HEI HEALTH EFFECTS INSTITUTE

Oxidant and Acid Aerosol Exposure in Healthy Subjects and Subjects with Asthma

Part I: Effects of Oxidants, Combined with Sulfuric or Nitric Acid, on the Pulmonary Function of Adolescents with Asthma

Jane Q. Koenig, David S. Covert, William E. Pierson, Quentin S. Hanley, Viviana Rebolledo, Karen Dumler, and Steve E. McKinney
Departments of Environmental Health, Pediatrics, and Biostatistics, University of Washington, Seattle, WA

Part II: Effects of Sequential Sulfuric Acid and Ozone Exposures on the Pulmonary Function of Healthy Subjects and Subjects with Asthma

Mark J. Utell, Mark W. Frampton, Paul E. Morrow, Christopher Cox, Paul C. Levy, Donna M. Speers, and F. Raymond Gibb
Departments of Medicine, Environmental Medicine, and Biostatistics, University of Rochester School of Medicine and Dentistry, Rochester, NY

Includes the Commentary of the Institute's Health Review Committee

Research Report Number 70
November 1994

HEI HEALTH EFFECTS INSTITUTE

The Health Effects Institute, established in 1980, is an independent and unbiased source of information on the health effects of motor vehicle emissions. HEI studies all major pollutants, including regulated pollutants (such as carbon monoxide, ozone, nitrogen dioxide, and particulate materials) and unregulated pollutants (such as diesel engine exhaust, methanol, and aldehydes). To date, HEI has supported more than 120 projects at institutions in North America and Europe.

Typically, HEI receives half its funds from the U.S. Environmental Protection Agency and half from 28 manufacturers and marketers of motor vehicles and engines in the United States. Occasionally, revenues from other public or private organizations either support special projects or provide resources for a portion of an HEI study. For this study, the Institute acknowledges the cooperation and support of the National Toxicology Program (NTP), which consists of four charter agencies of the U.S. Department of Health and Human Services. The NTP sponsored the inhalation component of this project as part of its studies on the toxicologic and carcinogenic effects of ozone. However, in all cases HEI exercises complete autonomy in setting its research priorities and in disbursing its funds. An independent Board of Directors governs the Institute. The Research Committee and the Review Committee serve complementary scientific purposes and draw distinguished scientists as members. The results of HEI-funded studies are made available as Research Reports, which contain both the Investigators' Report and the Review Committee's evaluation of the work's scientific and regulatory relevance.

HEI Statement

Synopsis of Research Report Number 70

The Effects of Inhaled Oxidants and Acid Aerosols on Pulmonary Function

BACKGROUND

Oxidants gases, such as ozone and nitrogen dioxide, and acid aerosols, such as sulfuric and nitric acids, are components of photochemical smog. Epidemiologic studies suggest that episodes of asthma are exacerbated by smog events. However, controlled human exposure studies have not been able to determine the specific pollutants that are responsible for these findings, nor do people with asthma appear to respond more intensely to ozone or nitrogen dioxide than healthy people in laboratory settings. However, some evidence indicates that people with asthma may be more sensitive to acid aerosols than healthy subjects.

Current risk estimates for air pollutants are calculated largely on the basis of the effects observed in humans exposed to individual gases in laboratory studies. Little is known about any interactive effects of multiple pollutants, or whether a pollutant can sensitize the airways and exacerbate an individual's response to a subsequent pollutant exposure. HEI sponsored the two studies, summarized here, to examine the effects of exposing healthy subjects and subjects with asthma to combined oxidant and acid pollutants.

