplasia	0.0*	1.8*.*	0.2##	2.5*.*##
Bronchiolocentric inflammation	0.0*	1.8*	0.5##	2.2##
Angiocentric inflammation	0.0*.*	0.8*.*	2.0**	2.8*
Alveolar hyperplasia	0.0	0.7#	0.5##	2.3*.*##
Alveolar septal thickening	0.0*	1.0*	0.3##	2.0##

^aLung sections were taken from rats ($n = 6$ for each of the four groups) 15 days after injection of VEH or MCT and 72 hr after INH of air or ROFA. Slides were read by a pathologist in a blind and random manner, and the following code was used for scoring: 0, not present, 1, minimal, 2, mild, 3, moderate, 4, severe. Results are expressed as mean scores. *.*, *.*, *.*# Groups that are significantly different are denoted by the same symbol.

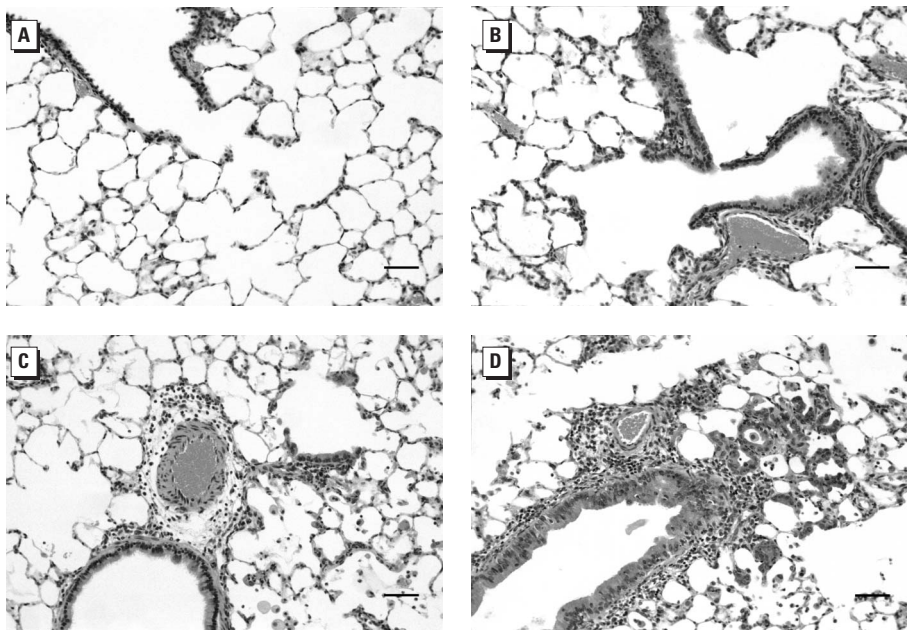


Figure 9. Light micrographs of lung tissue from rats 15 days after injection of VEH or MCT and 72 hr after INH of air or ROFA. (A) VEH-air. (B) VEH-ROFA. (C) MCT-air. (D) MCT-ROFA. Scale bars, 70 μ m.

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