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Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study

Terry Gordon, Christine Nadziejko, Lung Chi Chen, and Richard Schlesinger

Includes a Commentary by the Institute's Health Review Committee

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HEI STATEMENT
Synopsis of Research Report 93

Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study

INTRODUCTION

Particulate matter (PM) is the term used to define the complex mixture of small particles in the atmosphere. The size, chemical components (such as metal, organic, and salt content), and other physical and biological properties of these particles are highly variable from place to place and from season to season. Particle properties are also influenced by their source (which may be either natural processes or human activities such as driving vehicles) as well as by changes the particles undergo in the atmosphere. Epidemiologic studies have indicated that exposure to PM is associated with increases in morbidity and mortality, particularly in individuals with compromised cardiopulmonary function. A plausible mechanism linking particles and pathophysiologic effects has not been established, however. Thus, toxicologic studies in appropriate animal models and in humans are critical in trying to establish how PM may exert its adverse health effects.

The Health Effects Institute funded the study described in this Research Report as part of a program intended to identify critically needed information.

APPROACH

Dr Terry Gordon and colleagues at the New York University School of Medicine conducted an exploratory study to test the effects of exposure to PM derived from New York City air on the rodent cardiopulmonary system. They hypothesized that PM would have greater, possibly fatal, effects in animals with compromised cardiopulmonary function than in normal animals. To maximize possible effects, they concentrated particles up to 10 times their level in ambient air, using for the first time in an exposure study an instrument known as the *Gerber concentrator*, which concentrates particles of 0.2 to 2.5 µm in diameter. Gordon and colleagues exposed animals for up to 6 hours to these particles in concentrations that ranged from approximately 150 to 900 µg/m^3. They exposed normal rats and hamsters, rats injected with monocrotaline to induce right-heart hypertrophy and pulmonary hypertension, and hamsters with a genetic cardiomyopathy. The investigators evaluated changes in heart rate and electrocardiogram intervals, mechanical pulmonary function, and inflammatory parameters. The majority of experiments involved normal and monocrotaline-injected young rats.

RESULTS AND INTERPRETATION

The investigators found little or no effect of concentrated ambient PM exposure on cardiac, mechanical pulmonary, or inflammatory measures in the rats and hamsters they studied. One of the few significant effects of concentrated PM was a small increase in heart rate of young rats in the 6 hours after exposure, but this increase was not found on all exposure days. Similar small effects were occasionally noted in monocrotaline-injected animals. Thus, the results indicated that the compromised rats and hamsters appeared no more sensitive to PM effects than age-matched control animals.

Previous studies have demonstrated that some types of PM can induce cardiac effects that may be fatal in monocrotaline-injected rats, but the effects reported in the current study were not life threatening. The current study used concentrated ambient particles, which might be expected to increase the likelihood of observing effects, but the study also used small numbers of animals (6 maximum) and small numbers of experiments, factors expected to reduce the likelihood of observing effects of exposure. Differences between the chemical composition and dose of PM components (such as metals) in other studies also may explain the discrepant findings.

A recent study reported cardiac effects of concentrated ambient PM from Boston air in dogs, and particularly in dogs with induced coronary occlusion. In addition to the different species and models of cardiac conditions, differences in the levels of various PM

This Statement, prepared by the Health Effects Institute, is a summary of a research project sponsored by HEI from 1995 to 1999. This study was conducted by Dr Terry Gordon and colleagues of the New York University Medical Center, Tuxedo, New York. The following Research Report contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

components at distinct geographic locations may explain the differences in results. Comparing the particles used in this study with those used in Boston is difficult because Gordon and colleagues determined particle mass and sulfate but did not fully characterize the particles to which the rodents were exposed.

No firm conclusions can be reached from this study about the sensitivity of these rodent models to concentrated PM, and the appropriateness of using these models—right-heart failure and pulmonary hypertension in particular—to represent a high-risk subset in the human population is uncertain.

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HEALTH EFFECTS INSTITUTE

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Research Report 93

Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study

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STATEMENT Health Effects Institute

This Statement is a nontechnical summary of the Investigators' Report and the Health Review Committee's Commentary.

PREFACE

INVESTIGATORS' REPORT

When an HEI-funded study is completed, the investigators submit a final report. This Investigators' Report is first examined by three outside technical reviewers and a biostatistician. The report and the reviewers' comments are then evaluated by members of the HEI Health Review Committee, who had no role in selecting or managing the project. During the review process, the investigators have an opportunity to exchange comments with the Review Committee and, if necessary, make revisions.

COMMENTARY Health Review Committee

The Commentary about the Investigators' Report is prepared by the HEI Health Review Committee and staff. Its purpose is to place the study into a broader scientific context, to point out its strengths and limitations, and to discuss remaining uncertainties and implications of the findings for public health.

PREFACE

In 1994, HEI initiated a research program to investigate the complex issues associated with the health effects of exposure to particulate matter (PM)* in the air. This program was developed in response to growing concern about the potential public health significance of reported associations between daily fluctuations in levels of PM and changes in daily morbidity and mortality in time-series epidemiology studies. These results were questioned for a variety of reasons, including the lack of support from experimental studies and the lack of a mechanism to explain how such effects would occur. To address these issues HEI undertook two research initiatives in 1994: (1) the Particle Epidemiology Evaluation Project (Samet et al 1995, 1997), which evaluated six of the time-series epidemiology studies that had reported effects of PM on mortality; and (2) a program of toxicologic and epidemiologic studies (funded from RFA 94-2, *Particulate Air Pollution and Daily Mortality: Identification of Populations at Risk and Underlying Mechanisms*), which aimed to understand better how PM might cause toxicity and what factors might affect susceptibility. In all, HEI has issued five requests for research on PM and funded 34 studies or reanalyses over the last five years.

This Preface provides general regulatory and scientific background information relevant to studies funded from RFA 94-2, including the study by Terry Gordon which is described in the accompanying Report and Commentary. All of the studies from RFA 94-2 have been completed and are either under review by HEI or have been published. The *HEI Program Summary: Research on Particulate Matter* (Health Effects Institute 1999) provides information on studies funded since 1996.

BACKGROUND

Particulate matter (PM) is the term used to define a complex mixture of anthropogenic and naturally occurring airborne particles. The size, chemical composition, and other physical and biological properties of PM depend on the sources of the particles and the changes the particles undergo in the atmosphere. In urban environments, these particles derive mainly from combustion, including mobile sources such as motor vehicles and stationary sources such as power plants. The most commonly used descriptor of particle size is *aerodynamic diameter*. Based on this parameter, ambient particles tend to fall into three size classes (often defined as modes): ultrafine or nuclei mode (particles less than 0.1 μm in diameter); fine or accumulation mode (particles between 0.1 and 2.5 μm in diameter), and coarse (particles larger than 2.5 μm in diameter). Fine and ultrafine particles are dominated by emissions from combustion processes while coarse particles are mostly generated by mechanical processes from a variety of noncombustion sources. Generally, the ultrafine and fine fractions are composed of carbonaceous material, metals, sulfate, nitrate and ammonium ions. The coarse fraction is composed mostly of mechanically generated particles and consists of insoluble minerals and biologic aerosols, with smaller contributions from primary and secondary aerosols and sea salts (US Environmental Protection Agency [EPA] 1996).

A number of early epidemiologic studies indicated that human exposure to high concentrations of PM, such as London fog, had deleterious effects (such as increased number of deaths), particularly in children, the elderly, and those with cardiopulmonary conditions (Firket 1931; Ciocco and Thompson 1961; Logan 1953; Gore and Shaddick 1968). Because of this apparent relation to increased mortality, the EPA has regulated the levels of ambient PM since 1971, when the Clean Air Act was first promulgated. This act authorized the EPA to set National Ambient Air Quality Standards (NAAQSs) for a number of potentially harmful air pollutants (including PM) in order to protect the health of the population, particularly those thought to be sensitive.

The first NAAQS for PM was based on controlling total suspended PM or particles up to 40 μm in diameter. In 1978, the standard was revised to regulate inhalable particles, or particles than can deposit in the respiratory tract and therefore have greater potential for causing adverse health effects. These are particles with an aerodynamic diameter of 10 μm or less (PM_{10}). More recent epidemiologic studies, published in the early 1990s, indicated a relatively consistent association between small short-term increases in PM levels and increases in both mortality and morbidity from respiratory and cardiovascular diseases (reviewed by the Committee of the Environmental and Occupational Health Assembly, American Thoracic Society [Bascom et al 1996]).

Some studies also suggested that long-term exposure to low levels of PM is associated with adverse effects (Dockery et al 1993; Pope et al 1995). These latter studies also pointed to a possible role of fine particles (less than

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

Table 1. Current NAAQSs for PM (set in 1997)

	PM_{10}	$PM_{2.5}$
Daily Standard	150 μg/m^3	65 μg/m^3
Annual Standard	50 μg/m^3	15 μg/m^3

2.5 μm in aerodynamic diameter [$PM_{2.5}$]). In 1997, the EPA considered the evidence for the effects of fine particles sufficient to promulgate a fine particle standard while retaining the PM_{10} standard (US Environmental Protection Agency 1997) (see Table 1). The next review of the PM NAAQS is scheduled to be completed by the year 2002. Scientific information for that review must be peer reviewed and available by the late spring of 2000.

RESEARCH PROGRAM FROM RFA 94-2

The wealth of epidemiologic data published in the early 1990s suggested an association between PM and health effects, but aspects of these fundings were not well understood. Problems involved uncertainties in the exposure estimates, confounding by weather or other factors, the role of copollutants, and the mechanisms by which particles may cause effects. Moreover, although the epidemiologic findings were consistent across different communities exposed to distinct mixes and levels of pollutants, they were not well supported by either human chamber studies or animal inhalation studies aimed at delineating pathologic changes that might result in death. Failure of the experimental studies to provide support for the epidemiologic findings was attributed to insufficient statistical power, use of particles not representative of ambient particles, or use of animals not representative of the individuals susceptible to increased mortality.

By the mid 1990s, it became apparent that the research to advance our understanding of the association between exposure to particles and daily mortality found in the epidemiologic studies needed to focus on identifying (1) susceptible populations, (2) mechanisms by which particles may lead to increased mortality, and (3) characteristics of the particles responsible for the effects. It was recognized that both epidemiologic and experimental studies would be required.

The HEI program initiated in 1994 was aimed at addressing these research needs. Six epidemiologic and toxicologic studies were funded through RFA 94-2, and three additional studies were added through the preliminary application process. As a group, the five epidemiologic studies investigated: (1) social and medical factors that might increase the risk of mortality when particulate pollution increases (Mark Goldberg of the National Institute of Scientific Research, University of Quebec); (2) components of particulate pollution that might account for its effect on mortality (Morton Lippmann of the New York University School of Medicine and Erich Wichmann of the GSF Insitute of Epidemiology and Ludwig Maximilian University); and (3) cause of death (Harvey Checkoway of the University of Washington and Mark Goldberg) or possible pathophysiologic mechanisms that might lead to death in people exposed to particulate air pollution (Douglas Dockery of Harvard School of Public Health [see Dockery et al 1999]).

The four experimental studies tested the hypothesis that older animals or animals with preexisting lung or heart disease or respiratory infections are more sensitive to the acute effects of particles than healthy animals. They investigated possible mechanisms leading to mortality such as inflammation, changes in immune response, or changes in cardiac and respiratory function. Three of these studies used for the first time concentrated ambient particles (CAPs) (John Godleski of Harvard School of Public Health [see Godleski et al 2000], and Terry Gordon and Judith Zelikoff of New York University School of Medicine). In these CAPs studies, particles in the range of about 0.1 to 2.5 μm are concentrated while those greater than 2.5 μm are removed and those under 0.1 μm remain at the ambient concentration. CAPs exposures represent a significant fraction of ambient PM and provide a reasonable approach to mimicking the exposure to PM in epidemiology studies. The fourth experimental study (Günter Oberdörster of the University of Rochester School of Medicine and Dentistry) focused on evaluating the effects of different ultrafine particles that have been hypothesized to be more toxic than fine particles.

CONTINUING RESEARCH

Many of the key questions identified in the early 1990s are still relevant and much research is ongoing to address them. The research strategies have evolved, however, as results from previous studies have provided insights into which animal models and which endpoints may be the most helpful to evaluate. In addition, advances in exposure assessment and statistical methods have pointed to new approaches for conducting epidemiologic studies. Since RFA 94-2, HEI has funded a number of research projects that build on the new findings and approaches. These studies will be completed over the next two years (2000–2002).

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INVESTIGATORS' REPORT

Effects of Concentrated Ambient Particles in Rats and Hamsters: An Exploratory Study

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ABSTRACT

Considerable controversy surrounds the biological plausibility of adverse effects from exposure to ambient particulate matter (PM)*, chiefly because these adverse effects have been observed at particle mass concentrations below those that have been shown to produce effects in healthy animals and human volunteers in the laboratory. To address this research gap, we examined the potential for concentrated ambient PM to produce pulmonary and cardiovascular changes in compromised rodent models.

Normal healthy and monocrotaline-treated rats received single or multiple exposures to concentrated ambient PM, and their responses were tested using functional, cellular, biochemical, and histological endpoints. Analyses determined that no changes in pulmonary function or structure occurred after exposure to concentrated ambient PM. Cardiac arrhythmias did not increase after PM exposure in normal or monocrotaline-treated rats. Increased atrial conduction time, accompanied by a decrease in the duration of the T wave portion of the electrocardiogram (ECG) waveform, was observed in PM-exposed monocrotaline-treated rats in one experiment. In addition, on several but not all exposure days, small yet statistically significant increases in heart rate and peripheral blood cell differential counts were observed in normal and monocrotaline-treated rats within 6 hours after exposure to concentrated ambient PM. The observed changes in cardiovascular parameters in rats returned to control values by 24 hours after exposure.

In a hamster cardiomyopathy model, no adverse cardiac or pulmonary changes were detected after exposure to concentrated ambient PM. Thus, these studies found that cardiopulmonary effects could be produced in rats, but not in hamsters with cardiomyopathy, exposed to concentrated ambient PM. None of the changes occurred on every exposure day and none appeared to be life threatening. Thus, the cardiac changes may reflect changes in homeostasis that could affect individuals who are critically ill, and these findings do not resolve the biological plausibility of adverse health effects associated with ambient PM in epidemiologic studies.

INTRODUCTION

Both cross-sectional and time-series epidemiologic studies have demonstrated that increases in morbidity and mortality are associated with exposure to ambient PM that is less than or equal to 10 μm in aerodynamic diameter (PM_{10}). A key unanswered question in PM_{10} health research is the biological plausibility of the association between PM_{10} and adverse health effects observed in regions throughout North America and Europe. In addition to the question of which components of airborne PM_{10} are responsible for the adverse effects, little is known regarding the identity of individuals at risk. The plausibility of an association between PM_{10} and increases in morbidity and mortality has been severely questioned because these adverse health effects have been observed at very low PM_{10} concentrations, often below the current National Ambient Air Quality Standard. Schwartz (1994) has suggested that these effects appear to have no concentration threshold. With regard to mortality, a 1% increase has been estimated for each 10-μg/m^3 increase in PM_{10} concentration (Ostro 1993).

* A list of abbreviations and other terms appears at the end of the Investigators' Report.

This Investigators' Report is one part of Health Effects Institute Research Report Number 93, which also includes a Preface, a Commentary by the Health Review Committee, and an HEI Statement about the research project. Correspondence concerning the Investigators' Report may be addressed to Dr Terry Gordon, Nelson Institute of Environmental Medicine, New York University School of Medicine, 57 Old Forge Road, Tuxedo NY 10987.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Award R824835 to the Health Effects Institute, it has not been subjected to the Agency's peer and administrative review and therefore may not necessarily reflect the views of the Agency, and no official endorsement by it should be inferred. The contents of this document also have not been reviewed by private party institutions, including those that support the Health Effects Institute; therefore, it may not reflect the views or policies of these parties, and no endorsement by them should be inferred.

The physical-chemical characteristics of PM that are responsible for the increases in morbidity and mortality generate particular concern. Although these increases occur in many geographical locations, suggesting a general particle effect, it is becoming increasingly clear that individual physical-chemical characteristics underlie the toxic properties of PM. At present, factors being examined include particle number and surface area, acidity, metal content, and oxidative potential. Understanding which physical or chemical properties of PM are responsible for the excess in morbidity and mortality is critical for remediation purposes.

It is unclear what characteristics make specific individuals vulnerable to the ill effects of such small increases in PM_{10} concentration. Logic dictates, and epidemiology suggests, that such small changes in PM_{10} concentration are unlikely to affect healthy individuals and that people with compromised health are the probable victims. In fact, controlled studies using healthy animals have not previously demonstrated effects that are likely to lead to life-threatening consequences. Epidemiologic studies have begun to explore this issue of sensitive subpopulations, and initial findings are consistent with the observations from the 1952 and 1962 London episodes, which found that pollution-associated mortality is age dependent and that the elderly are more susceptible (Schwartz 1994).

We examined in animal models whether the potential sensitivity of individuals with compromised pulmonary and/or cardiovascular health can account for the increased morbidity and mortality associated with ambient PM. Two established animal models of pulmonary and cardiovascular disease were chosen on the basis of their relevance to humans with compromised health. The majority of endpoints that were studied were adapted from techniques used on human subjects so as to maintain clinical relevance.

First, an estimated 14 million individuals in the United States suffer from chronic obstructive pulmonary disease (COPD), and when these patients are over the age of 50, a significant fraction also have secondary pulmonary hypertension. Because individuals with pulmonary hypertension are susceptible to both respiratory and cardiac insults, these individuals also may be especially susceptible to adverse health effects from inhaled PM. Monocrotaline-induced pulmonary hypertension was used in our studies as a model for secondary pulmonary hypertension. This model has been used by numerous investigators to study the pathophysiology of primary and secondary pulmonary hypertension (Reindel et al 1990). Importantly, Costa and Dreher (1997) have recently shown that rats with monocrotaline-induced pulmonary hypertension have an increased mortality rate after tracheal instillation of fine particles.

Second, although some PM-associated mortality may be explained by coexisting respiratory disease, irritating particulate pollutants may have cardiac effects even in the absence of respiratory disease. To establish a link between inhalation of PM and cardiac failure, we used an inbred strain of Syrian hamster that develops progressive cardiac failure and arrhythmias resulting in death by 11 months of age. The key lesion of this congenital myopathy is necrotic injury and scarring in the heart wall that lead to an enlarged chamber and a thin left ventriclar wall followed by low cardiac output, raised preload, and increased lung water. The histologic appearance of the cardiac tissue and the alterations in cardiac function are similar to changes seen in humans with compensated congestive heart failure (Panchal and Trippodo 1993).

SPECIFIC AIMS

The biological plausibility of an association between airborne PM and increases in morbidity and mortality has been questioned because severe adverse health effects have been observed at very low PM concentrations. The studies reported here tested the hypothesis that specific subpopulations with compromised health have an increased vulnerability to PM and sought to identify the physiologic pathways of the vulnerability. The study used two models of compromised health: (1) monocrotaline-induced pulmonary hypertension as a rat model of cor pulmonale and (2) congestive heart disease as a hamster model of cardiomyopathy. The animals were exposed to concentrated ambient PM in New York City (Manhattan). Our exposure system was based on a centrifugal particle concentrator designed by Gerber (1979). Adverse effects of PM on the respiratory and cardiovascular systems were measured by noninvasive telemetric techniques as well as biochemistry, histopathology, and pulmonary function testing. Because time-series analyses in epidemiologic studies suggest that a major portion of the morbidity and mortality associated with PM episodes is acute (ie, having a short or no lag time), particular emphasis was placed on detecting changes in indices relevant to cardiac and pulmonary failure (eg, pulmonary injury and inflammation and cardiac arrhythmias) during single or short-term repeated exposures.