APPROACH

Drs. Koenig and Utell each conducted studies in which human volunteers received either combined or sequential exposures to oxidant gases and acid aerosols. In each case, standard pulmonary function tests were performed and symptoms were recorded. Dr. Koenig exposed 28 adolescents with asthma to each of the following substances in a random sequence: filtered air (control exposure); 0.12 parts per million (ppm) ozone + 0.3 ppm nitrogen dioxide; 0.12 ppm ozone + 0.3 ppm nitrogen dioxide + 68 μg of sulfuric acid per cubic meter of air; 0.12 ppm ozone + 0.3 ppm nitrogen dioxide + 0.05 ppm nitric acid. Each exposure lasted for 90 minutes while the subject exercised intermittently; exposures to the different combinations of substances were separated by at least one week.

Dr. Utell examined the effects of sequential exposures to sulfuric acid and ozone on pulmonary function in 30 subjects with asthma and 30 healthy subjects between the ages of 18 and 45. Subjects were exposed first to either sulfuric acid or sodium chloride (as a control exposure) at a concentration of 100 $\mu g/m^3$. Twenty-four hours later they were exposed to ozone (0.08, 0.12, or 0.18 ppm) for three hours, including intermittent exercise. Each two-day exposure protocol was separated from the next one by at least two weeks.

RESULTS AND IMPLICATIONS

Dr. Koenig found no significant effects of exposure to any combination of the test atmospheres on the pulmonary function of the adolescents with asthma. Interpretation of these negative results is limited, however, because 6 of the 28 subjects were not able to complete the study. These individuals had moderate to severe asthma, and may represent a particularly sensitive subgroup of subjects with asthma. Dr. Utell found a statistically significant decrease in one pulmonary function measurement (forced vital capacity) in subjects with asthma exposed to 0.18 ppm ozone. This decrease was greater with exposure to sulfuric acid before exposure to ozone, but no evidence of aggravation of asthma was apparent. Exposure to ozone or sulfuric acid aerosol caused no change in symptoms or pulmonary function in healthy subjects.

These results need to be interpreted in the context of the investigators' protocols, which were directed toward simple mixtures of two or three components and do not reflect the complex mixtures found in ambient air. Results from both studies suggest that subjects with asthma are heterogeneous in their responses to air pollutants. As a group, they did not show consistently enhanced responses to air pollutants; however, some subpopulations of individuals with asthma may be more susceptible to certain pollutant exposures. Future studies should be directed toward identifying and studying such subpopulations.

This Statement, prepared by the Health Effects Institute (HEI) and approved by its Board of Directors, is a summary of two research projects sponsored by HEI from 1988 to 1993. Dr. Jane Q. Koenig and colleagues of the University of Washington in Seattle, WA, conducted the first study, Effects of Oxidants, Combined with Sulfuric or Nitric Acid, on the Pulmonary Function of Adolescents with Asthma; and Dr. Mark J. Utell and associates from the University of Rochester School of Medicine and Dentistry in Rochester, NY, conducted the second study, Effects of Sequential Sulfuric Acid and Ozone Exposures on the Pulmonary Function of Healthy Subjects and Subjects with Asthma. The following Research Report contains both the detailed Investigators' Reports and a Commentary on the studies prepared by the Institute's Health Review Committee.

Library of Congress Catalog No. for the HEI Research Report Series: WA 754 R432.

The paper in this publication meets the minimum standard requirements of the ANSI Standard Z39.48-1984 (Permanence of Paper) effective with Report Number 21, December 1988, and with Report Numbers 25, 26, 32, 51, and 65 Part IV excepted. Reports 1 through 20, 25, 26, 32, 51, and 65 Part IV are printed on acid-free coated paper.

TABLE OF CONTENTS

Research Report Number 70

Oxidant and Acid Aerosol Exposure in Healthy Subjects and Subjects with Asthma

(continued on next page)

Oxidant and Acid Aerosol Exposure in Healthy Subjects and Subjects with Asthma

Part I: Effects of Oxidants, Combined with Sulfuric or Nitric Acid, on the Pulmonary Function of Adolescents with Asthma

Jane Q. Koenig, David S. Covert, William E. Pierson, Quentin S. Hanley, Viviana Rebolledo, Karen Dumler, and Steve E. McKinney