This research tested the following hypothesis: PM can cause life-threatening physiological alterations in subpopulations with preexisting cardiopulmonary disease. Concentrated ambient PM would thus be expected to cause greater adverse effects in a rat model of pulmonary hypertension and a hamster model of congestive heart failure than in healthy animals.

METHODS AND STUDY DESIGN

F344 rats were treated with monocrotaline to produce pulmonary hypertension and right heart hypertrophy as a model of pulmonary hypertension. Bio TO-2 Syrian hamsters with spontaneous cardiomyopathy were used as a model of cardiac failure. Both test animals and their respective controls were exposed to filtered air or concentrated ambient PM (the concentrations were 132 to 919 $\mu g/m^3$) for a single 3-hour exposure or for 3 daily 6-hour exposures and examined for changes in various biological endpoints.

TEST ANIMALS

F344 rats (200 to 250 g, specific pathogen-free, males from Charles River Laboratories, Boston MA) were quarantined for a minimum of 1 week prior to study. The rats were housed in polycarbonate cages with corncob bit bedding within a laminar flow animal isolator equipped with a high-efficiency particulate air (HEPA) filter. Rats were treated with a single injection of monocrotaline (40 mg/kg intraperitoneally; Sigma Chemical, St Louis MO) and held for a minimum of 10 days to induce pulmonary hypertension. Right heart hypertrophy was confirmed at necropsy. Control rats of the same weight were sham-injected with sterile saline.

Hamsters (Bio TO-2, 6 to 8 months of age, male) with cardiomyopathy and weight-matched normal control hamsters (Bio-F1B, male) were purchased from Bio Breeders (Fitchburg MA). Hamsters were housed in polycarbonate cages with corncob bit bedding and quarantined for a minimum of 2 weeks prior to exposure.

All rats and hamsters were provided with food (Purina Rodent Chow, Purina Mills, St Louis MO) and water ad libitum except during exposure. The animals were kept on a 12-hour on/off light cycle.

PULMONARY FUNCTION MEASUREMENTS

Lung volumes and diffusing capacity of carbon monoxide (DLCO) were measured in rats and hamsters at 3 and 24 hours after a single 3-hour exposure to air or concentrated ambient PM. The methods have been described (Takezawa et al 1980). Briefly, animals were anesthetized with a combination of intramuscular ketamine hydrochloride and xylazine (100 mg/kg and 15 mg/kg, respectively), and the trachea was cannulated with a blunt needle. Measurements were made in triplicate in a specific sequence and completed within 2 to 3 hours after anesthesia. Measurements of vital capacity (VC) and inspiratory capacity (IC) were followed by total lung capacity (TLC) and DLCO. Airway pressure was monitored continuously with a differential pressure transducer (MP45, Validyne Engineering, Northridge MA) attached to the tracheal cannula by a three-way T-connector. VC was determined by reducing airway pressure to –10 cm H_2O by gentle suction and then recording the volume used to inflate the lungs to an airway pressure of 25 cm H_2O. To determine IC, the animal was first hyperventilated with a small animal respirator (Harvard Apparatus, Holliston MA) for 10 to 20 seconds at 12 cm H_2O. During the ensuing brief apnea, the lungs were inflated to an airway pressure of 25 cm H_2O. The volume required was recorded as the IC. TLC was calculated on the basis of neon dilution by inflating the lung 5 times with a test gas containing 0.5% neon in a volume equivalent to the measured VC. Functional residual capacity (FRC) and residual volume (RV) were computed by standard formulas (Morris et al 1984).

DLCO measurements were performed following the last lung volume maneuver. First, apnea was again induced by hyperventilation. The lungs were then inflated with a volume of certified test gas (0.5% Ne, 0.4% CO; AIRCO, Leetsdale PA), equivalent to IC. Lung inflation was maintained for 6 seconds, and the concentration of Ne and CO in the end expiratory sample (50% of the withdrawn volume) was analyzed on a gas chromatograph (Carle AGC series 100, Hach Co, Loveland CO) calibrated with the test gas. DLCO and the apparent alveolar volume (VA) were calculated using standard formulas (Morris et al 1984). The average of 3 measurements of VC, IC, TLC, and DLCO was used in data analyses.

LAVAGE FLUID MEASUREMENTS

At 3 or 24 hours after exposure, animals were killed with 175 mg/kg of pentobarbital and exsanguinated by transecting the inferior vena cava. Rats and hamsters were tracheostomized, cannulated, and lavaged twice with 10 and 5 mL, respectively, of sterile, pyrogen-free, phosphate-buffered saline (Gibco-BRL, Gaithersburg MD). Total cell counts in the lavage fluid were done with a hemocytometer. Lavage fluid was centrifuged at 400 × *g* for 10 minutes, and aliquots of the supernatant were analyzed for protein content and lactate dehydrogenase (LDH) activity. Protein was determined on diluted aliquots (stored at –20°C) of lavage fluid with a commercially available microassay kit (BioRad, Berkeley CA). The samples were assayed in duplicate and compared to a standard curve prepared with bovine serum albumin (Sigma). LDH activity was determined in duplicate with a commercially available kit (Sigma) on the same day on which the lavage procedure was performed. LDH activity was expressed as Berger-Broida (BB) units: 1 BB unit is equivalent to 0.48 IU

of activity. Cell differentials (100 cells/slide for each animal) were prepared by cytocentrifugation and stained (Hemacolor, EM Diagnostic Systems, Gibbstown NJ).

PERIPHERAL BLOOD CELL COUNTS

After the animals were killed, blood was collected in heparinized containers to count white blood cells (WBC) (Coulter Counter ZM, Luton Beds, England) and platelets (Coulter counter T540). Blood smears were made on slides using standard procedures and differential WBC counts were performed on 100 cells/slide for each animal.

HISTOLOGY

Rat lungs were fixed with neutral buffered formalin (Fisher Scientific) and embedded in paraffin. Sections (4 μm thick) were stained with hematoxylin and eosin. Slides were examined in random order without knowledge of the treatment group.

TELEMETRY MONITORING

Heart rate, temperature, and ECG intervals were monitored in all animals by telemetry using hardware and software from Data Sciences International (St Paul MN). The ECG/temperature transmitters (model TA11-CTA-F40) were inserted in the peritoneal cavity under aseptic conditions and methohexital sodium (Brevital; 25 mg/kg) anesthesia. To establish a lead II ECG configuration, the negative lead of the transmitter was placed over the right clavicle and the positive lead was placed in the left groin. The animals were allowed to recover for a minimum of 1.5 weeks prior to exposure experiments.

On the day of exposure, the animals were brought to the exposure room and housed individually in cages that were placed on Data Sciences receivers (model JA1020). The telemetry data were transmitted to a computer located in the exposure room. Except where noted otherwise, data were collected by the schedule shown in Table 1.

Each heart rate and temperature measurement was the average of a 10-second recording. Each ECG waveform series was sampled at an acquisition rate of 1,000 Hz for 10 seconds and stored digitally. Up to 12 animals (6 air-exposed and 6 PM exposed) were monitored before and after each exposure, but only 6 of these animals (3 air exposed and 3 PM exposed) could be monitored during exposure due to space constraints caused by the size of the receivers. The hourly mean heart rate and temperature for each animal were calculated prior to statistical analysis. By excluding data greater than 3 SDs from the mean, aberrant heart rate data (caused by electrical noise or other artifacts) were removed prior to averaging, but subsequent statistical testing showed the results to be unaffected by removal of the aberrant heart rate data.

In order to optimize the analyses of heart rate in some experiments, baseline values were collected for 24 hours or more during an acclimation period in the laboratory where subsequent exposures and ECG data were collected. In the analyses, the postexposure heart rate was normalized for circadian rhythm effects by subtracting the baseline heart rate (of the identical time of the preceding day) from the postexposure rate for each animal. These normalized values are presented as change in heart rate (beats per minute [bpm]) at each postexposure time period. The normalized values were then used in statistical comparisons to identify the effect of air or PM on heart rate.

ECG WAVEFORM ANALYSIS

Rats

The stored ECG waveforms were displayed on the computer screen using Data Sciences software and visually inspected. Extensive examination of ECG waveforms obtained from normal and monocrotaline-treated rats before, during, and after exposure to air or PM showed that arrhythmias were extremely rare in normal or monocrotaline-treated F344 rats. For example, only two arrhythmias (one skipped beat and one premature atrial contraction) were visually detected in 12 monocrotaline-treated rats ($n = 6$ for air and $n = 6$ for PM) monitored for 24 hours. The arrhythmia counts in rats were judged to be too low to allow statistical analysis and the rare arrhythmias that were seen were not clinically significant.

This same data set of 12 monocrotaline-treated rats was then analyzed for potential PM-induced changes in ECG waveform intervals. Several characteristic indices of the ECG waveform were measured in each animal (Figure 1). These parameters were calculated from the 10-second waveform recordings collected at 30-minute intervals before exposure and after exposure. The mean of each parameter (intervals, segments, or duration) within each

Table 1. Data Collection Schedule

	Before Exposure (1 hour)	During Exposure (3 hours)	After Exposure (18 hours)
Heart rate	Every 5 min	Every 5 min	Every 5 min
Temperature	Every 5 min	Every 5 min	Every 5 min
ECG waveforms	Every 30 min	Every 5 min	Every 30 min

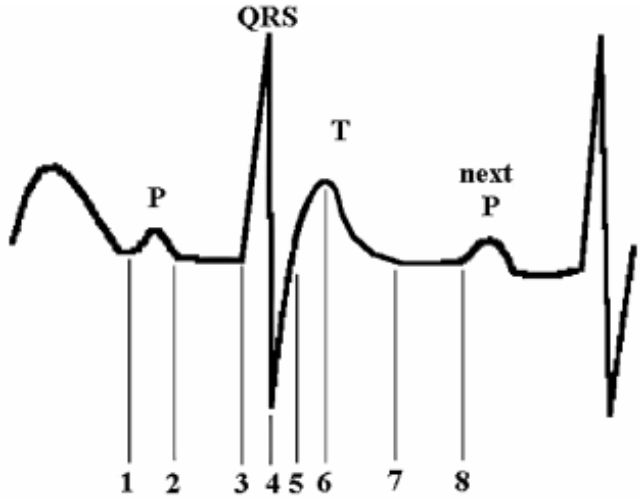

Figure 1. ECG waveform of a rat. Interval measurements and segment lengths are represented by the differences between the 8 numeric marks on the waveform. PP Interval = 8–1; P wave duration = 2–1; PR interval = 3–1; PR segment = 3–2; QRS duration = 4–3; ST segment = 5–4; RaT interval = 6–3; SaT segment = 6–4; RT interval = 7–3; ST interval = 7–4; T wave duration = 7–5; aTP segment = 8–6; PT interval = 7–1.

10-second ensembled waveform was determined using software developed by Dr W Penn Watkinson and Sean Dodd at the US Environmental Protection Agency (Watkinson et al 1985). Although several features of the rat ECG are similar to the human ECG, certain portions do not have true correlates with the human ECG. For example, the rat T wave begins before the QRS complex ends. Despite this difference, the waveform in Figure 1 is labeled in a manner similar to the human ECG and these labels are used in Dr Watkinson's analysis software. This software superimposes all the waveforms in a 10-second recording to create a single ensembled ECG complex. The parameters were then automatically measured on the ensembled ECG complex. For quality control purposes, a pilot study was done to confirm that the measurements made with this method agreed with manual measurements of ECG complexes selected at random within a 10-second recording. In addition, the ensembled waveform for each recording period was printed out, verified, and corrected (if necessary) by a veterinarian with expertise in rodent ECGs (Dr Keiichiro Sato, Takeda Chemical Corporation, Osaka, Japan).

Hamsters

As has been reported previously (Desjardins et al 1996; Hano et al 1991), both normal hamsters and hamsters with cardiomyopathy were found to have frequent sinus arrhythmia in the form of skipped beats and brief abrupt intervals of bradycardia. Because these changes make quantitative analysis of the ECG waveforms impractical, each of the 10-second ECG recordings was scored for arrhythmias using the following scheme:

1. Is the tracing adequate for analysis?

   No: Too much noise or low signal/noise ratio. Burst was not analyzed.

   Yes: Continue as follows.
2. Are there any sinus arrhythmias in the 10-second recording?

   Skipped beat: RR interval is twice or more than the adjacent, usually previous, beat.

   Rhythm change: RR interval is twice or more than the adjacent, usually previous, beat, and the beats with an extended RR interval continue for 2 beats or more.
3. Are there any abnormalities in P waves or QRS complex?

   Waveform abnormality in P waves: notching or splitting.

   Waveform abnormality in QRS complex: notching or splitting.
4. Are there any premature contractions?

   Premature atrial contraction (PAC).

   Premature ventricular contraction (PVC).
5. Is the 10-second interval free of all of the above abnormalities?

   Normal.

The number of bursts scored as containing arrhythmia was divided by the total number of bursts scored.

OPERATION OF CENTRIFUGAL CONCENTRATOR

The centrifugal aerosol concentrator (a gift of the US Naval Research Laboratory, Bethesda MD) was originally designed to test the sensitivity of a nephelometer (Gerber 1979). Like the virtual impactor particle concentrator developed by Sioutas and colleagues (1995), the centrifugal concentrator uses inertia of the particles to separate particles from air and direct the concentrated particle stream to an exposure chamber. A high-volume blower was used to deliver ambient air to the inlet manifold of the centrifugal concentrator and the entrained particles traveled along a concentric annulus formed by a stationary solid outer cylinder and a porous inner cylinder rotating at 10,000 rpm (Figure 2). Suction applied at one end of the porous shaft caused the dispersion medium (air) to pass through the porous cylinder and into the shaft. Because the rotational velocity of airborne particles was comparable to that of the rotating cylinder near its surface, the particles moved radially outward due to the centrifugal force as well as laterally along the cylinder and inward due

to the suction of air into the rotating porous cylinder. The particles reached their highest concentration near the outlet manifold. Except for coarse particle loss due to impaction and ultrafine particle loss due to diffusion in the concentrator, the increase in particle concentration was the ratio of the flow rates for the inlet air to the air delivered to the exposure chamber.

For its use in this project, the system was modified to expose animals under positive pressure conditions (approximately 0.4 cm H_2O) (Gordon et al 1999). For all animal exposures, the centrifugal concentrator was operated at 10,000 rpm with an output of 5.6 to 10 L/minute. An inlet blower (model DR068, EG & G Rotron, Saugerties NY) delivered ambient air through a 1-inch stainless steel tube to the concentrator inlet at a nominal flow rate up to 124 L/minute. The inlet blower had a Teflon seal and an isolated motor so that foreign particles would not be introduced into the exposure system. An exhaust pump (model 3CW, Westmoor, Sherrill NY) drew air from the annular region of the concentrator into the rotating porous inner cylinder at a nominal flow rate up to 114 L/minute. Calibrated orifice meters were used to monitor inlet and exhaust flow rates. Flow from the inlet blower and into the exhaust pump was balanced so that the concentrated aerosol stream was delivered to the animal exposure chamber with a minimum flow of 6 L/minute at a slightly positive pressure (0.4 cm H_2O). An inline stainless steel hot wire anemometer (model 2011, TSI, St Paul MN) was used to continuously monitor flow to the chamber (CH Technology, Westwood NJ). Noise of blowers and pumps was abated by enclosing high-speed instruments in ventilated, soundproofed enclosures and placing a laboratory-built muffler on the outlet side of the exhaust pump.

The concentrator system was located at the New York University (NYU) School of Medicine in the Manhattan borough of New York. Because the objective of animal studies was to examine the adverse effects of typical urban air on the pulmonary and cardiovascular health of compromised animals, the concentrator system was located in an eighth-floor research laboratory above a pedestrian walkway to avoid intake of fresh exhaust of gasoline and diesel engines. Ambient particles were drawn by the inlet blower into a stainless-steel tube that extended approximately 2 feet outward from the window (Figure 3).

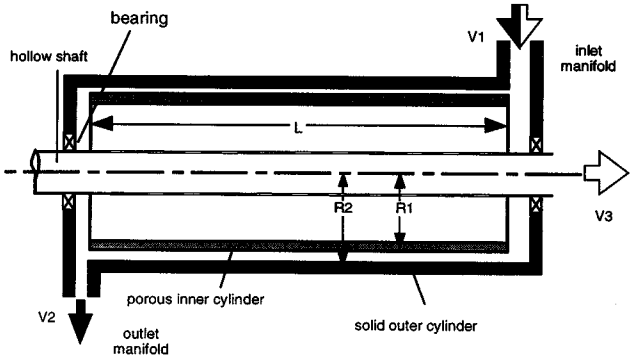

Figure 2. Gerber centrifugal concentrator. V1, V2, and V3 are at the inlet to the concentrator (flow rate 124 L/minute), the outlet of the concentrator directed to the exposure chamber (flow rate 10 L/minute), and the exhaust from the porous filter (flow rate 114 L/minute), respectively. The length (L) of the porous filter is 30 cm, and R1 and R2 are 4.445 and 5.125 cm, respectively. Not drawn to scale.

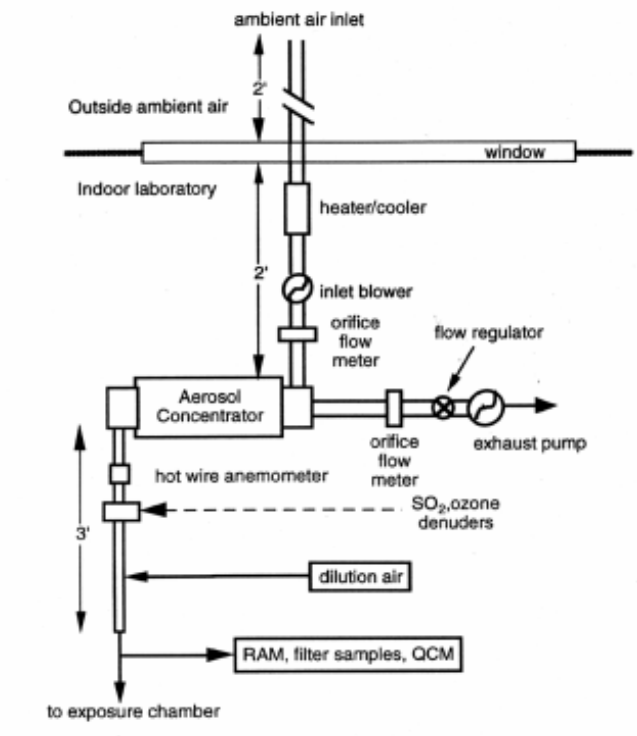

Figure 3. Exposure system for animal inhalation studies using the centrifugal concentrator. Filter samples of ambient air were taken at 15 L/minute at a port immediately downstream of the orifice flow meter on the inlet line and filter samples of the chamber air were taken at 1.5 L/minute from an unused nose-only port of the exposure chamber (not shown). Not drawn to scale.

Honeycomb denuders coated with potassium iodide and lead were located downstream of the anemometer to remove ozone and sulfur dioxide (95% and 92% removal efficiency, respectively). Sampling the airstream with a condensation nuclei counter (model 3020, TSI) upstream and downstream of the denuders determined that less than 5% of the particles were lost in the gas denuders. For the control chamber exposure, ambient air was drawn from the outside environment with a stainless steel diaphragm pump and delivered to the nose-only exposure chamber after passing through gas denuders (identical to those used on the concentrated ambient PM chamber) and a HEPA filter.

EXPOSURE ATMOSPHERE MONITORING

To determine PM mass concentration, gravimetric filter samples (37-mm Teflon filters, Graseby/Anderson, Smyrna GA) of the concentrator output and input were taken from one of the stainless steel delivery ports of the nose-only exposure chamber (1.57 L/minute) and a sampling port immediately before the centrifugal concentrator (15.14 L/minute). Weights were measured before and after the experiment with an electronic microbalance (model 30, Cahn Instruments, Cerritos CA) that was located in a controlled humidity chamber (35% ± 5% RH) and calibrated daily with a class 1.1 calibration weight. Prior to weighing, filters and multiple filter blanks were conditioned for 48 hours in the controlled humidity chamber. Size distributions of ambient and concentrated particles were determined once during each exposure period with a piezoelectric cascade impactor (QCM, California Measurements, Sierra Madre CA) operating at 0.24 L/minute. The cumulative results were plotted on a log-probability graph and the mass median aerodynamic diameter (MMAD) and geometric standard deviation (σ_g) were calculated. Particle number concentration was measured with the condensation nucleii counter immediately upstream of the centrifugal concentrator and at the exposure chamber (0.3 L/minute).

A real-time aerosol monitor (RAM-1) (2 L/minute, MIE, Bedford MA), initially calibrated against Arizona road dust by the manufacturer, was zeroed daily with a HEPA filter and used to monitor output of the centrifugal concentrator at ambient temperature and relative humidity. The RAM-1 reading and the flows from the concentrator, the inlet blower, and the exhaust pump were recorded at 15-minute intervals during the exposure period. Ambient CO was measured once during each exposure period with a Miran 1A infrared analyzer (30 L/minute; Foxboro Instruments, Foxboro MA) that was zeroed daily with hopcalite (activated copper oxide and magnesium oxide). The Miran analyzer was calibrated against serial dilutions of a certified test gas from 0 to 100 ppm CO (AGL, Newburgh NY).

ANALYSIS OF H^+, SULFUR SPECIES, AND OTHER ANIONS

After sample collection, filters were extracted in a mild solution (pH 4.0), which yielded a high-efficiency extraction of sulfate and its associated H^+. Nitrate and sulfate ions were determined in the extraction medium by ion chromatography. A model 4000i Dionex ion chromatography unit (Sunnyvale CA) equipped with a 4 × 250 mm fast-run anion separator A3 and an anion membrane suppressor were employed to analyze the Teflon filter samples for these ions. A 100-µL sample injection loop eluted with 3 mM $NaHCO_3$ and 2.4 mM $NaCO_3$ eluent and suppressed with 0.03N H_2SO_4. Samples were analyzed on the 30 $\mu\Omega^{-1}$/cm full-scale range setting on the conductivity meter. External standards used for calibration containing 0.1 to 10 µg/mL of each ionic species were routinely analyzed. The typical precision of the method expressed as the coefficient of variation for five control standards was 8.9% for sulfite and 2.7% for sulfate. The detection limit as determined by the method of Gabriels (1970) was 0.10 µg/mL for sulfate. All reagents were certified analytic reagent–grade chemicals (Fisher Scientific) and standards were traceable in the National Institute of Standards and Technology (NIST). Dilutions for reagents and standards were made up in fresh 18-MΩ H_2O.

The strong acidity analysis of the extraction sample utilized the pH determination methods previously documented in detail by Koutrakis and colleagues (1988), except that a model 611 Orion pH meter with log-R compensation was used with a Ross model 816300 combination probe (both from Orion Research, Boston MA).

ENDOTOXIN

Cellulose acetate filter samples were collected, extracted, and analyzed for endotoxin levels using sterile techniques (Gordon et al 1992). After sampling, filter media were immediately placed in sterile, pyrogen-free glass containers and stored at 4°C until the endotoxin was extracted. To extract endotoxin from the filters, 30 mL of sterile, pyrogen-free water (Baxter) were added to each sample. The samples were placed in a 68°C water bath for 30 minutes. The extracts were decanted and placed at 4°C to cool and were stored until analysis. Samples were assayed the same day as extraction. Test tubes, pipets, pipet tips, filters, water, and microplates were routinely analyzed to ensure that there was no prior pyrogen contamination. Endotoxin concentrations were quantitated with a Limulus amebocyte

lysate assay (QC1000, Whittaker Bioproducts, Walkersville MD) using a spectrophotometric microplate method.

The assay results were compared with a standard NIST-traceable endotoxin and expressed in terms of endotoxin units (EU) or nanograms (10 EU was assumed to equal 1 ng). No endotoxin above background levels could be detected in the concentrated PM samples. Based on the volume of air sampled during an exposure and the lowest endotoxin standard used in the assay, the lowest concentration of measurable endotoxin was 0.22 ng/m^3. The actual detection limit, however, influenced by the background level of endotoxin contamination on blank filters, was greater than 10 ng/m^3.

VALIDATION OF EXPOSURE SYSTEM

The aerosol concentrator was validated using monodisperse and polydisperse fluorescent test particles and ambient PM.

Validation with Fluorescent Test Particles

The fluorescent test particles were generated from a stock solution of 2 mL oleic acid, 95 mL analytic-grade 100% ethanol, and 0.1 g fluorescein. By varying the concentration of oleic acid, monodisperse particles ranging from 0.15 to 8 µm were generated with a vibrating orifice generator (model 3450, TSI). At each monodisperse particle size, filter samples (0.45 µm cellulose acetate; Millipore, Bedford MA) were taken simultaneously before and after the centrifugal concentrator. Filter samples were sonicated for 10 minutes in 10 mL of an extraction solution that consisted of 10 mL of NH_4OH diluted up to 1 L with 17.8 MΩ water. Total fluorescence was measured using excitation and emission wavelengths of 480 and 514 nm, respectively (model LS-5, Perkin-Elmer, Norwalk CT). The stock oleic acid/ethanol solution was also nebulized with a 3-jet Laskin nebulizer to generate polydisperse test particles. To minimize the concentrating effect of ethanol evaporation in the laboratory-built Laskin nebulizer, a peristaltic pump was used to keep a constant level of fluid in the nebulizer. Mercer 7-stage cascade impactors (Intox Products, Albuquerque NM) operated at 5.5 L/minute with 22-mm glass substrates and a cellulose acetate final filter simultaneously sampled the polydisperse aerosol before and after the centrifugal concentrator. The outputs of the vibrating orifice generator and the Laskin nebulizer were diluted with HEPA-filtered air prior to introducing them into the intake of the inlet blower of the concentrator system.

In defining the operating conditions of the aerosol exposure system, particle loss in the inlet blower was first characterized using monodisperse oleic acid particles generated with the vibrating orifice generator (0.5, 0.73, 0.99, 1.98, 2.96, 3.73, and 4.01 µm). For each particle size, a minimum of 2 filter samples (0.45 µm cellulose acetate) were taken simultaneously before and after the inlet blower. Filter samples were sonicated for 10 minutes in 10 mL of the extraction solution and total fluorescence was measured.

The effect of rotational speed on the concentrating factor for different size particles was determined using polydisperse oleic acid particles. Rotation of the centrifugal concentrator was maintained at 5,000 to 12,500 rpm. Because increasing the rotational speed of the porous shaft increased the resistance to air entering the shaft, the inlet and exhaust control valves were adjusted as necessary to keep the flow rate to the exposure chamber at a constant 10 L/minute and the exhaust and inlet flow rates at approximately 114 L/minute and 124 L/minute, respectively. Simultaneous filter samples (0.45 µm cellulose acetate) were taken to monitor the particles entering and exiting the centrifugal concentrator.

The effect of components of the concentrator system on particle size distribution was determined upstream and downstream of the inlet blower and after the centrifugal concentrator. The rotational speed was held constant at 10,000 rpm, and the flow rate to the exposure chamber was 10 L/minute. At each of the 3 locations, polydisperse fluorescein-tagged oleic acid aerosols were sampled with a 7-stage Mercer cascade impactor (Intox Products, Albuquerque NM) operated at 5.5 L/minute with 22-mm glass substrates and a cellulose acetate final filter. Appropriate substrate and filter blanks were included in the extraction/fluorescence measurements.

Validation with Ambient Particles

For the final testing and validation with ambient particles, the centrifugal concentrator was operated at 10,000 rpm with an output of 5.6 L/minute at 0.4 cm H_2O directed into the nose-only exposure chamber. To determine PM mass and anion concentrations, filter samples were taken from a stainless steel delivery port of the nose-only exposure chamber and a sampling port immediately before the centrifugal concentrator. These filters were designated *ambient* or *concentrated*, respectively. Particle size analysis of the urban particles was determined with the piezoelectric cascade impactor.

STATISTICAL METHODS AND DATA ANALYSIS

All experiments consisted of an air-exposed group and a PM-exposed group. In some experiments, air-exposed and

PM-exposed animals were killed at 3 or 24 hours; in other experiments, all animals were killed at a single timepoint. Because our main objective was to compare air-exposed with PM-exposed animals, we analyzed the lavage fluid and blood data using statistical tests (two-tailed) designed to compare two groups and analyzed each timepoint separately. Because the number of animals in many experiments was too small to determine whether the lavage and blood data were normally distributed, all lavage and blood data were analyzed by the nonparametric Mann-Whitney *U* test. When data are normally distributed, the Mann-Whitney *U* test has 95% of the power of the two-tailed Student *t* test for detecting a significant difference in the group mean (Fisher and van Belle 1993).

Analysis of variance (ANOVA) with repeated measures was used to analyze the heart rate data that was normally distributed. In these experiments, heart rate was monitored for 10-second intervals (bursts) during the 1 hour before exposure and during the 18 hours after exposure. Our objective was to determine whether group mean heart rate of PM-exposed animals differed from the air-exposed animals after PM exposure. Data were analyzed by determining the hourly mean for each of the animals before and after exposure. As described elsewhere, in some experiments, the hourly means were calculated during a 24-hour baseline period for each animal and were subtracted at each timepoint after exposure to remove trends due to circadian rhythm. ANOVA with repeated measures was used to determine statistical differences between air-exposed and PM-exposed animals. Because examination for a PM effect over the entire 18 hours after exposure could wash out changes in short-term heart rate (ie, important changes in heart rate due to PM exposure may last only a few hours), we applied the ANOVA with repeated measures to three 6-hour intervals in each experiment. This decision was a compromise between the overly conservative analysis of a single 18-hour period and the numerous calculations that would result from separate, individual analyses of all 18 postexposure hours. The 6-hour interval was chosen based upon a visual analysis of the data (see Figures 8 and 9). For all statistical tests, significant differences were accepted for $P \leq 0.05$.

Quality of the gravimetric filter analyses was assured by interlaboratory comparison of the weighing of filters. A set of 12 filters was first weighed using the microbalance conditioned for controlled relative humidity and temperature at New York University Sterling Forest and then weighed at the Harvard School of Public Health filter laboratory in collaboration with George Allen. The postexperiment filter weights ($n = 6$ each for samples from the inlet and outlet of the centrifugal concentrator) were measured at NYU after a minimum 48-hour conditioning period and then reweighed at the Harvard laboratory. There was good agreement between the gravimetric analyses performed at the 2 labs: 6.6% ± 13.3% (mean ± SD).

RESULTS

VALIDATION OF CENTRIFUGAL CONCENTRATOR

To test the concentrating factor of the centrifugal concentrator across a range of particle sizes, it was necessary to generate both monodisperse and polydisperse oleic acid–fluorescein aerosols and sample them immediately before and after the concentrator. Where the particle sizes overlapped, the particle-concentrating factor was similar for the monodisperse and the polydisperse particles used in validation of the centrifugal concentrator (Figure 4). In the size range of interest, the size distribution of a laboratory-generated oleic acid–fluorescein aerosol was not significantly affected by the inlet blower of the exposure system (compare the size distribution before and after the inlet blower in Figure 5). Particles larger than 3 µm, however, were efficiently removed by the inlet blower (data not shown).

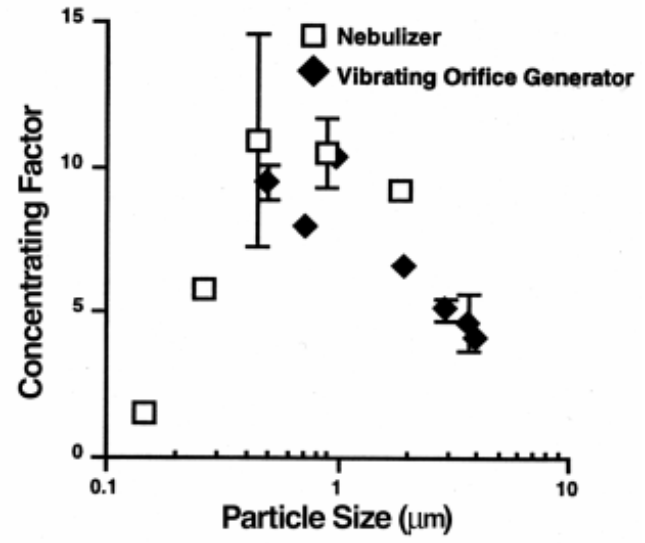

Figure 4. The concentrating factor for monodisperse and polydisperse oleic acid–fluorescein particles sampled immediately before and after the centrifugal concentrator. Open squares represent data points for polydisperse particles produced by a Laskin nebulizer and collected on impactor stages. Closed diamonds represent data points for monodisperse particles produced by a vibrating orifice generator and collected on a filter. Standard error bars are too small to be visible for some data points.

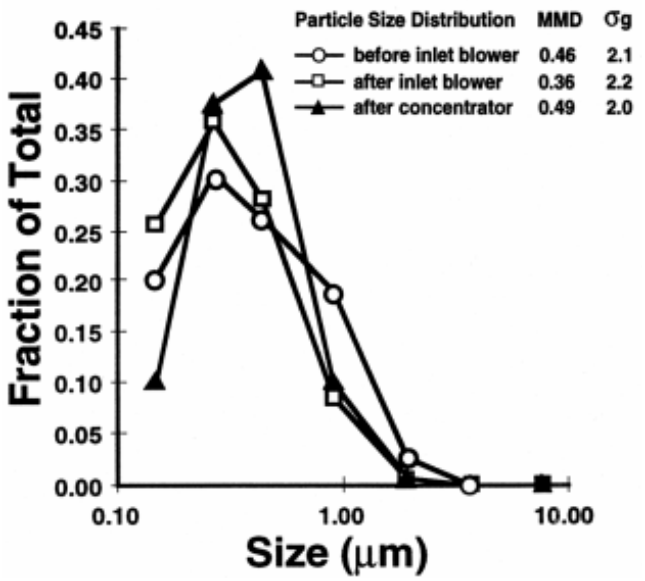

Figure 5. Particle size distribution of oleic acid–fluorescein particles at different sites in the concentrator system. Open circles represent data points for impactor samples taken immediately upstream of the inlet blower. Open squares represent data points for samples taken immediately downstream from the inlet blower. Closed triangles represent data points for samples taken from the outlet of the centrifugal concentrator.

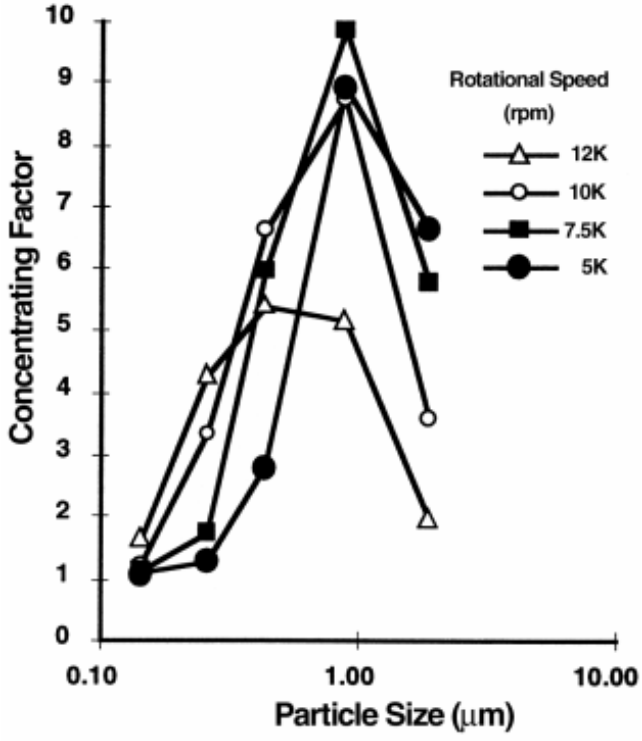

Figure 6. Effect of rotational speed on concentrating factor for oleic acid–fluorescein particles of different sizes. The flow rate from the concentrator was held constant at 10 L/minute, and the inlet and exhaust flow rates ranged from 108 to 114 L/minute and 120 to 124 L/minute, respectively.

Experiments were undertaken to determine the optimal rotational speed of the centrifugal concentrator using polydisperse, oleic acid–fluorescein particles that were generated with a Laskin nebulizer. We confirmed earlier findings (Gerber 1986) that rotational speed alters the upper and lower cut-off diameters in terms of which particles are being concentrated (Figure 6). For example, using fixed inlet and outlet air flow rates, increasing the rotational speed to 12,500 rpm shifted the size of particles that were effectively concentrated to the left (ie, smaller particles were more efficiently concentrated). The effect of rotational speed on the concentrating factor for ambient PM was also examined. We determined that, as would be predicted by the submicron size, ambient PM was concentrated more effectively by increasing the rotational speed of the concentrator (data not shown).

We also examined the effect of the inlet and outlet flow rates on the performance of the aerosol concentrator. Changing the inlet and suction flow rates while keeping the air flow to the exposure chamber at a constant rate of 10 L/minute had little effect on the size distribution of polydisperse, laboratory-generated particles and caused only a modest change in the concentrating factor (data not shown). Significant changes in concentrating factor were obtained by decreasing the outlet flow rate below 10 L/minute. Thus, at least for the current concentrator design, one can increase the concentration of PM delivered to the chamber by decreasing the flow rate to the chamber or by increasing the rotational speed of the concentrator. As predicted, the ratio of the concentrator inlet/output airflows had a strong influence on the concentrating factor. We observed that decreasing the airflow of particle-laden ambient air to the exposure chamber (below the standard 10 L/minute) could increase the particle concentrating factor by over 30-fold (data not shown). The utility of this technique, however, was limited by the requirement for sufficient airflow (V2) both to sample and monitor the chamber atmosphere properly and to provide air for the animal exposure.