ABSTRACT

Both peak flow decrements in children at summer camps and increased hospital admissions for asthma have been associated with summer "acid haze," which is composed of ozone and various acidic species. The objective of this study was to investigate the pulmonary effects of acid summer haze in a controlled laboratory setting. Twenty-eight adolescent subjects with allergic asthma, exercise-induced bronchospasm, and a positive response to a standardized methacholine challenge enrolled in the study; 22 completed the study. Each subject inhaled one of four test atmospheres by mouthpiece on two consecutive days. The order of exposure to the four test atmospheres was assigned via a random protocol: air, oxidants (0.12 parts per million [ppm]* ozone plus 0.30 ppm nitrogen dioxide), oxidants plus sulfuric acid at 70 $\mu g/m^3$ of air, or oxidants plus 0.05 ppm nitric acid. Exposure to each of the different atmospheres was separated by at least one week. The exposures were carried out during alternating 15-minute periods of rest and moderate exercise for a total exposure period of 90 minutes per day. Pulmonary function was measured before and after exposure on both test days and again on the third day as a follow-up measurement. A postexposure methacholine challenge was performed on Day 3. Low methacholine concentrations were chosen for the postexposure challenge to avoid provoking a response. The protocol was designed to detect subtle changes in airway reactivity. The statistical significance of the pulmonary function values was tested using paired *t* tests. First, we compared the difference between baseline and postexposure measurements after air exposure on Day 1 with the differences between baseline and postexposure measurements after Day 1 exposure to each of the other three atmospheres. Second, we compared the difference between baseline and postexposure measurements after the Day 2 air exposure with the differences between baseline and postexposure measurements after the Day 2 exposure to each of the pollutant atmospheres. Third, we compared the difference between baseline measurements on Day 1 of each exposure atmosphere with measurements after exposure to the same atmosphere on Day 2 to detect delayed effects.

No changes in any of the pulmonary function parameters were statistically significant when compared with changes after clean air exposure. Six subjects left the study because of uncomfortable symptoms associated with the exposures. These all occurred after exposure to pollutant atmospheres and not after exposure to clean air. Although we have consistently observed significant pulmonary function effects in adolescents with asthma after acute exposure (30 to 45 minutes) to sulfuric acid, a recent study in our laboratory showed that the effect diminished with longer exposures (90 minutes). Thus, the data from this study are not inconsistent with other data from our laboratory showing no pulmonary function change after a 90-minute exposure to sulfuric acid at 70 $\mu g/m^3$. The reason for the disappearance of significant effects between 45 and 90 minutes of exposure is unknown. Further research is necessary to investigate this puzzling result.

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

This Investigators' Report is Part I of Health Effects Institute Research Report Number 70, which also includes Part II: Effects of Sequential Sulfuric Acid and Ozone Exposures on the Pulmonary Function of Healthy Subjects and Subjects with Asthma, by M.J. Utell and associates; a Commentary by the Health Review Committee on both Investigators' Reports; and an HEI Statement about the research projects. Correspondence concerning this Investigators' Report may be addressed to Dr. Jane Q. Koenig, School of Public Health and Community Medicine, Department of Environmental Health SC-34, University of Washington, Seattle, WA 98195.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Agreement 816285 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 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 those parties, and no endorsement by them should be inferred.

INTRODUCTION

Exposures to combinations of pollutants in adolescent subjects have not been reported; however, others have studied combined exposures in adult subjects. None of these human studies demonstrated convincing additive or synergistic effects of the pollutant combinations. A study of combined sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) in healthy subjects showed no significant effects (Linn et al. 1980). Neither did a study of 0.15 ppm ozone (O_3), NO_2,