The ability of the centrifugal concentrator to concentrate ambient particles was examined for gravimetric and sulfate data. For validation purposes, the concentrating factor was also calculated for the RAM-1 readings. Table 2 presents the ambient and concentrated particle data for a subset of animal-exposure experiments that were conducted from February 1997 through April 1998. Using all the available data during this time period, the mean ± SD gravimetric concentrating factor of 19.5 ± 18.6 was similar to the concentrating factor of 14.5 ± 5.0 calculated with the

Table 2. Daily Concentrating Factors Determined by Gravimetric Analysis, RAM-1 Readings, and Sulfate Analysis

		Gravimetric			RAM-1			Sulfate			MMAD		σ_g		Particle Count (per $cm^3 \times 10^4$)	
Date	Start Time	Ambient ($\mu g/m^3$)	Concentrated ($\mu g/m^3$)	Concentrating Factor[a]	Ambient ($\mu g/m^3$)	Concentrated ($\mu g/m^3$)	Concentrating Factor	Ambient ($\mu g/m^3$)	Concentrated ($\mu g/m^3$)	Concentrating Factor	Ambient (μm)	Concentrated (μm)	Ambient	Concentrated	Ambient	Concentrated
2/26/97	10:40	7.3	400	54.5	33	574	17.4	5.7	15.6	2.7	0.195	0.29	2.36	1.93	ND[b]	4.24
3/19/97	11:00	19.0	176	9.3	10	169	16.9	0.5	12.7	25.2	0.575	0.495	3.03	1.46	ND	3.60
3/27/97	11:35	0.0	339		35	356	10.2	ND	14.1		0.44	0.49	2.2	1.88	ND	4.03
4/3/97	11:15	14.7	219	14.9	20	242	12.1	ND	19.8		ND	ND	ND	ND	ND	4.24
4/11/97	13:00	2.3	157	68.3	12	289	24.1	ND	28.7		ND	ND	ND	ND	ND	3.04
4/16/97	11:00	0.0	184		45	363	8.1	ND	46.3		0.305	ND	3.05	ND	2.79	4.59
4/22/97	08:00	0.0	217		38	387	10.2	ND	34.2		6.4	0.515	18.29	2.51	3.75	2.98
4/25/97	14:05	16.8	206	12.2	14	186	13.3	0.2	4.9	24.0	ND	ND	ND	ND	3.60	3.67
4/26/97	11:00	14.4	185	12.8	7	98	14.0	0.2	5.9	37.7	ND	0.485	ND	3.88	2.92	1.66
4/27/97	11:15	0.0	86		4	104	26.0	ND	12.1		ND	ND	ND	ND	2.10	1.91
6/5/97	10:05	2.9	134	46.2	13	157	12.6	0.1	0.6	7.8	ND	ND	ND	ND	2.52	2.49
6/10/97	10:30	6.6	162	24.5	21	247	11.8	0.6	7.2	12.8	ND	ND	ND	ND	3.05	2.36
10/6/97	12:40	9.5	466	49.1	52	671	12.9	1.8	67.6	38.5	0.195	0.18	1.7	1.7	3.14	3.02
10/29/97	14:35	21.0	184	8.8	20	167	8.4	2.0	17.9	9.0	ND	0.162	ND	1.4	3.61	3.87
11/11/97	11:10	6.1	134	22.0	18	123	6.8	0.2	12.2	64.0	ND	0.225	ND	1.6	2.71	1.75
12/9/97	09:25	32.0	350	10.9	20	299	15.0	2.3	17.2	7.4	0.24	0.54	3.8	2.2	4.61	5.92
12/17/97	10:15	24.0	170	7.1	19	213	11.2	1.7	14.8	9.0	1.29	0.68	3.1	3	5.00	4.92
1/21/98	10:40	23.0	191	8.3	13	216	16.6	2.5	23.2	9.4	0.61	0.285	2	1.7	4.92	4.19
1/27/98	09:35	19.0	212	11.2	13	162	12.5	1.5	18.0	11.8	6.1	0.48	5.3	2.6	4.29	3.83
2/23/98	09:10	35.0	220	6.3	24	345	14.4	2.8	NA[b]		0.49	0.23	8.8	2	4.60	4.22
3/4/98	10:25	27.0	162	6.0	17	259	15.2	1.5	21.1	13.8	0.9	0.42	8.2	4.8	3.36	4.10
3/16/98	10:15	27.0	191	7.1	10	189	18.9	1.8	16.5	9.4	0.82	0.42	6.1	4.8	3.77	3.66
4/6/98	13:30	12.0	32	2.7	5	82	16.4	NA	NA		3.2	1.2	2.7	2.8	3.19	1.76
4/7/98	10:05	9.0	57	6.3	5	101	20.2	NA	NA		1.2	0.54	3.5	2	1.08	2.49
4/8/98	10:05	13.0	275	21.2	19	399	21.0	NA	NA		0.174	0.425	2.2	2.9	3.85	3.93
Maximum				68.3			26.0			64.0	6.4	1.2	18.3	4.8	5.0	5.9
Minimum				2.7			6.8			2.7	0.2	0.2	1.7	1.4	1.1	1.7
Mean				19.5			14.6			18.8	1.4	0.4	4.8	2.5	3.4	3.5
SD				18.6			4.8			16.6	2.0	0.2	4.2	1.0	1.0	1.1

[a] Ratio of concentrated volume to ambient value.

[b] ND = not determined; NA = not available.

RAM-1 data, but it was much more highly variable. The ambient gravimetric concentrations were very low on several occasions. On the basis of detection limits for a 3-hour sampling, we culled those days in which the ambient gravimetric concentration was less than 10 μg/m^3. The recalculated gravimetric concentrating factor was 9.9 ± 4.6 versus the data from the RAM-1 of 14.4 ± 3.7 (n = 14) obtained on those days in which the ambient gravimetric concentration was greater than 10 μg/m^3 (Table 3). Importantly, this gravimetric concentrating factor for ambient particles agreed closely with the 10 × concentrating factor calculated using monodisperse and polydisperse, laboratory-generated, fluorescein–oleic acid particles under similar operating conditions. Although more variable than either the gravimetric or RAM-1 concentrating factors, the sulfate concentrating factor was 13.0 ± 10.6 (mean ± SD). The mean nitrate concentrating factor was greater than that for any other measurement parameter, although this was misleading due to the high degree of variability in the nitrate data (35.7 ± 56.4).

The gravimetric filter data were compared with the RAM-1 data to verify the utility of the RAM-1 as an onsite, real-time monitor of the ambient particle output of the centrifugal concentrator during 3-hour animal exposure studies. Figure 7 compares the gravimetric (filter) data with the RAM-1 data on exposure days spanning several seasons. Because of the limits of detection for both measurement techniques during 3-hour exposure periods at ambient particle levels, the only data presented and analyzed were the gravimetric and RAM-1 data obtained from the concentrator output (ie, delivered to the exposure chamber). As shown in Figure 7, the RAM-1 measurements (y axis) were useful in predicting the gravimetric filter concentrations (R^2 = 0.73) and provided a real-time surrogate measurement of the exposure atmosphere's particle concentration.

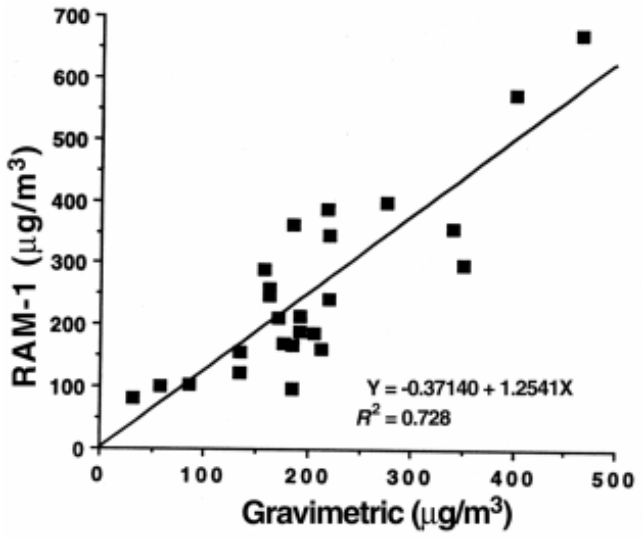

Figure 7. Gravimetric mass concentration compared to RAM-1 readings. The best fit line was derived by least-squares regression analysis.

MONOCROTALINE-TREATED RATS

The first animal study was done to validate the ability of monocrotaline to induce pulmonary hypertension in rats in our laboratory and to determine the time course of response. F344 rats were injected with 40 mg/kg monocrotaline intraperitoneally and the degree of pulmonary hypertension was determined by measuring the right ventricle weight/left ventricle weight ratio (the right ventricle muscle mass is known to increase in response to pulmonary hypertension). These data demonstrated that this dose of monocrotaline increased the size of the right ventricle (Table 4). The increase in right ventricle weight/left ventricle weight ratio was time-dependent and increased from week 2 to week 4 after injection.

The lungs of the monocrotaline-injected animals were lavaged to examine the time course of injury and inflammation that accompanied the monocrotaline-induced pulmonary hypertension. Increases in lavage fluid protein (an index of leakage of serum into air spaces of the gas-exchange region), neutrophils (an index of inflammation),

Table 3. Gravimetric, RAM-1, and Sulfate Concentrating Factors on Exposure Days with Ambient Concentrations Greater Than 10 μg/m^3 (n = 14 days)

	Gravimetric	RAM-1	Sulfate
Mean	9.9	14.4	13.0
SD	4.6	3.7	10.6
Maximum	21.2	21.0	38.8
Minimum	2.7	7.7	7.7

Table 4. Time Course of Cardiopulmonary Response to Monocrotaline Injection[a]

Parameter	2 Weeks	3 Weeks	4 Weeks
RtV (weight [g])[b]	0.111	0.132	0.190
RtV/LV[b]	0.252	0.270	0.354
LDH (BB units)	156.0	87.5	130.8
Protein (μg/mL)	582.1	685.8	1,579.4
PMN (%)	31	47	54

[a] Rats were injected with monocrotaline and killed at 2, 3, or 4 weeks after injection (n = 2/group).

[b] RtV = right ventricle; LV = left vetricle.

and LDH (an index of cell injury) increased in a time-dependent manner. On the basis of the right ventricle weight/left ventricle weight ratio data and the lavage results, a time window of 2 to 4 weeks after monocrotaline injection was chosen for air and PM exposures with the exception of one experiment conducted at 7 weeks. This decision was based on the development of pulmonary hypertension and lung injury or inflammation. Although the degree of pulmonary hypertension would increase over time and make the animals more compromised, any effect of the PM exposure on lavage fluid indices could be obscured by extensive lung injury and inflammation produced by monocrotaline with time after administration. The 2-week window was necessary to ensure that a day with sufficient ambient PM was available for generating the concentrated ambient PM exposure atmospheres.

Lavage Fluid Parameters

With one exception, no cellular or biochemical changes were observed in the lavage fluid obtained from the lungs of normal or monocrotaline-treated rats at 3 and 24 hours after a single exposure to air or concentrated ambient PM (Tables 5 and 6). In one experiment, monocrotaline-treated animals were exposed to 400 $\mu g/m^3$ PM, and total cell count, protein, and LDH were observed to be approximately twice the values of those of air-exposed animals. No cellular or biochemical changes in lavage fluid were observed in monocrotaline-treated rats exposed to 192 $\mu g/m^3$ concentrated ambient PM for 6 hours/day for 3 consecutive days (Table 6).

Histology

The effect of PM exposure on lung structure was examined using histopathologic techniques in normal and monocrotaline-treated rats that were exposed to air or PM (176 and 219 $\mu g/m^3$ for the normal and monocrotaline-treated rats, respectively) for 3 hours and killed at 3 or 24 hours after exposure (n = 3 rats/exposure group at each time point). Lung sections from normal rats exposed to PM showed no microscopic changes and could not be distinguished from air-exposed controls. Lung sections from monocrotaline-treated rats showed a variety of significant histologic changes, such as focal areas of alveolar inflammation with occasional granulomas, patchy areas with markedly increased numbers of alveolar macrophages, and marked hyperplasia of airway epithelium in the small airways. The extent and type of change varied considerably among animals. Lung sections from monocrotaline-treated animals exposed to PM could not be distinguished from air-exposed monocrotaline-treated animals, thus demonstrating an absence of any obvious pulmonary response to concentrated ambient PM.

Table 5. Lavage Fluid Measurements at 3 Hours After Single 6-Hour Exposure

Animal Model	Exposure ($\mu g/m^3$)	n	PMN[a] (%)	Cell Count[a] ($\times 10^6$)	Protein[a] ($\mu g/mL$)	LDH[a] (BB units)
Young normal	Air	6	1 ± 0	1.5 ± 0.2	158 ± 4	26 ± 3
	PM = filtered inlet	6	2 ± 1	1.9 ± 0.1	157 ± 4	25 ± 6
Young rats treated with monocrotaline[b]	Air	3	42 ± 9	0.7 ± 0.1	1,587 ± 559	141 ± 16
	PM = 157	3	31 ± 6	1.0 ± 0.1	1,625 ± 338	160 ± 24
	Sulfate = 29					
Young rats treated with monocrotaline[b]	Air	3	30 ± 4	0.8 ± 0.1	2,764 ± 780	279 ± 13
	PM = 217	3	20 ± 8	0.9 ± 0.3	2,525 ± 1233	215 ± 61
	Sulfate = 34					
6-Month-old rats treated with monocrotaline[b]	Air	3	23 ± 2	0.8 ± 0.1	1,306 ± 233	299 ± 9
	PM = 219	3	16 ± 4	0.9 ± 0	1,772 ± 450	311 ± 7
	Sulfate = 20					

[a] Results shown are mean ± SE. No significant differences were noted between air-exposed and PM-exposed group means.

[b] Animals were tested 4 weeks after being injected with monocrotaline.

Table 6. Lavage Fluid Measurements at 24 Hours After Exposure

Animal Model	Exposure ($\mu g/m^3$)	n	PMN[a] (%)	Cell Count[a] ($\times 10^6$)	Protein[a] (μg/mL)	LDH[a] (BB units)
Young normal rats	Air	6	2 ± 0	0.7 ± 0.1	173 ± 5	31 ± 2
	PM = 184	6	1 ± 1	0.7 ± 0.1	189 ± 13	32 ± 2
	Sulfate = 46					
6-Month-old normal rats	Air	6	1 ± 0	1.8 ± 0.2	150 ± 3	23 ± 1
	PM = 339	6	1 ± 0	1.5 ± 0.1	146 ± 3	20 ± 3
	Sulfate = 14					
Young rats treated with monocrotaline[b]	Air	6	25 ± 2	0.6 ± 0.1	3,450 ± 742	321 ± 17
	PM = 134	6	18 ± 2	0.5 ± 0.1	2,627 ± 509	288 ± 21
	Sulfate = 1					
Young rats treated with monocrotaline[c]	Air	3	42 ± 1	0.6 ± 0.1	1,284 ± 206	159 ± 21
	PM = 157	3	32 ± 6	0.7 ± 0.1	1,361 ± 231	171 ± 36
	Sulfate = 29					
Young rats treated with monocrotaline[b]	Air	6	23 ± 2	0.2 ± 0	835 ± 68	251 ± 14
	PM = 162	6	19 ± 2	0.3 ± 0	941 ± 79	224 ± 11
	Sulfate = 7					
Young rats treated with monocrotaline[c]	Air	3	25 ± 5	0.6 ± 0.1	1,097 ± 457	152 ± 62
	PM = 217	3	27 ± 4	0.9 ± 0.1	1,396 ± 215	197 ± 30
	Sulfate = 34					
6-Month-old rats treated with monocrotaline[c]	Air	3	27 ± 7	1.1 ± 0.4	2,131 ± 841	297 ± 40
	PM = 219	3	23 ± 10	1.0 ± 0.5	2,223 ± 1361	234 ± 79
	Sulfate = 20					
Young rats treated with monocrotaline[c]	Air	6	24 ± 5	0.6 ± 0.1	693 ± 137	146 ± 22
	PM = 400	6	31 ± 4	1.2 ± 0.2[e]	1,489 ± 251[e]	238 ± 24[e]
	Sulfate = 16					
Young rats treated with monocrotaline[d]	Air	5	20 ± 5	0.5 ± 0.1	506 ± 255	204 ± 16
	PM = 192	5	27 ± 6	0.5 ± 0.1	322 ± 59	198 ± 12
	Sulfate = 8					

[a] Results are shown as mean ± SE.

[b] Animals received a single 6-hour exposure at 3 weeks after monocrotaline injection.

[c] Animals received a single 6-hour exposure at 4 weeks after monocrotaline injection.

[d] Animals were exposed for 6 hours/day for 3 days at 2 weeks after monocrotaline injection.

[e] Difference between air-exposed and PM-exposed group means was significant ($P < 0.05$; Mann-Whitney U test).

Pulmonary Function

Lung volume indices and DLCO were examined in normal and monocrotaline-treated rats at 3 ($n = 3$) and 24 ($n = 6$) hours after exposure to air or 181 $\mu g/m^3$ concentrated ambient PM. Although small differences in lung volumes and DLCO were observed between monocrotaline-treated and normal rats (Table 7), no adverse effects of exposure to concentrated ambient PM were found for any of the parameters of pulmonary function that were examined. DLCO was greater in monocrotaline-treated animals exposed to PM ($P < 0.05$), but this change suggests an improvement in gas transfer.

Blood Parameters

Hematologic changes were observed at 3 hours following exposure to PM (Table 8). These changes were (1) evidenced by a sporadic increase in percentage of neutrophils and decrease in percentage of lymphocytes in circulating blood; (2) absent by 24 hours after exposure (Table 9); and (3) present in both normal and monocrotaline-treated rats and 6-month-old rats exposed to concentrated ambient PM. To verify that these peripheral blood changes were a result of particle exposure and not ambient gases or experimental conditions, additional animals were exposed to air or the filtered output of the centrifugal concentrator.

In one experiment, particles were removed by a HEPA filter placed on the outlet of the concentrator and, in another experiment, a HEPA filter was placed on the inlet of the concentrator. No significant differences in circulating blood neutrophil or lymphocyte changes were observed between air-exposed or filtered PM–exposed rats.

Archived blood smears were first used to determine the effects of PM exposure on platelet number. Platelets can be seen in blood smears, but the number of platelets per unit area is a function of the thickness of the smear. The red blood cell (RBC) number was found to be unchanged by PM exposure and, thus, the ratio of platelets to RBCs in blood smears was determined as an index of platelet number. Platelet/RBC ratios were determined in blood smears from F334 rats exposed to air or PM (176 μg/m^3) for 3 hours. At 3 hours after exposure, the platelet/RBC ratio was 0.065 ± 0.013 (mean ± SE, n = 3) for air-exposed animals and 0.119 ± 0.004 for PM-exposed animals ($P < 0.02$), indicating that platelet number increased significantly. The platelet/RBC ratio was unchanged at 24 hours after exposure.

Based on these pilot data, platelet number was then measured with a Coulter counter in two other experiments. Normal rats were exposed to filtered air or PM (350 μg/m^3) for 3 hours. Platelet number was 501 ± 20 × 10^3/mm^3 (n = 6) in air-exposed animals and 572 ± 15 in PM-exposed rats ($P < 0.02$), an increase of 15%. The platelet/RBC ratio obtained from the blood smears of PM-exposed rats was also significantly higher than the ratio obtained from the blood smears of the air-exposed rats. An exposure of rats to air or 212 μg/m^3 PM failed to lead to a subsequent increase in platelets. The data were insufficient to determine whether the difference in results of the two experiments reflected differences in PM concentration or PM composition. Also, since physiologically induced acute changes in platelet number are usually transient, assays of additional, more stabile coagulation parameters, such as fibrinogen levels, may give more reproducible results.

Heart Rate and ECG Waveforms

Small increases in heart rate were observed in normal young rats and monocrotaline-treated rats exposed to concentrated ambient PM (Table 10 and Figure 8), although these changes did not occur on all exposure days. In normal young rats, the increase in heart rate was restricted to the first 6 hours after exposure in one experiment (132 μg/m^3 PM) and to the first 3 hours after exposure in another experiment (184 μg/m^3 PM). These changes were not observed in 6-month-old normal rats after a single exposure to 339 μg/m^3 PM (Table 10) or after 3 daily 6-hour exposures to PM (Table 11).

Table 7. Pulmonary Function in Rats After Single 6-Hour Exposure to Air or PM

Pulmonary Function Parameter	Normal Rats, Air Exposure[a]	Normal Rats, PM Exposure[a]	Monocrotaline-Treated Rats, Air Exposure[a]	Monocrotaline-Treated Rats, PM Exposure[a]
3 Hours After Exposure[b]				
VC	8.0 ± 0.4	8.1 ± 0.3	10.3 ± 0.5	9.8 ± 0.8
IC	7.2 ± 0.3	7.1 ± 0.3	8.8 ± 0.4	8.3 ± 0.6
TLC	10.9 ± 0.6	9.6 ± 0.9	12.6 ± 0.5	12.6 ± 0.5
RV	2.9 ± 0.6	1.4 ± 1.0	2.3 ± 0.3	2.8 ± 0.5
FRC	3.7± 0.5	2.7 ± 1.1	3.8 ± 0.2	4.4 ± 0.4
DLCO	0.135 ± 0.009	0.147 ± 0.009	0.161 ± 0.005	0.168 ± 0.014
DLCO/VA	0.014 ± 0.001	0.014 ± 0.001	0.013 ± 0.000	0.013 ± 0.000
24 Hours After Exposure[c]				
VC	8.8 ± 0.4	9.3 ± 0.3	9.1 ± 0.6	8.9 ± 0.4
IC	8.2 ± 0.3	7.9 ± 0.3	7.7 ± 0.6	7.7 ± 0.3
TLC	11.7 ± 0.7	12.0 ± 0.6	13.3 ± 1.2	12.2 ± 0.6
RV	2.8 ± 0.4	2.7 ± 0.5	4.2 ± 0.7	3.3 ± 0.3
FRC	4.0 ± 0.5	4.1 ± 0.5	5.6 ± 0.7	4.5 ± 0.4
DLCO	0.144 ± 0.007	0.142 ± 0.009	0.119 ± 0.013	0.150 ± 0.006[d]
DLCO/VA	0.013 ± 0.000	0.013 ± 0.001	0.010 ± 0.001	0.013 ± 0.001

[a] All data are expressed as mean ± SE.

[b] n = 3/group.

[c] n = 6/group.

[d] The monocrotaline-treated group exposed to PM was significantly different from the monocrotaline-treated group exposed to air ($P < 0.05$; Mann-Whitney U test).

Table 8. White Blood Cell Differential Measurements at 3 Hours After Single 6-Hour Exposure to Air or PM

Animal Model	Exposure ($\mu g/m^3$)	*n*	PMN[a] (%)	Lymphocytes[a] (%)	Total WBC[a] (cells/mL)
Young normal rats	Air	6	27 ± 2	72 ± 2	2,832 ± 468
	PM filtered[b]	6	30 ± 3	69 ± 3	3,125 ± 277
Young normal rats	Air	6	38 ± 4	61 ± 4	2,622 ± 219
	PM filtered[b]	6	41 ± 3	58 ± 3	2,501 ± 278
Young normal rats	Air	6	23 ± 2	77 ± 1	4,653 ± 428
	PM = 113	6	36 ± 5[c]	62 ± 5[c]	3,757 ± 460
	Sulfate = 7				
Young normal rats	Air	6	—	—	2,300 ± 552
	PM = 170	6	—	—	2,417 ± 206
	Sulfate = ND[d]				
Young normal rats	Air	3	35 ± 4	64 ± 4	1,948 ±441
	PM = 176	3	55 ± 4[c]	44 ± 4[c]	2,000 ± 446
	Sulfate = 13				
Young normal rats	Air	6	31 ± 4	68 ± 4	4,683 ± 751
	PM = 212	6	29 ± 3	70 ± 3	4,683 ± 484
	Sulfate = 18				
Young normal rats	Air	6	28 ± 4	73 ± 4	2,054 ± 125
	PM = 247	6	24 ± 2	76 ± 2	1,791 ± 187
	Sulfate = ND				
Young normal rats	Air	6	28 ± 4	72 ± 5	2,383 ± 353
	PM = 350	6	28 ± 3	72 ± 3	2,467 ± 260
	Sulfate = 17				
Young rats treated with monocrotaline[e]	Air	3	38 ± 6	60 ± 6	3,464 ± 488
	PM = 157	3	49 ± 4	50 ± 5	2,123 ± 321
	Sulfate = 29				
Young rats treated with monocrotaline[e]	Air	3	29 ± 5	69 ± 5	4,830 ± 594
	PM = 217	3	41 ± 3[c]	58 ± 3[c]	5,095 ± 270
	Sulfate = 34				
Young rats treated with monocrotaline[f]	Air	3	36 ± 3	61 ± 2	2,547 ± 157
	PM = 919	3	48 ± 2[c]	50 ± 1[c]	3,038 ± 783
	Sulfate = 97				
6-Month-old rats treated with monocrotaline[e]	Air	3	48 ± 5	51 ± 5	1,909 ± 183
	PM = 219	3	75 ± 2[c]	24 ± 2[c]	2,736 ± 368[c]
	Sulfate = 20				

[a] Data are expressed as mean ± SD.

[b] A HEPA filter was placed on either the inlet or the outlet of the centrifugal concentrator.

[c] Air-exposed and PM-exposed group means were significantly different ($P < 0.05$; Mann-Whitney *U* test).

[d] ND = Not detected.

[e] Animals were tested 4 weeks after being injected with monocrotaline.

[f] Animals were tested 2 weeks after being injected with monocrotaline.

Table 9. White Blood Cell Differential Measurements at 24 Hours After Exposure to Air or PM

Animal Model	Exposure ($\mu g/m^3$)	*n*	PMN[a] (%)	Lymphocytes[a] (%)	Total WBC[a] (# cells/mL)
Young normal rats	Air	3	32 ± 2	67 ± 2	1,949 ± 505
	PM = 176	3	31 ± 5	69 ± 2	1,716 ± 362
	Sulfate = 13				
6-Month-old normal rats	Air	6	48 ± 7	51 ± 6	3,968 ± 352
	PM = 339	6	48 ± 5	52 ± 5	3,006 ± 367[b]
	Sulfate = 14				
Young rats treated with monocrotaline[c]	Air	6	24 ± 3	76 ± 3	3,229 ± 185
	PM = 134	6	24 ± 4	75 ± 4	3,231 ± 347
	Sulfate = 1				
Young rats treated with monocrotaline[d]	Air	3	37 ± 3	63 ± 3	3,986 ± 167
	PM = 157	3	31 ± 4	68 ± 4	4,438 ± 193
	Sulfate = 29				
Young rats treated with monocrotaline[c]	Air	6	15 ± 4	82 ± 2	4,247 ± 251
	PM = 162	6	13 ± 4	80 ± 2	2,656 ± 142[b]
	Sulfate = 7				
Young rats treated with monocrotaline[d]	Air	3	20 ± 2	80 ± 2	4,830 ± 594
	PM = 217	3	27 ± 4	72 ± 4	5,095 ± 270
	Sulfate = 34				
6-Month-old rats treated with monocrotaline[d]	Air	3	40 ± 5	60 ± 5	1,889 ± 210
	PM = 219	3	31 ± 3	67 ± 2	1,725 ± 58
	Sulfate = 20				
Young rats treated with monocrotaline[d]	Air	6	37 ± 2	62 ± 2	3,699 ± 744
	PM = 400	6	40 ± 2	60 ± 2	3,006 ± 538
	Sulfate = 16				
Young rats treated with monocrotaline[e]	Air	5	49 ± 7	46 ± 7	4,121 ± 763
	PM = 192	5	49 ± 5	50 ± 5	4,891 ± 709
	Sulfate = 8				

[a] Results shown are mean ± SE.

[b] Air-exposed and PM-exposed group means were significantly different ($P < 0.05$; Mann-Whitney *U* test).

[c] Animals received a single 6-hour exposure at 3 weeks after monocrotaline injection.

[d] Animals received a single 6-hour exposure at 4 weeks after monocrotaline injection.

[e] Animals were exposed 6 hours/day for 3 days at 2 weeks after monocrotaline injection.

Table 10. Heart Rate in Rats Undergoing Single 6-Hour Exposure to Air or PM[a]

Animal Model	Exposure (μg/m^3)	*n*	1 Hour Before Exposure: Mean Rate (bpm)	*P*[b]	1–6 Hours After Exposure: Mean Rate (bpm)	*P*[b]	7–12 Hours After Exposure: Mean Rate (bpm)	*P*[b]	13–18 Hours After Exposure: Mean Rate (bpm)	*P*[b]
Normal rats	Air	6	409.0 ± 6.6	0.337	407.8 ± 5.1	0.031	396.5 ± 6.6	0.507	381.0 ± 6.7	0.990
	PM = 132 Sulfate = 7	6	399.3 ± 6.9		427.0 ± 5.7		402.8 ± 6.4		381.1 ± 6.1	
Normal rats	Air	6	358.5 ± 10.5	0.717	374.2 ± 5.7	0.097[c]	365.4 ± 5.7	0.266	375.1 ± 4.0	0.365
	PM = 184 Sulfate = 46	6	363.0 ± 6.0		389.7 ± 7.4		376.5 ± 7.4		383.1 ± 7.5	
6-Month-old normal rats	Air	5	353.0 ± 6.1	0.390	371.5 ± 7.6	0.878	348.7 ± 3.2	0.727	386.9 ± 5.6	0.061
	PM = 339 Sulfate = 14	6	361.8 ± 7.3		370.3 ± 3.1		351.0 ± 5.4		363.0 ± 7.3	
Young rats treated with monocrotaline[e]	Air	6	350.3 ± 3.1	0.134	392.5 ± 6.7	0.266[d]	375.9 ± 4.0	0.816	375.2 ± 1.7	0.892
	PM = 400 Sulfate = 16	6	338.8 ± 6.3		401.3 ± 4.1		377.5 ± 6.0		376.2 ± 7.4	
Young rats treated with monocrotaline[f]	Air	3	401.3 ± 10.3	0.305	393.3 ± 21.6	0.840	381.4 ± 18.0	0.938	371.0 ± 16.1	0.771
	PM = 137 Sulfate = 13	4	423.3 ± 14.5		388.3 ± 13.2		379.1 ± 20.1		378.1 ± 15.5	

[a] Results are shown as the mean ± SE heart rate; data were collected at 5-minute intervals in the 1 hour before exposure and in the 18 hours after exposure.

[b] The *P* value between the air and PM exposure groups was determined by ANOVA with repeated measures for each 6-hour segment in the postexposure period.

[c] For the first 3 hours after exposure ($P = 0.05$).

[d] For the first hour after exposure ($P = 0.01$).

[e] Animals were exposed 4 weeks after monocrotaline injection.

[f] Animals were exposed 7 weeks after monocrotaline injection.

Table 11. Change in Heart Rate from Baseline Values Undergoing 3 Consecutive Days of 6-Hour Exposures of Rats to Air or PM

Animal Model	Day	Exposure (μg/m^3)	*n*	1–6 Hours After Exposure: Change[a]	*P*[b]	7–12 Hours After Exposure: Change[a]	*P*[b]	13–18 Hours After Exposure: Change[a]	*P*[b]
6-Month-old normal rats	1	Air	5	16.9 ± 6.9	0.993	12.3 ± 4.9	0.508	–1.48 ± 1.40	0.759
		PM = 170	5	16.8 ± 2.7		6.4 ± 7.0		–3.64 ± 6.64	
	2	Air	5	12.3 ± 7.5	0.557	–3.3 ± 6.1	0.612	4.4 ± 3.4	0.901
		PM = 223	5	7.3 ± 3.5		–8.5 ± 7.7		5.4 ± 6.7	
	3	Air	5	88.2 ± 32.9	0.821	36.0 ± 19.0	0.796	51.8 ± 32.3	0.553
		PM = 152	5	79.2 ± 20.1		19.4 ± 59.1		17.8 ± 43.7	

[a] The heart rate was monitored at 5-minute intervals to establish a 24-hour baseline before animals began exposure sequence. At each postexposure hour, the change in mean hourly heart rate was calculated by subtracting the mean hourly baseline heart rate from the same chronological time of the 24-hour baseline period. Results are presented as mean ± SE.

[b] The *P* values between the air and PM exposure groups were determined by ANOVA with repeated measures for each 6-hour segment after exposure.

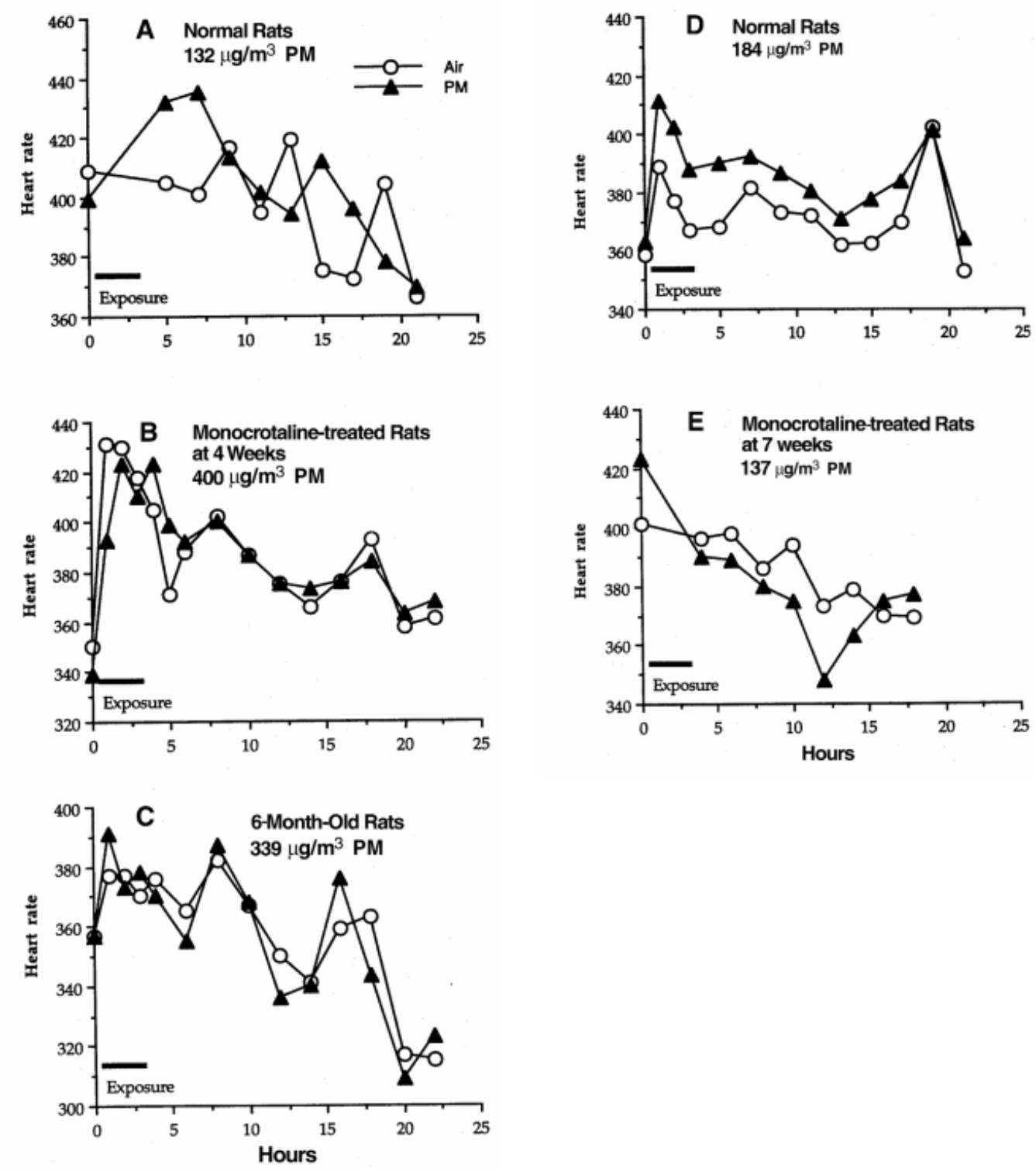

Figure 8. Effect of air and PM exposure on heart rate (beats per minute) in normal, monocrotaline-treated, and 6-month-old rats. The open circles (air-exposed animals) and closed triangles (PM-exposed animals) represent the mean heart rate in the 1 hour before exposure, the hourly mean during exposure (in some experiments), and the 2-hour means after exposure. The bar represents the 3-hour exposure period. Panels A–D include results for 6 animals for each exposure (air and PM); panel D includes results for 3 animals exposed to air and 4 animals exposed to PM.

To investigate the reproducibility of the PM-induced increase in heart rate further, a crossover experiment was performed. Baseline heart rate values were obtained in 12 normal young rats over a 24-hour period. These rats were then exposed to air or 325 μg/m^3 PM for 3 hours (n = 6/group). After a 2-month interval, a new 24-hour baseline for heart rate was obtained, the exposure groups were reversed (ie, the air rats became PM rats and vice versa), and they were exposed to air or PM. To correct the postexposure heart rate values for circadian rhythm, the change in each animal's mean hourly heart rate was calculated by subtracting its mean hourly baseline heart rate value from the same chronologic time of the preceding 24-hour baseline period. In the first experiment, heart rate was significantly increased in the 7 to 12 hours after exposure (P = 0.041, Table 12 and Figure 9A). The heart rate also increased during the 1 to 6 hours after exposure (P = 0.088). When the exposure groups were reversed in the second exposure, no increase in heart rate was observed after exposure to PM (Figure 9B). These results suggest that aging (the rats were nearly 6 months old at the time of the second exposure) blunted the response to PM, as has already been observed, or that the composition of the concentrated ambient PM differed at the time of the 2 exposures.

In general, the magnitude of the increase in heart rate response in monocrotaline-treated rats exposed to PM did not appear to differ from that of normal rats, although the increase in young monocrotaline-treated rats did not reach statistical significance. A change in heart rate was not observed in very sick rats exposed to PM at 7 weeks after injection with monocrotaline, although a limited increase

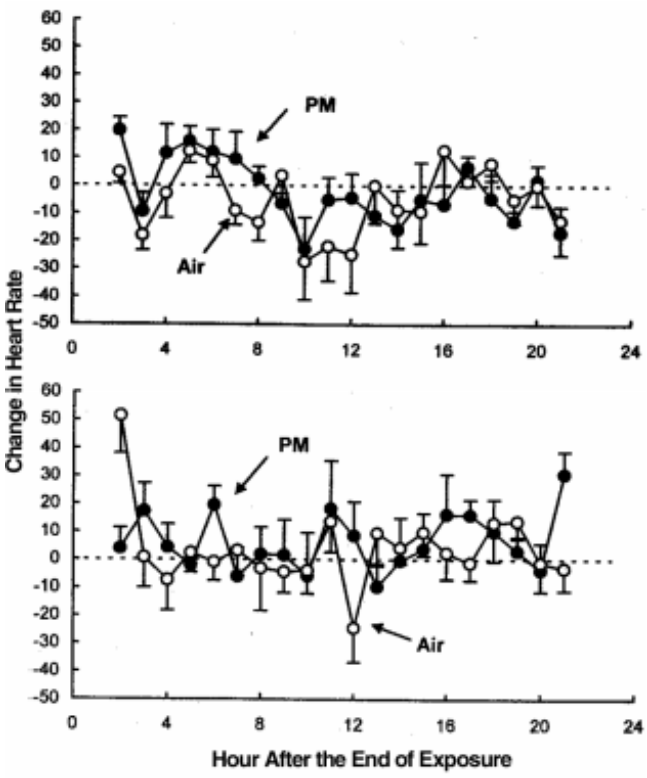

Figure 9. Effect of air and PM exposure on heart rate in normal animals exposed on 2 separate occasions (crossover design). At each postexposure hour, the change in mean hourly heart rate was calculated by subtracting the mean hourly baseline heart rate from the same chronological time of the 24-hour baseline (change in heart rate in beats per minute). Top: Results of the first exposure in which the animals were exposed to air (n = 6) and PM (n = 6). Bottom: Results of the second exposure in which the animal exposure groups were reversed (ie, the animals exposed to air in the first exposure inhaled PM and vice versa).