or SO_2, alone or in combination, in seven healthy adult male subjects exposed for two hours during intermittent exercise (Kagawa 1983). Pulmonary function responses in healthy subjects during intermittent exercise after exposure to 0.5 ppm SO_2 and 0.5 ppm NO_2 combined with zinc ammonium sulfate at 20 $\mu g/m^3$ for two hours and 15 minutes were not significantly different from responses after exposure to the gases combined with a sodium chloride aerosol (Kleinman et al. 1985). Stacy and coworkers (1983) found no significant pulmonary function changes in healthy adult subjects after exposure to various combinations of O_3, NO_2, SO_2, sulfuric acid (H_2SO_4), and ammonium sulfate or ammonium nitrate aerosols. Kulle and associates (1982) saw no significant effect of preexposure to 0.3 ppm O_3 on a subsequent exposure to H_2SO_4 at 100 $\mu g/m^3$ in 12 healthy adult subjects. Kulle and coworkers (1984) also studied pulmonary function and bronchial reactivity in healthy subjects after exposure to ammonium sulfate and SO_2 and again found no significant changes. Kleinman and colleagues (1985) did report that a mixture of SO_2 and O_3 (both at 0.37 ppm), combined with H_2SO_4 at 100 $\mu g/m^3$ may have produced a slightly greater effect than the exposure to O_3 alone, but the effect was not significant. The subjects in a study by Stacy and associates (1983) had a 50% greater change in specific airway resistance after exposure to O_3 and H_2SO_4 than after O_3 alone; however, this change also was not significant. Thus, studies of pollutant combinations seem to suggest that the combined exposures enhance pulmonary effects, but no conclusive significant data are available. A higher concentration of O_3 (0.40 ppm) has resulted in inflammatory responses in healthy subjects as measured by bronchoalveolar lavage (Koren et al. 1989).

However, animal and epidemiologic data suggest that combinations of pollutants may cause synergistic lung responses. Dr. Jerold Last has conducted several studies of the combined effects of O_3 and respirable aerosols in animals. As little H_2SO_4 as 40 $\mu g/m^3$, combined with 0.20 ppm O_3, produced a greater effect in rats than 0.2 ppm O_3 alone (Warren and Last 1987). The end points measured were lavageable protein, total lung protein, and collagen synthesis. This concentration of O_3 is approximately twice that used in our present study.

At atmospheric concentrations of O_3 that would not be predicted by laboratory studies to cause effects, epidemiologic studies have measured increased hospital admissions for asthma in the general population (Bates and Sizto 1983, 1987), and decreased pulmonary function in children (Lioy et al. 1985; Spektor et al. 1988) . Researchers have speculated that some other atmospheric species may be interacting with O_3 to cause the effect that led to the term summer "acid haze" (Bates and Sizto 1987). Sulfuric acid or other compounds that contain hydrogen ions (H^+) are the most likely candidates.

Several questions based on the above summary of controlled human exposures to pollutant combinations remain unanswered. First, all of the above studies were conducted with healthy, relatively young adults. Thus, no groups that might be especially sensitive to inhaled irritants have been studied. Subjects with asthma are considerably more responsive to one category of air pollution (the sulfur oxides SO_2 and H_2SO_4) than are healthy subjects (Sheppard et al. 1981; Koenig et al. 1983a,b; Linn et al. 1983; Horstman et al. 1986). Therefore, it seems important to conduct exposure studies using combinations of pollutants with subjects with well-documented asthma.

Second, the test atmospheres investigated have not been modeled to resemble closely urban ambient air. When conditions of air stagnation and elevated pollutant levels occur, they often last for several days. Therefore exposure to pollutant atmospheres on two consecutive days appears to be a realistic representation of urban conditions. The pollutant concentrations we studied are concentrations that are measured in the atmosphere (0.12 ppm O_3, 0.03 ppm NO_2, 0.05 ppm nitric acid [HNO_3], and H_2SO_4 at 70 $\mu g/m^3$). Our model test population showed little or no pulmonary effect after a single exposure to each of these atmospheres alone, with the exception of H_2SO_4.