Table 12. Change in Heart Rate from Baseline Values After Crossover Exposures of a Group of Normal Rats to Air or PM

			1–6 Hours After Exposure		7–12 Hours After Exposure		13–18 Hours After Exposure	
	Exposure (μg/m^3)	n	Change[a]	P[b]	Change[a]	P[b]	Mean	P[b]
First exposure	Air	6	12.9 ± 1.9	0.088	–15.3 ± 3.6	0.041	0.6 ± 2.9	0.134
	PM = 325	6	21.2 ± 3.9		–4.3 ± 3.0		–5.9 ± 2.7	
Second exposure	Air	6	25.1 ± 4.9	0.661	–3.1 ± 4.4	0.230	6.4 ± 3.1	0.954
	PM = 339	6	28.1 ± 4.9		3.1 ± 2.1		6.1 ± 5.1	

[a] Each animal had two single 3-hour exposures delivered in a crossover pattern: In the first series, six animals were exposed to air and six were exposed to PM. After 2 months, the groups were reversed (eg, rats exposed to air first were exposed to PM), and the exposures were repeated. The heart rate was recorded at 5-minute intervals before the exposure (to establish a 24-hour baseline) and after the exposure. The change in mean hourly heart rate was calculated by subtracting the mean hourly heart rate after exposure from the baseline heart rate at the same chronological time during the 24-hour baseline period. Results are shown as mean ± SE.

[b] The P value between the air and PM exposure groups was determined by ANOVA with repeated measures for each 6-hour segment after exposure.

in heart rate was observed in monocrotaline-treated rats exposed to 400 μg/m^3 PM at 4 weeks after injection (Table 10 and Figure 8). This latter increase in heart rate reached statistical significance only in the first hour after exposure. Analyses also revealed no alterations in ECG waveform intervals in these PM-exposed monocrotaline-treated rats (Figure 10) or normal rats (data not shown).

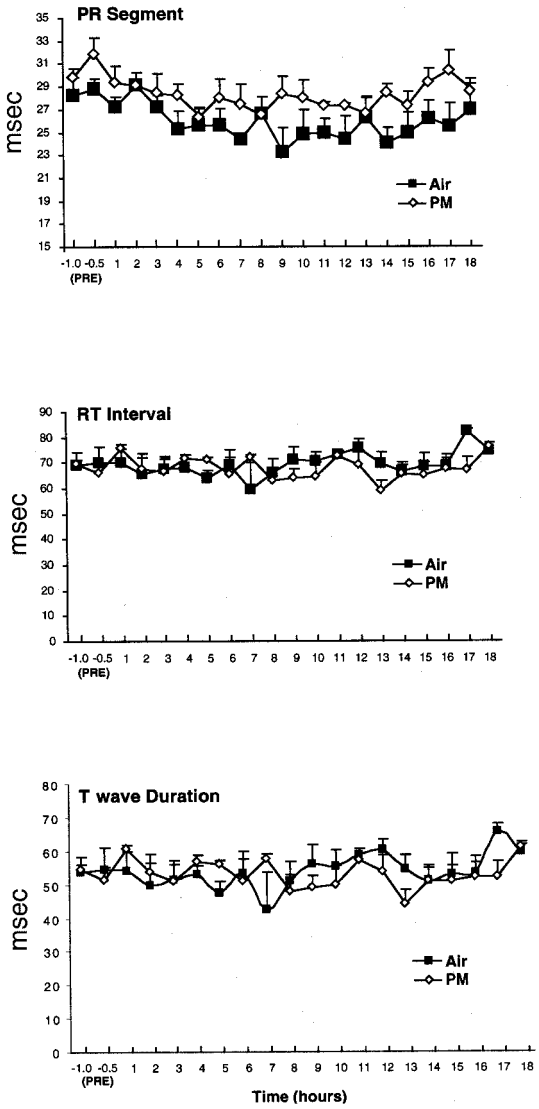

Figure 10. Effect of exposure to air (n = 6) and PM (n = 6) on interval measurements and segment lengths in ECG waveforms of monocrotaline-treated rats. The measurements represented by PR segment, RT interval, and T wave duration are described in Figure 1.

ECG tracings from all rat experiments were visually scanned for arrhythmias. Skipped beats were rare in normal and in monocrotaline-treated rats, and no differences were evident between air-exposed and PM-exposed rats. For example, only 2 arrhythmias (1 skipped beat and 1 premature atrial contraction in PM-exposed rats) were observed in the ECG tracings from 12 monocrotaline-treated rats that were exposed to air or PM (n = 6/group) for 3 hours and monitored for 1 hour before exposure and for 18 hours after exposure (approximately 23,000 heart beats). These arrhythmias were not attributed to adverse health effects.

HAMSTERS WITH CARDIOMYOPATHY

Lavage Fluid Parameters

The biochemical or cellular indices in lavage fluid were highly variable and more closely related to the degree of cardiomyopathy (ie, heart size) than exposure (Figure 11). No adverse changes in lavage fluid parameters were observed in normal hamsters or hamsters with cardiomyopathy exposed to concentrated ambient PM, although a decrease in the percentage of polymorphonuclear leukocytes (PMNs) and LDH was seen in 8- and 10-month-old hamsters with cardiomyopathy, respectively, exposed to concentrated ambient PM (Table 13).

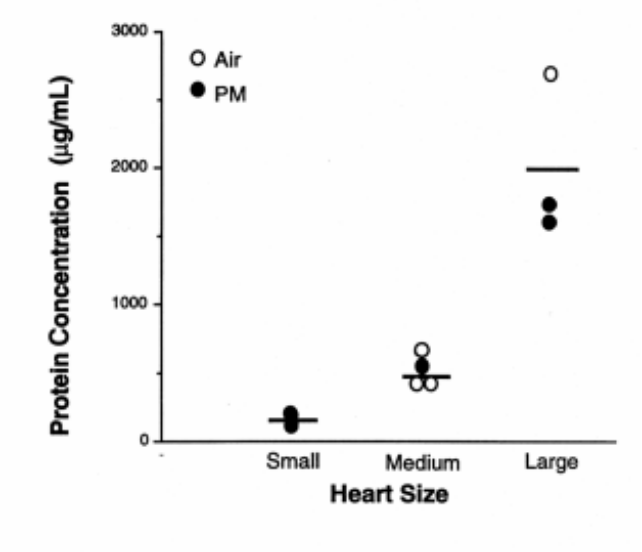

Figure 11. Effect of air and PM exposure on lavage fluid protein in hamsters with various stages of cardiomyopathy (as indicated by heart size).

Table 13. Lavage Fluid Measurements in Hamsters

Animal Model	Exposure ($\mu g/m^3$)	*n*	PMN[a] (%)	Cell count[a] ($\times 10^6$)	Protein[a] (μg/mL)	LDH (BB units)
Normal hamsters	Air	3	5 ± 1	0.5 ± 0.1	118 ± 13	12 ± 2
	PM = 134 Sulfate = 12	5	6 ± 1	0.5 ± 0.1	129 ± 8	21 ± 9
8-Month-old hamsters with cardiomyopathy	Air	4	31 ± 8[b]	0.9 ± 0.4	1,060 ± 553	83 ± 51
	PM = 184 Sulfate = 18	5	12 ± 2[b]	0.7 ± 0.1	843 ± 767	111 ± 53
10-Month-old hamsters with cardiomyopathy	Air	3	23 ± 15	0.5 ± 0.2	660 ± 265	65 ± 18[b]
	PM = 466 Sulfate = 68	3	6 ± 2	1.2 ± 0.6	256 ± 25	20 ± 6[b]

[a] Results are shown as mean ± SE.

[b] Statistically significant difference between air-exposed and PM-exposed group means ($P < 0.05$; Mann-Whitney *U* test).

Pulmonary Function

To examine the effect of PM on pulmonary function, hamsters with cardiomyopathy were exposed to filtered air or concentrated ambient PM for 3 hours and anesthetized for pulmonary function testing at 3 ($n = 3$) or 24 ($n = 6$) hours after exposure. No exposure-related effects were observed in these hamsters (Table 14).

Table 14. Pulmonary Function in Hamsters with Cardiomyopathy After Single 3-Hour Exposure to Air or PM

Pulmonary Function	Air[a]	PM[a]
3 Hours After Exposure[b]		
VC	5.5 ± 0.1	5.0 ± 0.4
IC	4.9 ± 0.2	4.4 ± 0.4
TLC	6.9 ± 0.4	6.2 ± 0.5
RV	1.4 ± 0.2	1.2 ± 0.2
FRC	2.0 ± 0.2	1.8 ± 0.2
DLCO	0.071 ± 0.004	0.072 ± 0.008
DLCO/VA	0.011 ± 0.001	0.011 ± 0.002
24 Hours After Exposure[c]		
VC	5.0 ± 0.2	4.9 ± 0.3
IC	4.3 ± 0.2	4.3 ± 0.2
TLC	6.1 ± 0.4	6.2 ± 0.1
RV	1.1 ± 0.2	1.4 ± 0.2
FRC	1.8 ± 0.2	1.9 ± 0.1
DLCO	0.083 ± 0.011	0.102 ± 0.018
DLCO/VA	0.013 ± 0.002	0.016 ± 0.003

[a] All data are expressed as mean ± SE.

[b] $n = 3$/group.

[c] $n = 6$/group.

Blood Parameters

Differential and WBC counts were determined in normal hamsters and hamsters with cardiomyopathy 24 hours after exposure to air or concentrated ambient PM. In 10-month-old hamsters with cardiomyopathy ($n = 3$/group), the WBC count was depressed ($P < 0.05$) after exposure to PM (Table 15). No changes in the percentage of PMNs or the percentage of lymphocytes accompanied this decrease in WBC, and there were no changes in blood cell indices in normal or 8-month-old hamsters with cardiomyopathy.

Table 15. Differential Measurements of White Blood Cells in Hamsters at 24 Hours After Exposure to Air or PM

Animal Model	Exposure (μg/m^3)	*n*	PMN[a] (%)	Lymphocytes[a] (%)	Total WBC[a] (cells/mL)
Normal hamsters	Air	3	29 ± 8	68 ± 8	2,398 ± 149
	PM = 134 Sulfate = 12	5	41 ± 4	58 ± 4	3,219 ± 324
8-Month-old hamsters with cardiomyopathy	Air	5	49 ± 9	50 ± 9	3,888 ± 352
	PM = 184 Sulfate = 18	5	45 ± 6	40 ± 9	3,990 ± 459
10-Month-old hamsters with cardiomyopathy	Air	3	52 ± 13	46 ± 13	4,132 ± 666
	PM = 466 Sulfate = 68	3	69 ± 10	29 ± 11	2,624 ± 8[b]

[a] Results shown as mean ± SE.

[b] The difference between air-exposed and PM-exposed group means is significant ($P < 0.05$; Mann-Whitney *U* test).

Heart Rate and ECG Waveforms

No changes in heart rate were observed in normal hamsters or in hamsters with cardiomyopathy at 8 months or 10 months after exposure to concentrated ambient PM (Table 16). The ECG waveforms of hamsters were irregular compared with those of rats. A method of analysis different than that used on the rat ECG data was therefore performed on the hamster data. The waveforms were analyzed for the number of bursts that contained all normal beats, skipped beats, P wave abnormalities, or QRS complex abnormalities. PVCs and PACs were extremely rare, and no differences between air-exposed and PM-exposed animals were observed (data not shown). Each endpoint was normalized to the total number of beats and is presented in Tables 17 through 19. The only statistically significant difference between air-exposed and PM-exposed hamsters in these ECG-derived endpoints was a decrease in skipped beats in the 8-month-old hamsters with cardiomyopathy at 7 to 12 hours after PM exposure. This difference between air-exposed and PM-exposed animals was also noted before exposure, suggesting that the observation was not exposure related. This effect was not observed in the normal or the 10-month-old hamsters with cardiomyopathy.

Table 16. Change in Heart Rate from Baseline Values After Exposure of Hamsters to Air or PM

Animal Model	Exposure (μg/m^3)	*n*	1–6 Hours After Exposure		7–12 Hours After Exposure		13–18 Hours After Exposure	
			Change[a]	*P*	Change[a]	*P*	Change[a]	*P*
Normal hamsters	Air	4	4.6 ± 7.4	0.118	–23.3 ± 7.6	0.495	–20.2 ± 10.1	0.432
	PM = 134 Sulfate = 12	5	27.6 ± 9.8		–14.5 ± 9.1		–7.3 ± 11.1	
8-Month-old hamsters with cardiomyopathy	Air	4	75.3 ± 4.1	0.001	28.5 ± 5.7	0.003	4.16 ± 12.1	0.090
	PM = 184 Sulfate = 18	5	16.7 ± 6.0		–2.4 ± 4.4		–21.5 ± 6.7	
10-Month-old hamsters with cardiomyopathy	Air	3	–2.4 ± 7.5	0.349	–13.9 ± 6.4	0.408	–15.4 ± 9.3	0.296
	PM = 466 Sulfate = 68	3	21.6 ± 21.5		11.0 ± 19.7		6.0 ± 15.1	

[a] Mean heart rate (± SE) during the 1-hour preexposure baseline period was subtracted from the mean hourly postexposure values.

Table 17. ECG Waveform Changes in Normal Hamsters Exposed to Air or PM

Parameter	Exposure	Before Exposure[a]	During Exposure[a]	0–6 Hours After Exposure[a]	7–12 Hours After Exposure[a]	13–18 Hours After Exposure[a]
Normal bursts	Air	0.94 ± 0.06	0.95 ± 0.07	0.90 ± 0.07	0.95 ± 0.03	0.85 ± 0.05
	PM	0.88 ± 0.07	0.86 ± 0.04	0.95 ± 0.03	0.84 ± 0.07	0.82 ± 0.06
Skipped beats	Air	0.06 ± 0.06	0.05 ± 0.03	0.10 ± 0.07	0.05 ± 0.03	0.09 ± 0.07
	PM	0.11 ± 0.07	0.14 ± 0.04	0.05 ± 0.03	0.16 ± 0.07	0.14 ± 0.04

[a] Mean ± SE; $n = 3$ for air-exposure group and $n = 5$ for PM-exposure group. The number of bursts scored as containing an arrhythmia was divided by the total number of scoreable bursts. P wave abnormalities were not observed. QRS-segment abnormalities were too few to analyze (only observed at 13 to 18 hours for 1 air-exposed animal and 1 PM-exposed animal).

Table 18. ECG Waveform Changes in 8-Month-Old Hamsters with Cardiomyopathy Exposed to Air or PM

Parameter	Exposure	Before Exposure[a]	During Exposure[a]	0–6 Hours After Exposure[a]	7–12 Hours After Exposure[a]	13–18 Hours After Exposure[a]
Normal bursts	Air	0.38 ± 0.16	0.22 ± 0.09	0.54 ± 0.18	0.45 ± 0.19	0.26 ± 0.13[b]
	PM	0.50 ± 0.22	0.30 ± 0.30	0.58 ± 0.18	0.61 ± 0.19	0.71 ± 0.12[b]
Skipped beats	Air	0.25 ± 0.10[b]	0.06 ± 0.06	0.19 ± 0.06	0.23 ± 0.07	0.39 ± 0.05
	PM	0[b]	0.03 ± 0.03	0.10 ± 0.06	0.07 ± 0.03	0.19 ± 0.08
P wave abnormalities	Air	0.13 ± 0.13	0.01 ± 0.01	0.06 ± 0.06	0.15 ± 0.15	0.11 ± 0.11
	PM	0	0.31 ± 0.31	0	0	0
QRS-segment abnormalities	Air	0.44 ± 0.26	0.72 ± 0.10	0.32 ± 0.23	0.41 ± 0.21	0.42 ± 0.20
	PM	0.50 ± 0.50	0.67 ± 0.33	0.36 ± 0.20	0.33 ± 0.19	0.17 ± 0.13

[a] Mean heart rate ± SE; $n = 4$ for air exposure and $n = 5$ for PM exposure. The number of bursts scored as containing an arrhythmia was divided by the total number of scoreable bursts.

[b] Significant difference between air and PM exposure groups ($P < 0.05$; Mann-Whitney U test).

Table 19. ECG Waveform Changes in 10-Month-Old Hamsters with Cardiomyopathy Exposed to Air or PM

Parameter	Exposure	Before Exposure[a]	During Exposure[a]	0–6 Hours After Exposure[a]	7–12 Hours After Exposure[a]	13–18 Hours After Exposure[a]
Normal bursts	Air	0.67 ± 0.24	0.37 ± 0.32	0.63 ± 0.18	0.59 ± 0.18	0.75 ± 0.20
	PM	0.67 ± 0.07	0.72 ± 0.26	0.92 ± 0.08	0.81 ± 0.06	0.66 ± 0.06
Skipped beats	Air	0	0.02 ± 0.02	0	0	0
	PM	0.07 ± 0.07	0.01 ± 0.01	0	0	0.03 ± 0.03
P wave abnormalities	Air	0.33 ± 0.24	0.61 ± 0.31	0.32 ± 0.18	0.35 ± 0.21	0.25 ± 0.20
	PM	0.13 ± 0.13	0.07 ± 0.07	0.19 ± 0.10	0.19 ± 0.06	0.21 ± 0.13
QRS-segment abnormalities	Air	0	0	0	0.06 ± 0.06	0
	PM	0.13 ± 0.13	0.23 ± 0.23	0.11 ± 0.11	0	0.10 ± 0.10

[a] Mean ± SE; $n = 3$/group. The number of bursts scored as containing an arrhythmia was divided by the total number of scoreable bursts.

DISCUSSION AND CONCLUSIONS

Considerable controversy surrounds the association between ambient PM and increases in cardiopulmonary morbidity and mortality. In order to support the biologic plausibility of the adverse health effects of ambient PM, mechanistic links between PM exposure and adverse health need to be established. Important as well is the need for susceptible individuals and disease entities to be identified. These factors are especially critical for regulatory purposes in defining acceptable ambient concentrations of PM. The focus of the present study was to examine whether subpopulations of individuals with preexisting cardiopulmonary disease are more susceptible than healthy individuals to the adverse effects of inhaled PM. Because of ethical issues involved in the exposure of individuals with moderate to severe disease to PM under controlled conditions, we used animal models of cardiopulmonary disease to examine the question of susceptible individuals. F344 rats with monocrotaline-induced pulmonary hypertension and right heart hypertrophy were used as a model of pulmonary hypertension. Bio TO-2 Syrian hamsters undergoing spontaneous cardiomyopathy were used as a model of cardiac failure.