Our laboratory has been conducting controlled human exposures to ambient air pollutants for 12 years. We have developed a successful model of susceptible populations by testing adolescent subjects with asthma or other allergic conditions. Using this model we have documented the increased responsiveness of subjects with asthma, compared with healthy subjects, to ambient concentrations of SO_2 (Koenig et al. 1980, 1981, 1983b). We also have shown that adolescent subjects with asthma respond to very low concentrations of H_2SO_4 (Koenig et al. 1983a, 1989; Hanley et al. 1992). The pulmonary responses of adolescents with asthma and those of healthy adolescents without any signs or symptoms of hyperresponsive airways have been studied after inhalation of oxidant pollutants (O_3 and NO_2). These pollutants, at the concentrations studied (0.12 and 0.18 ppm O_3, and 0.12, 0.18, and 0.30 ppm NO_2), elicited no consistent changes in lung function in our model susceptible population or in healthy adolescent subjects (Koenig et al. 1985, 1987). Also, 60-minute exposures during intermittent exercise to a combination of 0.12 ppm O_3 and 0.30 ppm NO_2 did not produce significant pulmonary function

changes in adolescent subjects with asthma (Koenig et al. 1988). However, sequential exposure to 0.12 ppm O_3 and 0.1 ppm SO_2 demonstrated that the previous exposure to O_3 potentiated the pulmonary functional response to an otherwise subthreshold concentration of SO_2 (Koenig et al. 1990). Thus, short-term exposure (45 minutes) to 0.12 ppm O_3, in adolescent subjects with asthma, does appear to elicit changes in bronchial airways, although it does not cause pulmonary function decrements.

AIMS

The objective of this study was to investigate the pulmonary effects of inhaling pollutant combinations chosen to mimic acid summer haze in adolescent subjects with asthma, a group shown to serve as an excellent model of susceptible individuals. We conducted a systematic study of combined O_3 + NO_2 alone, and mixed with H_2SO_4 or HNO_3. These test atmospheres were studied during 90-minutes exposures on two consecutive days. This combination of pollutants is an accurate representation of atmospheric exposures to elevated pollutant levels that occur during periods of air stagnation. The subjects' pulmonary functions also were measured on the third day to detect persistent or delayed effects. A postexposure methacholine challenge at low concentration was performed on the day after the two days of exposure. The subjects were studied during one season (summer) to prevent the possible confounding effects of seasonal factors such as pollens.

We hypothesized that two consecutive days of O_3 + NO_2 exposure would produce a greater change in pulmonary function than single-day exposures; that is, increasing the time (T) of exposure without changing the concentration (C) would increase the dose ($C \times T$) sufficiently to elicit a statistical change in pulmonary function. We based our expectation of an enhanced effect with two-day exposures on our hypothesis that some type of subtle change in bronchial hyperresponsiveness would occur after Day 1 of exposure, as was previously reported in similar subjects exposed to 0.12 ppm O_3 (Koenig et al. 1990). That change could be inflammation, increased activity of some cytokine that acts as a mediator of inflammation, enhanced nervous system activity through exposed receptors or stronger synaptic activity, a change in the pH of airway lining fluid, or some more obscure mechanistic change. We also hypothesized that the addition of an acid compound (H_2SO_4 or HNO_3) to the O_3 + NO_2 mixture would increase the toxicity of the test atmospheres.

METHODS

SUBJECT SELECTION

Twenty-two adolescent subjects with allergic asthma from the clinical practice of Dr. Pierson and his associates at the Northwest Asthma and Allergy Center completed this study. Approximately 10 subjects were studied in each of three summer seasons from 1989 through 1991. This schedule was adopted to diminish seasonal effects. Six other subjects started the study but left at various intervals. Subjects' ages were from 12 to 19 years; both females and males participated. They fulfilled the following four screening criteria: (1) reversible obstructive airways disease documented with spirometry; (2) personal history of allergic asthma; (3) presence of exercise-induced bronchospasm after an exercise tolerance test; and (4) airway responsiveness to a standardized methacholine challenge. Exercise-induced bronchospasm was defined as a drop of more than 15% in forced expiratory volume in one second (FEV_1) after six minutes of exercise on a Quinton (Seattle, WA) treadmill at 85% or more of maximum oxygen consumption (Eggleston et al. 1979); FEV_1 was measured at 1, 3, 5, 10, 15, and 20 minutes after exercise.