Although other investigators have found life-threatening effects in animals exposed to concentrated ambient PM, we observed only a limited number of adverse changes in normal and compromised animals after a single exposure to concentrated ambient PM at concentrations as high as 919 $\mu g/m^3$. No apparent PM-related effects were observed in rats or hamsters exposed for 6 hours/day for 3 consecutive days. Moreover, no major pulmonary effects were observed in either compromised animal model. Histologic findings were unremarkable in the lungs of normal rats exposed to concentrated ambient PM, and no visible changes were observed in the lung tissue from PM-exposed rats treated with monocrotaline. Similarly, no adverse effects on lung volume measurements or diffusing capacity were observed in normal or compromised animals exposed to concentrated ambient PM. Adverse changes in the lung were observed only in monocrotaline-treated rats exposed to 400 $\mu g/m^3$ concentrated ambient PM. In these animals, modest increases in protein, total cells, and LDH activity were observed in lavage fluid.

These changes did not occur in monocrotaline-treated rats exposed to lower PM concentrations and, therefore, may reflect a dose response. These isolated effects noted in lavage fluid may also have been due to a change in the composition of ambient PM on that exposure day. Because the H^+ concentration was below the detection limit and the sulfate concentration was greater on exposure days in which no adverse effects were seen, we have not determined what chemical constituents might be involved. Ongoing analyses of metal content on collected filter samples may provide more information. Moreover, regardless of the statistical significance of these changes noted in lavage fluid, the biologic significance of these changes is limited in explaining PM-associated increases in mortality.

The lack of major changes in the pulmonary response to inhaled concentrated ambient PM suggests that the life-threatening effects of PM identified in epidemiologic studies are unlikely to be due to lung inflammation or injury. A large number of controlled-exposure studies have demonstrated that environmental and occupational air pollutants (including ozone, nitrogen oxides, zinc oxide, cigarette smoke, and endotoxin) produce marked pulmonary injury and inflammation in human subjects. These pollutants, however, are not associated with acute life-threatening illness at the concentrations that were used to produce these effects in the laboratory. Thus, it is unlikely that direct pulmonary injury is mechanistically involved in the acute mortality changes associated with episodic exposure to ambient PM.

Potentially significant PM-induced changes in cardiac endpoints, suggestive of a stress response, were observed in our experiments. On some exposure days, small but statistically significant increases in heart rate were observed for several hours after a 3-hour exposure to concentrated ambient PM. The increases in heart rate associated with PM have been reported in studies by 3 groups of investigators (Liao et al 1999; Peters et al 2000; Pope et al 2000). Pope and associates (2000) reported results from a panel study of 90 elderly subjects in Utah Valley, an area that has periodic episodes of increased PM during the winter. A small (5 to 10 bpm) increase in daily heart rate was found to be significantly associated with increased PM levels on the previous 1 to 5 days. Heart rate response to PM was similar in healthy subjects and in subjects with chronic cardiac or respiratory disease. Peters and colleagues (2000) reanalyzed heart rate data from the MONICA study, an epidemiologic study of randomly selected adults aged 25 to 64 in southern Germany. The study period included a severe air pollution episode in which sulfur dioxide reached 200 $\mu g/m^3$ and total suspended particles ranged from 60 to 176 $\mu g/m^3$. Analysis of data from over 2,500 adults indicated that the pollution episode was associated with a small but significant increase (2 to 3 bpm) in daily heart rate. The association between increased heart rate and PM exposure was not affected by adjustment for weather, age, or other cardiovascular risk factors. In a similar fashion, Liao and colleagues (1999) demonstrated in a panel study in a retirement center that change in heart rate

variability was consistently associated with variations in daily PM levels.

Taken together, these animal and human studies indicate that increased levels of ambient outdoor PM can cause small increases in heart rate in normal and compromised individuals and that this effect is unlikely to be caused by copollutants or weather conditions. Are these small increases in heart rate biologically relevant? How could these changes possibly relate to mortality? A case can be made that small increases in heart rate are biologically significant and do represent an adverse heath effect. PM-induced increases in heart rate could be caused by an alteration in the autonomic nervous system. Changes in heart rate, regardless of the cause, are ultimately mediated by changes in input from the sympathetic and parasympathetic nerves that innervate the heart. Release of acetylcholine by parasympathetic nerves slows the heart rate (by reducing the firing rate of the sinoatrial node, the pacemaker of the heart), and release of epinephrine from sympathetic nerves increases the heart rate. A neural connection is known to exist between the respiratory tract and the autonomic nervous system. Activation of afferent neural receptors found in the airway epithelium by mechanical and chemical stimuli (such as inhalation of smoke, ether, or ammonia) has been shown to cause significant stimulation of the sympathoadrenal system.

In general, a small increase in heart rate could be mediated by a reduction in the parasympathetic input to the heart, whereas a moderate increase in heart rate could involve increased activity of sympathetic cardiac nerves as well as decreased parasympathetic input. Large increases in heart rate may be mediated by increased activity of the sympathetic nervous system and secretion of epinephrine from the adrenal gland into the blood stream. Such a change in catecholamines could be responsible for the transient increases in neutrophils, via demargination, observed in our studies. Thus, because our studies found no PM-related pulmonary inflammation or injury, direct pulmonary toxicity probably did not cause the observed cardiac changes. Therefore, PM-activated neural receptors in the respiratory tract may have caused these systemic effects.

We observed no life-threatening arrhythmias in normal or compromised animals after exposure to concentrated ambient PM. Our results contradict those reported by investigators examining the cardiac effects of inhaled or instilled residual oil fly ash. Others (Killingsworth et al 1997) have observed deaths in monocrotaline-treated rats exposed to fuel oil fly ash. Watkinson and associates (1998) found that instilled residual oil fly ash produced serious cardiac arrhythmia in normal rats and serious cardiac arrhythmia and deaths in monocrotaline-treated rats. At the present time, no peer-reviewed publications have reported cardiac changes produced by concentrated ambient PM in rodents. Godleski and colleagues have presented, at annual HEI conferences and other national meetings, evidence of life-threatening ECG changes and increased deaths in compromised animals (rats with sulfur dioxide–induced chronic bronchitis or high-dose monocrotaline–induced pulmonary hypertension) exposed to concentrated ambient PM. Such life-threatening effects after exposure to concentrated ambient PM have not been observed in our laboratory or in studies conducted with monocrotaline-treated rats at the US Environmental Protection Agency in North Carolina (Dr Dan Costa, personal communication, 1999).

Although differences in the source of concentrated ambient particles, the type of concentrator, or both may influence the health outcomes observed in the different laboratories, our animal models of compromised health may be less sensitive to the toxicity of concentrated ambient PM. Not surprisingly, the heart rate and blood cell effects of PM were not observed on all exposure days. Moreover, no gravimetric concentration response was evident, suggesting that day-to-day changes in particle composition may be responsible, at least in part, for the variable response. Epidemiologic studies have reported, however, that adverse cardiopulmonary effects of ambient PM occur in several regions of North and South America, Europe, and Asia. The particle composition of these regions varies a great deal, suggesting that the adverse health effects are due to a general particle effect rather than the specific chemical constituents of ambient PM. The results of our controlled-exposure animal studies appear to contradict findings of the epidemiologic studies. Cardiac changes were not observed on all exposure days and did not appear to be dose related. A similar day-to-day variation in response to concentrated ambient PM, however, has been reported by Godleski and colleagues (2000).

We propose that, despite the apparent general particle effect observed in epidemiologic studies, particle composition is critical in the production of adverse changes in the cardiopulmonary system. Important differences in response to key components of ambient particles have been reported in inhalation toxicology studies. For example, sulfate, a major component of ambient particles in the northeastern United States, exists in several forms that vary in their toxicological potency. Metals are trace components of ambient PM and also display significant differences in their toxicologic properties. The valence state of the metal compound as well as the element influence the pulmonary toxicity of metals. Importantly,

Dreher and colleagues (1996) have reported differential toxicity of the soluble metals present in residual oil fly ash. Thus, our findings of day-to-day variations in response to PM are consistent with the established differential toxicity of some major components of urban particles.

The lack of any significant increase in sensitivity to particle inhalation when comparing animals with compromised cardiopulmonary health to normal animals was unexpected. Numerous controlled inhalation studies using healthy animals and human subjects have demonstrated that few adverse pulmonary effects occur after exposure to concentrations of airborne particles much higher than those encountered in the ambient urban environment. For example, the exposure of healthy human subjects to concentrations of sulfuric acid as high as 1,000 $\mu g/m^3$ has resulted in no or minimal changes in nonspecific airway responsiveness and bronchoalveolar lavage indices of inflammation and injury (Utell et al 1984). Asthmatic subjects, however, have responded to approximately one tenth the concentration of sulfuric acid particles (Utell et al 1984; Koenig et al 1989). In one experiment, monocrotaline-treated rats showed evidence of pulmonary toxicity and inflammation. Such changes were not observed in experiments with normal rats. Thus, complicated studies with dose-response exposure regimens, including the exposure of normal and compromised animals within a given day, may be necessary to address adequately the issue of increased susceptibility to PM in individuals with compromised cardiopulmonary health.

IMPLICATIONS OF FINDINGS

The 2 main objectives in our study were to determine whether concentrated ambient PM can produce life-threatening cardiopulmonary changes in rodents and whether animals with compromised health are more sensitive than noncompromised animals to the adverse effects of concentrated ambient PM. Our studies demonstrated that little direct toxicity occurred in the lungs of normal or compromised animals exposed to concentrated ambient PM up to 6 times the current 24-hour National Ambient Air Quality Standard for $PM_{2.5}$. Cardiovascular system changes, in the form of increased heart rate and neutrophil and lymphocyte changes in peripheral blood, were observed in rats within a few hours after exposure to concentrated ambient PM. Together, these observations suggest that the systemic changes produced by inhaling ambient PM are not sequelae of direct injury and inflammation in the respiratory system. Although we did not measure the release of inflammatory mediators, such as cytokines, from the lung after PM exposure, biologically significant increases in these inflammatory mediators would be unlikely outside the respiratory system. Future research should therefore focus on other pathways, such as neural reflex pathways, by which inhaled particles might produce systemic effects. Identification of the mechanisms by which inhaled ambient PM produces systemic effects is a key research priority for establishing the biological plausibility of PM-associated increases in cardiac-based deaths.

The lack of life-threatening cardiopulmonary effects in our animals exposed to concentrated ambient PM could be interpreted to suggest that the epidemiologic findings of PM-associated adverse health effects are incorrect. Two common criticisms of the epidemiologic studies are that the databases have been analyzed in the same general, and possibly incorrect, manner and that these analyses are not sufficiently controlled for confounding factors such as copollutants, temperature, and season. We do not feel, however, that our findings should be used to imply that the epidemiologic findings are spurious.

More animal studies are necessary to confirm our work. These studies must include other, potentially more sensitive, animal models of compromised cardiopulmonary health. Moreover, investigators must consider the potential for seemingly small changes in homeostasis to result in catastrophic effects in certain individuals. Although the PM-associated increases in heart rate observed by others in human populations and by us in normal and compromised rats are small, such seemingly unimportant changes could produce increases in mortality in certain susceptible individuals. The association of increased mortality with small drifts in the homeostasis of cardiovascular indices, such as heart rate or coagulability, could be observed only in large population studies. Thus, except in laboratory experiments using thousands of animals, studies may not be large enough to identify the mechanisms underlying PM-induced increases in mortality.

Our studies also demonstrated that adverse cardiac effects did not occur on all exposure days and that a dose response was not apparent. Thus, the chemical composition of the concentrated particles was likely to be the dominant influence on particle toxicity. Reports by other investigators have shown similar findings in animals exposed to inhaled concentrated ambient PM and in animals in which ambient particles collected on sampling media were instilled via the trachea. If future controlled laboratory exposures of animals and human subjects confirm the day-to-day variability in the toxicity of inhaled concentrated ambient PM, this finding would strongly suggest that PM-monitoring programs instituted by government agencies should not be restricted to measurement of gravimetric mass concentrations. Substantial effort should be

made to address the chemical characterization of ambient urban particles. In particular, these monitoring programs should be closely coordinated with ongoing and future epidemiologic studies in an effort to provide epidemiologists with the research tools needed to identify the components of PM responsible for the increases in morbidity and mortality.

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OTHER PUBLICATIONS RESULTING FROM THIS RESEARCH

Gordon T, Gerber H, Fang CP, Chen LC. 1999. A centrifugal particle concentrator for use in inhalation toxicology. Inhalation Toxicol 11:101–117.

ABBREVIATIONS AND OTHER TERMS

ANOVA	analysis of variance
BB units	Berger-Broida units; 1 BB = 0.48 IU
bpm	beats per minute
COPD	chronic obstructive pulmonary disease
DLCO	diffusing capacity of carbon monoxide
ECG	electrocardiogram
EPA	US Environmental Protection Agency
EU	endotoxin units
FRC	functional residual capacity

σ_g	geometric standard deviation
HEPA	high-efficiency particulate air (filter)
IC	inspiratory capacity
LDH	lactate dehydrogenase
MMAD	mass median aerodynamic diameter
MΩ	megaohm
NIST	National Institute of Standards and Technology
PAC	premature atrial contraction
PBL	peripheral blood
PM	particulate matter
PM_{10}	This term is commonly used to denote particles less than or equal to 10 µm in aerodynamic diameter; technically, it refers to the cut size of the sampler inlet or impactor stage for which collection efficiency is 50%.
PMN	polymorphonuclear leukocyte
PVC	premature ventricular contraction
RAM-1	real-time aerosol monitor
RBC	red blood cell
RV	residual volume
TLC	total lung capacity
V_A	alveolar volume
VC	vital capacity
WBC	white blood cell
Ω^{-1}	reciprocal ohm

INTRODUCTION

Epidemiologic studies have indicated that exposure to low levels of particulate matter (PM)*, even below the standards set by the US Environmental Protection Agency, is associated with an increase in morbidity and daily mortality, particularly in individuals with compromised cardiopulmonary function (reviewed in US Environmental Protection Agency 1996). A plausible mechanism linking low-level particle exposure and pathophysiologic effects, however, has not been established. Assessing the effects of PM in appropriate animal models is critical in studying how PM may exert adverse health effects.

In 1994, HEI issued RFA 94-2 to address these and other outstanding issues in PM research. In response, Terry Gordon and colleagues at the New York University School of Medicine proposed to study the effects of exposure to PM on the cardiac and pulmonary function of normal rats and hamsters and rats and hamsters with cardiopulmonary disease. Gordon hypothesized that animals with compromised cardiopulmonary function would be more sensitive than normal animals to the effects of PM. He proposed that PM would affect cardiac function, and that this would be mediated indirectly, via the induction of responses in the airways. In addition to monitoring PM-induced cardiac changes, he proposed to evaluate changes in the airways histologically, biochemically, and by measuring respiratory function. To maximize possible PM effects, he proposed using the Gerber particle concentrator, which delivers particles concentrated up to 10 times their level in ambient air. Because epidemiologic studies suggested that responses to PM occurred with little or no lag time, Gordon proposed to measure changes in cardiac and airway responses during or shortly after single or repeated exposures. The HEI Research Committee funded the proposal because it thought the study provided an innovative approach to assessing the health effects of PM in potentially relevant animal models.†

This Commentary is intended to aid HEI sponsors and the public by highlighting the strengths of the study, pointing out alternative interpretations, and placing the report into scientific perspective.

JUSTIFICATION FOR THE STUDY

Although epidemiologic studies have described an association between exposure to short-term increases in PM and short-term increases in morbidity and mortality due to cardiopulmonary causes, a plausible pathophysiologic mechanism for such an effect has not yet been established. Prior to the current application, several approaches tried to define such a link by investigating the potentially toxicologic effects, particularly to the airways, of different components of PM in controlled animal exposures in a number of species. In general, the results of these studies showed only small effects of low levels of pollutants even following chronic exposure, and unless exposure was to very high levels of pollutants, responses did not lead to acute mortality (Mauderly 1994). A number of studies tested the effects of sulfuric acid particles, which comprise a significant fraction of ambient PM. For example, Gearhart and Schlesinger (1989) showed that inhalation of 250 $\mu g/m^3$ sulfuric acid for 1 hour/day for 5 days/week over 4, 8, or 12 months decreased mucociliary clearance of particles from the bronchial area of the lung in rabbits. Amdur and Chen (1989) reported that a 3-hour aerosol exposure of 20 to 60 $\mu g/m^3$ sulfuric acid adsorbed onto 1 to 5 mg/m^3 zinc oxide impaired the oxygenation of blood across the guinea pig alveolar capillary membrane and increased indicators of an inflammatory response in bronchoalveolar lavage (BAL). In a long-term study of a respirable sulfite aerosol in beagle dogs, Heyder and colleagues reported few changes in respiratory lung function, a small decrease in alveolar macrophage phagocytic capacity, and a small increase in BAL inflammatory parameters (Heyder et al 1992).

The paucity of effects induced by low levels of PM suggested either that the animals chosen to study might not be sensitive to PM effects, or that the acid aerosols that were investigated were missing some more active constituent. To address these issues, research focused on pollutant effects in animals that modeled human populations with inflammatory lung diseases, as epidemiologic studies suggested these individuals might be particularly sensitive to

* A list of abbreviations and other terms appears at the end of the Investigators' Report for your reference.

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† Dr Terry Gordon's 3-year study, *Health Effects of Ambient Particulate Matter in Compromised Rodent Models*, began in July 1995 with total expenditures of $340,254. The Investigators' Report from Dr Gordon and colleagues was received for review in December 1998. A revised report, received in July 1999, was accepted for publication in August 1999. During the review process, the HEI Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Report and in the Review Committee's Commentary.

PM effects. One model of inflammatory lung disease is the monocrotaline-injected rat, which develops right-heart hypertrophy and pulmonary hypertension. Costa and colleagues (1994) showed that intratracheal instillation of high concentrations of residual oil fly ash, a highly toxic combustion-generated urban particulate, increased inflammation in the airways and led to the death of these animals. The study did not, however, suggest a mechanism that linked the inflammatory response to the pulmonary and cardiac effects of PM.

To explain the possible link between PM and cardiovascular effects in the monocrotaline-treated rat, in the application for the current study Gordon and colleagues proposed to evaluate whether acute cardiac effects might occur as a consequence of rapid responses to PM in the airways. They also proposed to study an additional model of human cardiopulmonary disease, hamsters with cardiomyopathy, in which they suggested that evidence for similar pathophysiologic effects of PM might be found. To test a physiologically relevant PM mixture that reflected many of the components found in ambient air, Gordon and colleagues proposed to study the effects of concentrated PM in the size range of 0.2 to 2.5 μm, derived from New York City air. Gordon and colleagues were among the first to attempt to assess the relationships among cardiac, inflammatory, and pulmonary parameters in the response to inhaled concentrated PM in both normal rodents and in rodents with cardiopulmonary conditions thought to model relevant human diseases.

TECHNICAL EVALUATION

AIMS AND OBJECTIVES

The aim of the study was to test the hypothesis that PM causes life-threatening physiological alterations in rodents with conditions thought to model humans with cardiopulmonary disease: rats injected with monocrotaline to induce pulmonary hypertension (a model of cor pulmonale) and hamsters with a genetic cardiomyopathy (a model ofing congestive heart failure). The investigators proposed to determine whether inhalation of concentrated PM derived from New York City air caused greater effects in those rodents with cardiopulmonary conditions than in healthy animal controls. The investigators evaluated changes in heart rate, electrocardiogram (ECG) tracing, inflammatory indicators, and pulmonary function.