The subject's response to the methacholine challenge was the most important criterion for documenting bronchial hyperresponsiveness. The challenge was based on criteria recommended by Chai and associates (1975) and by Shapiro and coworkers (1982). The concentrations of methacholine used were 0.0, 0.025, 0.25, 2.5, 5.0, 10.0, and 25 mg/mL of phosphate-buffered saline. A subject inhaled a series of concentrations of methacholine by mouth with nose clips in place for two minutes during tidal breathing. After each concentration, FEV_1 was measured. If a 20% drop in FEV_1 was not observed, the subject inhaled the next larger concentration until a 20% drop was measured. The sustained drop in FEV_1 of 20% or more below baseline values then was defined as a positive response, and noted as the provocative dose of methacholine at which FEV_1 drops by 20% (PD_{20}) for that subject. Some subjects had been screened for other studies using slightly different methacholine concentrations and those PD_{20} values were used for those subjects.

Each subject inhaled each test atmosphere for 90 minutes via a rubber mouthpiece during intermittent moderate exercise on two consecutive days. All exposures were carried out at approximately 22°C and 65% relative humidity. Each two-day exposure to one atmosphere was separated from exposure to the next atmosphere by at least one week. In year 1, the coach and the technician calculating the pulmonary function values did not know the identity of the test

atmospheres; however, the recorder did. This was corrected for exposures in years 2 and 3, during which none of the three knew the exposure atmosphere. The subjects never knew the identity of the exposure atmosphere.

TEST ATMOSPHERES

The sequence of exposures was determined randomly. There were four test atmospheres in this study: (1) filtered air; (2) 0.12 ppm O_3 + 0.30 ppm NO_2; (3) 0.12 ppm O_3 + 0.30 ppm NO_2 + H_2SO_4 at 70 $\mu g/m^3$; and (4) 0.12 ppm O_3 + 0.30 ppm NO_2 + 0.05 ppm HNO_3.

AIR CHEMISTRY

Figure 1 shows a schematic diagram of the system used to generate and monitor the exposure atmospheres. Compressed air was passed through a series of filters to remove particulate and gaseous impurities. Before each exposure, a bag sample was collected and tested for hydrocarbons and carbon monoxide by infrared spectral analysis. Relative humidity was controlled by the addition of distilled water from a metered source into a humidification column. The air was heated immediately upstream of the humidification column to vaporize the water droplets. An absolute filter removed any residue particles.

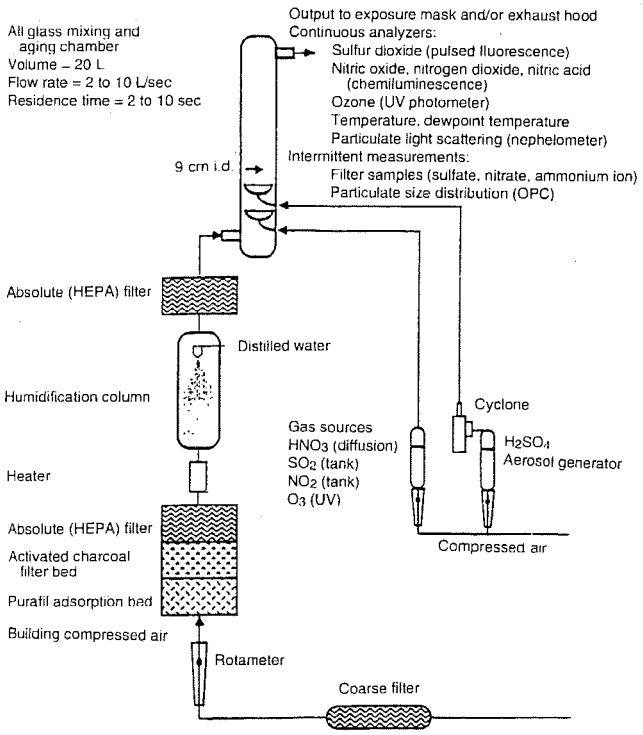


Figure 1. A schematic design of the gas-aerosol generation and monitoring system. HEPA = high-efficiency particulate air filter; UV = ultraviolet; i.d. = internal diameter; OPC = optical particle counter.

The main air flow then was mixed turbulently with a small flow of the chosen test atmosphere and routed to the subject. Ozone was produced by ultraviolet (UV) irradiation of clean air (OREC Model 03V1-0, Ozone Research and Equipment Co., Phoenix, AZ) and monitored with a UV photometric analyzer (Model CSI 3100, Columbia Scientific Industries, Austin, TX). On some occasions, the reference standard instrument, a Dasibi 1003 (Dasibi Corp., Glendale, CA), was used to monitor O_3 during the exposures.

Nitrogen dioxide was supplied from a gas cylinder (0.397 mol% in a volume:volume ratio of NO2:nitrogen; Scott-Marrin, Riverside, CA), and the output concentration was monitored with a chemiluminescent analyzer for oxides of nitrogen (Model 8840, Monitor Labs, Inc., San Diego, CA).

The H_2SO_4 aerosol was generated by bubbling air through a glass frit in a 5% (by volume) aqueous solution of H_2SO_4. The mass median aerodynamic diameter (MMAD) of the H_2SO_4 aerosol produced by this system was 0.6 μm with a geometric standard deviation of 1.5 (Royco 220, Menlo Park, CA). An integrating nephelometer (Meteorology Research Inc., Altadena, CA), which measures the light scattering due to aerosol particles, was used as a continuous measure of aerosol mass concentration. Filter sampling was used to check the mass concentration for each exposure. The filters subsequently were analyzed for the presence of sulfate, nitrate, and ammonium ion with an ion chromatograph (Dionex 2020i, DX-100, Sunnyvale, CA).

The HNO_3 gas was generated by passing a controlled flow of clean air over a reagent-grade aqueous solution of HNO_3. This concentrated vapor was mixed turbulently into a dilution chamber and delivery system of all glass and polyethylene. The HNO_3 gas concentrations at the subject's mouthpiece were determined with a Thermoelectron (Hopkinton, MA) NO_x analyzer (Series 14) modified to determine HNO_3 gas concentrations, a method first described by Joseph and Spicer (1978). The technique relies on the fact that a nylon filter (Membrana Corp.) absorbs HNO_3 gas without disturbing nitrogen oxides gases. Both sides of a split-stream sample were fed to a molybdenum converter for reduction to nitric oxide; one stream was first directed through a nylon filter cartridge, so that the difference in nitric oxide concentrations was due to HNO_3 gas. The unit was calibrated with HNO_3 permeation tubes at 1.5 and 3.0 $\mu mol/m^3$, and also by comparison with air samples collected on a nylon filter and analyzed by ion chromatography. These filter analyses were conducted on all O_3 + NO_2–exposure days to allow us to detect the presence of HNO_3 contamination.

The acceptable ranges for each test atmosphere were set as follows: O_3, ± 5%; NO_2, ± 5%; H_2SO_4, ± 20%; and HNO_3, ± 35%.

To confirm that outdoor O_3 levels would not be a confounding factor, we obtained outdoor O_3 data from one monitoring station approximately 25 miles from the university where the study was carried out. The monitor is in the photochemical belt (where elevated O_3 levels are found) that exists 20 to 40 miles east of Seattle. Most of our subjects live north of Seattle, which is west of the photochemical belt. The four highest O_3 values (in ppm) for each summer of the study were, for 1989: 0.09, 0.08, 0.07, and 0.07; for 1990: 0.126, 0.123, 0.108, and 0.096; and for 1991: 0.109, 0.100, 0.091, and 0.086. Adaptation to concentrations of O_3 below 0.12 ppm has not been reported, and we do not believe that it influenced the outcome of this study. Summer camp data show persistent, rather than adaptive, effects of daily O_3 concentrations near the National Ambient Air Quality Standard (NAAQS) of 0.12 ppm (Lioy et al. 1985; Spektor et al. 1988).