STUDY DESIGN

Rats and hamsters were exposed to concentrated PM (132–919 μg/m^3) nose-only for 3 hours, or in some experiments for 6 hours, on either 1 or 3 different days. All experiments consisted of a concentrated PM-exposed group and a control, filtered air–exposed group. Each group used 5 or 6 rats and 3 to 5 hamsters. In some experiments, groups were killed at 3 and 24 hours following exposure, while in other experiments groups were killed at only 1 of these time points.

The investigators focused on concentrated PMs responses in normal and monocrotaline-injected young rats. Responses in hamsters and in older rats, with or without monocrotaline treatment, were evaluated in only a few experiments. The limited number of experiments performed and the small group size are factors that reduced the possibility of observing positive results.

METHODS

Ambient Particle Concentrator

The fine particle concentrator used was a Gerber centrifugal aerosol concentrator, which can concentrate ambient PM approximately tenfold in the size range 0.2 to 2.5 μm mean mass diameter. The concentrator is described in Figures 2 and 3 of the Investigators' Report. Ambient air was collected via an inlet valve on the eighth floor of a New York University building in New York City. Air was introduced to the concentrator at a flow rate, V_1, by a blower with a Teflon seal and an isolated motor to prevent foreign particles from being introduced into the system. The air volume then traveled along a concentric annulus formed by a stationary outer cylinder and a porous inner cylinder rotating at high speed. A portion of the total air volume was pulled through the porous cylinder and into a porous shaft by a pump attached to one end of the shaft at a flow rate V_3 ($V_3 < V_1$). Airborne particles within the annulus move radially due to centrifugal force and laterally along the surface of the cylinder, finally exiting the concentrator at a flow rate, V_2 (V_1–V_3). If the air moving into the porous cylinder and shaft contains no particles, the increase in particle concentration of the air exiting the system is V_2/V_1. Particles may move inward toward the porous shaft and be removed from the system, however, if their sedimentation velocity is less than the velocity of air leaving the shaft. The sedimentation velocity depends on particle diameter and density as well as the angular velocity and dimensions of the rotor.

The upper size limit of particles collected in this system was determined to be approximately 2.5 μm. The Gerber concentrator concentrates particles around 1.0 μm with

most efficiency, while smaller particles are collected with much lower efficiency; particles below 0.2 µm are not concentrated.

The investigators characterized only the particle mass and sulfate level of the ambient air and concentrated particles. More information about the physical and chemical composition of the PM, such as its metal components or carbon levels, would have been helpful in evaluating the results of this study and comparing these results with others. Difficulty in fully characterizing the PM components, however, may be a general problem with the Gerber concentrator. It exposes animals to only a small volume of air at a low flow rate, and it is likely that only a small fraction of the input air can be removed to provide samples for all PM characterization analyses.

Animals

The investigators evaluated concentrated PM effects on young or 6-month-old male F344 rats, previously injected with either saline (controls) or 40 mg/kg monocrotaline to induce right-heart hypertrophy and pulmonary hypertension. Since preliminary experiments indicated that the hypertrophic period (increased ratio of right ventricle/left-heart weight) lasted from 2 until 4 weeks after monocrotaline injection, the investigators exposed the rats to concentrated PM during that time period. The investigators also tested the effects of concentrated PM on 8- to 10-month-old male cardiomyopathic (Bio TO-2) and weight-matched control (Bio-F1B strain) hamsters. The Bio TO-2 hamster strain develops progressive cardiac failure and arrhythmias and dies after the age of 11 months.

Pulmonary Function

The investigators sequentially measured pulmonary mechanical function in anesthetized animals at 3 and 24 hours after a 3-hour exposure to concentrated PM or air using previously described methods (Takezawa et al 1980). Vital capacity (VC) and inspiratory capacity were measured first, followed by total lung capacity and diffusing capacity of carbon monoxide (DLCO). The investigators computed functional residual volume and residual volume by standard formulas (Morris et al 1984).

Cellular And Biochemical Parameters

Leukocyte counts were determined in BAL fluid and peripheral blood (PBL) at the time of killing, which was 3 or 24 hours following exposure. Peripheral blood platelet to red blood cell ratios were determined in smears, and platelet number was assessed by Coulter counting. Gordon and colleagues also determined protein and lactate dehydrogenase (LDH) content of lavage fluid, as markers of cellular damage reflecting an inflammatory response in the airways.

Lung Histology

The investigators evaluated paraffin-embedded sections of rat lungs (4 µm thick; stained with hematoxylin and eosin).

Telemetry Monitoring

To monitor heart rate, temperature, and ECG, the investigators inserted a transmitter into the peritoneal cavity of each test animal. The negative lead of the transmitter was placed in the right clavical region and the positive lead in the left groin region. Heart rate and temperature were monitored every 5 minutes starting 1 hour before until up to 18 hours following the exposure. ECG waveforms were monitored over 10 second periods every 15 minutes before exposure, every 5 minutes during exposure, and every 30 minutes in the 18 hours after exposure. The investigators reported comparisons between concentrated PM and air exposure for normal bursts, skipped beats, P wave abnormalities, and QRS abnormalities in cardiomyopathic hamsters; and for PR segment, RT interval, and T wave duration in monocrotaline-treated rats. The investigators calculated hourly mean heart rate and temperature for each animal.

RESULTS

The investigators' key findings are summarized in Table 1.

COMMENTS ON KEY FINDINGS

Cardiac Effects

- No ECG changes were detected in any rat or hamster group; no arrhythmia induction was evident in either control or monocrotaline-treated rats.
- Heart rate in young healthy and monocrotaline-treated rats increased approximately 5% increase within 6 hours after exposure; on some, but not all, exposure days.

Inflammatory Responses

- Lavage fluid at 3 or 24 hours after concentrated PM exposure showed no biochemical or cellular changes in any rat or hamster group tested.
- Sporadic changes were noted 3 or 24 hours after concentrated PM exposure:

  At 3 hours after exposure, the percentage of circulating neutrophils increased and the percentage of lymphocytes decreased in young healthy rats (2/5 days), young, monocrotaline-treated rats (2/3 days), and old monocrotaline-treated rats (1 experiment). The total WBC did not change in any group, including those showing changes in leukocyte percentages. In experiments showing changes in PBL leukocyte composition at 3 hours after exposure, leukocyte percentages at 24 hours after exposure were similar for groups receiving concentrated PM or air.

  At 24 hours after exposure, circulating neutrophil and lymphocyte percentages were similar in all PM and control groups. Total WBC decreased in the young, monocrotaline-treated rats (1/6 experiments) and healthy old rats (1 experiment) receiving concentrated PM.

  At 24 hours after exposure of hamsters, the total WBC or leukocyte composition in control or 8-month-old cardiomyopathic animals did not change; total WBC in 10-month-old cardiomyopathic animals decreased.

  In 3 experiments in normal rats exposed to concentrated PM, 1 animal showed an increased platelet/RBC ratio at 3 hours but not at 24 hours after exposure, 1 animal showed a 15% increase in platelet number at 3 hours after exposure, and 1 animal showed no change.

- Inflammatory changes in the structure of rat lung tissue were attributed to monocrotaline injection. No effects of concentrated PM were observed.

Pulmonary Mechanics

No change observed in lung volumes or DLCO in any group except for an increase in DLCO in old, monocrotaline-injected rats, suggesting an enhanced capacity of gas transfer.

Table 1. Effects of Concentrated PM in Rats and Hamsters

	Young Rats		Old Rats		Hamsters	
Parameters	Healthy	Monocrotaline Injected	Healthy	Monocrotaline Injected	Healthy	Monocrotaline Injected
Cardiac						
ECG changes	—	—	—	NT	—	—
Heart rate	↑ (2)	↑ (2)	— (1)	NT	— (1)	— (1)
Inflammatory Response						
BAL 3 hr after exposure	— (1)	— (2)	NT	—	—	— (1)
BAL 24 hr after exposure	— (1)	— (6)	— (1)	— (1)		
PBL (number or composition)	inconsistent changes (6)	inconsistent changes (3)	NT	— (1)	— (1)	changed (1)
Lung histology	—	—	NT	—	NT	NT
Pulmonary Mechanics						
Lung capacity (various measurements)	—	—	—	—	—	—

[a] Numbers in parentheses indicate the number of experiments performed. – = no effect; ↑ = small increase; NT = not tested.

SUMMARY OF RESULTS

The investigators found that concentrated PM had little or no effect on the parameters they measured. This included no change in ECG waveforms in either control or monocrotaline-treated rats, and no detectable changes in cardiac, pulmonary function, or inflammatory parameters of healthy hamsters or those with cardiomyopathy.

Some concentrated PM–induced effects on rat cardiac and inflammatory parameters were observed sporadically, but it is possible that these were within fluctuations expected of multiple testing. In experiments in which changes were reported in the period immediately after exposure (small increases in heart rate in first 6 hours and small changes in the percentages of circulating leukocytes at 3 hours), these effects were transient. They were not observed at 24 hours after exposure. Changes in concentrated PM–induced responses were similar in both control and monocrotaline-treated rats.

DISCUSSION

The premise for this exploratory study was that particles concentrated from ambient air would have life-threatening acute effects on rodents with preexisting cardiopulmonary disease. One of the strengths of the study was that it examined PM effects in 2 rodent models—rats with pulmonary hypertension and hamsters with a genetic predisposition to cardiomyopathy—which may model human subpopulations potentially susceptible to PM effects (discussed in Kodavanti et al 1998). A further strength of the study was that it used genetically identical animals, which should be more uniform in their responses than outbred populations.

The investigators found, however, that concentrated PM had little or no effect on the parameters they measured. They point out correctly that their results are based on small numbers of animals, and they suggest that responses might have been detected if group sizes had been larger. In contrast, some previous studies have indicated that rats and hamsters respond to different types of PM. For example, rats and hamsters synthesize proinflammatory cytokines in response to particles resuspended from filters (Li et al 1997) and as a consequence of smoke inhalation, respectively (Brain et al 1998). In addition, inhalation or instillation of the highly toxic residual oil fly ash into monocrotaline-injected rats has been shown to induce ECG changes associated with fatal arrhythmias (Watkinson et al 1998), induction of proinflammatory cytokines (Killingsworth et al 1997; Kodavanti et al 1997), and death (Costa et al 1994; Killingsworth et al 1997; Watkinson et al 1998).

The current study differs in both methodology and design from these previous studies, and issues such as differences in the level and composition of PM or concentrated PM components at different geographical locations and the route of PM exposure are likely to account for the discrepant results. The current study was the first to use the Gerber concentrator to provide particles for toxicologic studies, and it is apparent that the day-to-day performance of the concentrator varied, as shown by the variability in the concentration factor (3 to 20, on days on which ambient PM could be reliably measured [above 10 $\mu g/m^3$]). In addition, although Gordon and colleagues did not chemically characterize the particles used in this study sufficiently to distinguish differences from day to day, results of other studies indicate that the composition of ambient air PM was likely to be distinct on different study days as a result of seasonal and daily variation (Altshuller 1980; Spengler and Thurston 1983; Godleski et al 2000). As a consequence, it is likely that animals in the current study were exposed to different levels of potentially critical components of the PM mixture on different days. This might explain the sporadic nature of the positive results: on some days, 1 or more of the critical PM components were present at too low a concentration in the concentrated PM to exert effects on animals.

In this context, it should be noted that the study focused on the effects of particulate rather than gaseous components of the pollutant mix: the metal housing of the concentrator removes ozone and sulfur dioxide, 2 gases in the ambient air that can induce inflammatory responses in humans and other species. In addition, because the inlet for collecting particles to be concentrated was situated on the eighth floor of a Manhattan building, collected samples are unlikely to have represented the PM composition at ground level where, for example, vehicle emissions might have been higher and might have produced greater effects in the animals. Another major variable is that Gordon and colleagues provided concentrated PM as an aerosol (nose only). This is a physiologically relevant mode of exposure, but probably resulted in lower delivery of particles to the airways compared with the high bolus concentration of particles used in instillation studies, such as those of Watkinson and coworkers (1998), which showed lethal effects of residual oil fly ash in rats at levels ranging from 250 to 2,500 µg.

Concentrated PM derived from Boston air using a different type of concentrator, the Harvard ambient particle concentrator (HAPC), which can concentrate particles up to 30 times their ambient level, has been shown to change cardiac and pulmonary function parameters in dogs over an exposure range similar to that used in the current study

(Godleski et al 2000). As in the current study, concentrated PM effects on canine cardiac and pulmonary measures in vivo were not observed on all days, and components of the PM mix—including mass, sulfate, and metal content—differed over a wide range from day to day (Godleski et al 2000). Godleski and colleagues also did not find inflammatory changes in the airways in their study. Differences between results from the Godleski and Gordon studies may be attributable to differences in the species studied, to differences in the route of exposure (via tracheostomy rather than inhalation), or to fundamental differences in particle composition between Boston and New York. Concentrated PM from Boston air has also been reported to affect rat and hamster cells in vitro (Goldsmith et al 1997, 1998; Imrich et al 1999).

Many of the pulmonary and inflammatory parameters that Gordon and colleagues selected to test for the effects of concentrated PM have been studied and continue to be used in contemporary studies of PM effects. The investigators, however, did not measure the production of cytokines or chemokines, which have been shown to change in response to PM in other studies. For example, Li and coworkers (1997) showed the production of tumor necrosis factor α from lung cells isolated from rats instilled with PM_{10} particles resuspended from filters. In addition, Killingsworth and associates (1997) showed that MIP-2 mRNA expression was increased in rat cells exposed to residual oil fly ash.

Gordon and colleagues were among the first to attempt to measure PM-related electrophysiologic changes in normal rodents and those with cardiopulmonary conditions. During the course of the Gordon study, Watkinson and associates (1998) showed that instilling residual oil fly ash at doses between 250 and 2,500 µg induced arrhythmias in monocrotaline-treated rats, with multiple changes noted in ECG waveform. Differences in such factors as the toxicity of the different PM components, group size (16 rats in the study of Watkinson and associates [1998], compared with a maximum of 6 in the current study), and the route of exposure may account for the differences in results seen in the 2 studies.

As part of their study, Gordon and colleagues measured changes in average heart rate over 5 minutes. This is not a particularly sensitive endpoint, and the response to particulate pollution would likely have been small. Overall, the investigators noted an increase of approximately 5% in the heart rate of young normal rats in the 6 hours following response to concentrated PM, which is similar to the changes in heart rate reported in a study of PM effects on an elderly population living at moderately high altitude (Dockery et al 1998; Pope et al 1999). Changes in more instantaneous measures such as heart rate variability, the fluctuations in an individual's heart rate (and an index of sympathetic to parasympathetic tone), may be a more sensitive measure. Concentrated PM–related changes in heart rate variability have been shown in recent studies of dogs with an induced coronary occlusion (Godleski et al 2000). In addition, an association between PM exposure and changes in heart rate variability was reported in 2 recent epidemiologic studies of elderly humans (Liao et al 1999; Pope et al 2000).

Gordon and colleagues' preliminary finding of concentrated PM increased platelet number is intriguing. It suggests a plausible and testable hypothesis for explaining PM effects on the cardiovascular system: a PM-induced increase in the number of platelets, which play a key role in blood clotting, may lead to increased likelihood of thromboembolism. Support for such an ischemia-inducing mechanism of PM effects was recently provided by Godleski and colleagues in their concentrated PM study in canines. Their results in a small number of dogs with induced coronary occlusion exposed to concentrated PM suggested that they may have developed one of the cardinal signs of myocardial ischemia—elevation of the ST segment—more rapidly and to a greater magnitude than control animals (Godleski et al 2000).

Questions remain as to whether the rodent models used in this study are appropriate models to study the potential effects of PM on susceptible human subpopulations. Patients with chronic obstructive pulmonary disease (COPD) comprise one group that may be susceptible to PM effects. The investigators indicate that the monocrotaline-injected rat models human cor pulmonale—right heart failure, secondary to pulmonary hypertension—and that a significant fraction of COPD patients aged 50 or older have secondary pulmonary hypertension. This may be an overestimate of the prevalence of pulmonary hypertension in individuals over 50 years old with COPD but perhaps not for those with end-stage disease (those with forced expiratory volume in 1 second [FEV_1] of less than 1 L). The morbidity and mortality data also do not clarify whether the subset of individuals with severe end-stage COPD is at greatest risk from PM. Therefore, the appropriateness of using a model of right heart failure and pulmonary hypertension to represent a high-risk subset in the population is open to question.

A further issue is the appropriateness of using rodent models to investigate cardiac responses. Even if concentrated PM induce changes in electrophysiologic responses in rodents, such changes would be difficult to extrapolate to humans because of differences in the potassium channels responsible for repolarization of the heart. For

example, the rat T wave begins before the QRS complex ends, and so the rat does not have an ST segment, an important parameter in human ECG readings. For these reasons, studies in rats are appropriate for initial studies only. Species such as the pig, dog, or nonhuman primate, whose cardiovascular systems are more closely analogous to the human's, are more appropriate for further extrapolations.

SUMMARY AND CONCLUSIONS

In this exploratory study, Gordon and colleagues addressed plausible mechanisms for linking the findings from epidemiologic studies with potential mechanisms of particle effects. They used the Gerber concentrator to expose rats and hamsters to a real-life pollutant mixture, ambient air particles in a concentrated form (concentrated PM). They also evaluated whether rats and hamsters with cardiopulmonary conditions would be more sensitive to PM effects than control animals, as they hypothesized that these conditions modeled populations of humans likely to be sensitive to PM effects.

The investigators found little or no effect of concentrated PM exposure in a range from approximately 150 to 900 $\mu g/m^3$ (for rats, corresponding to a dose of approximately 10 to 60 µg/animal, based on its ventilation rate over a 6-hour exposure) on cardiac, pulmonary mechanical, or inflammatory measures in the rats and hamsters they studied. On some exposure days, Gordon and colleagues observed an average increase of approximately 5% in the heart rate of young healthy rats within the 6 hours following concentrated PM exposure. Similar sporadic increases were noted in young, monocrotaline-treated rats in the first hours after concentrated PM exposure. These positive results might have been the result of variability in either the day-to-day performance of the concentrator or of the composition of the concentrated PM; alternatively, they might have been due to random fluctuations in the data. Whatever the reason, the results did not demonstrate an increase in sensitivity for PM effects of either rats injected with monocrotaline or hamsters with genetic cardiomyopathy.

In summary, the study indicated that neither normal rats and hamsters nor rats and hamsters with compromised cardiopulmonary systems experienced life-threatening changes in the cardiac, pulmonary, or inflammatory parameters measured. The small group size and limited number of experiments performed in the current study made it unlikely that positive responses would be found. Further, the negative findings do not necessarily contradict results from studies showing PM and concentrated PM effects in other animal models. Differences in levels of PM components, method of concentrated PM exposure, and the sensitivity of the endpoints examined may explain the lack of positive findings in the current study. In addition, the finding that rodents with cardiopulmonary conditions showed no greater effect of concentrated PM than normal animals does not contradict the results of epidemiologic studies that suggest that individuals with cardiopulmonary conditions are particularly sensitive to PM. The animal models chosen by Gordon and collaborators may not adequately represent the human subpopulations most susceptible to PM effects. Further studies will be required to identify animal models and health endpoints that may be more sensitive to PM effects.

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