PULMONARY FUNCTION MEASUREMENTS

All physiologic measurements except oral ammonia levels and peak flow were recorded with a thermal recorder or an *x-y* plotter when the subject was seated in a pressure-compensated volume-displacement body plethysmograph. The following measurements were recorded:

- Oral ammonia.
- Peak flow (measured with a Vitalograph [Kansas City, MO] peak flow meter).
- Total respiratory resistance (R_T), using the forced-pressure oscillatory technique at 3 Hz (Goldman et al. 1970); values are based on an average of 10 breaths.
- Thoracic gas volume at functional residual capacity (FRC) using the gas compression technique (DuBois et al. 1956). This measurement was made to verify that baseline and postexposure resistance measurements were recorded at approximately the same lung volume; FRC was not used to detect pollutant-induced effects.
- Maximum flow calculated at 50% ($\dot{V}_{max50}$) and 75% ($\dot{V}_{max75}$) of expired vital capacity from a maximum flow-volume ($\dot{V}_{max}$) curve.
- Forced expiratory volume in one second calculated from the same maximum flow–volume procedures as the $\dot{V}_{max}$ values discussed above. Forced vital capacity (FVC) also was calculated from this procedure.

Oral ammonia in exhaled air was measured by having subjects breathe for 10 minutes with a sampling probe inserted between their teeth (Larson et al. 1979). The sample flow at a rate of 1 L/min was diluted 2:1 with clean, dry air, and reduced to a pressure of 70 kPa to prevent water condensation. The ammonia in the air was scrubbed by 5 mL of 0.01 M solution of H_2SO_4 in distilled water, which then was analyzed for ammonium ion by an ion chromatograph (Dionex 2020i, Sunnyvale, CA). The FEV_1, FVC, and FRC values were measured in triplicate, and the mean value was used to assess functional changes. Measurement of FEV_1 was repeated if the triplicates were not within 10% of one another. Pressure and flow signals were processed by a microcomputer that calculated R_T (Goldman et al. 1970). Atypical baseline data were defined as follows: FEV_1, ± 10%; R_T, ± 100%; $\dot{V}_{max50}$, ± 50%. If an atypical baseline was observed, the subject was rescheduled for another test.

PROTOCOL

Each subject was exposed to four test atmospheres. The protocol for each atmosphere covered three days, and each three-day protocol was separated from the next one by one week. Thus, each subject participated in the study for a duration of at least four weeks.

Days 1 and 2

No medication was allowed within four hours of an exposure. The subjects scheduled for afternoon protocols were allowed to take their early morning medication. Individual subjects were tested at nearly the same time of day (morning or afternoon) to avoid confounding from diurnal variation in pulmonary function. The sequence of exposures was determined randomly. The protocol, which was identical on Days 1 and 2, followed this plan: (1) oral ammonia measurement and pulmonary function baseline values for this day's protocol (peak flow, R_T, FRC, maximal flow, FEV_1, and FVC); (2) a 90-minute exposure via a rubber mouthpiece to a randomly determined test atmosphere (with alternating periods of 15 minutes at rest and 15 minutes of moderate exercise on a treadmill); and (3) pulmonary function measurements 1 to 3 minutes after the exposure (referred to as postexposure 1 measurements), postexposure measurement of oral ammonia, and repeated pulmonary function measurements 15 minutes after exposure (referred to as postexposure 2 measurements) (see Table 1). The exercise level was adjusted to increase resting expired volume per minute ($\dot{V}_E$; also called minute ventilation) values approximately three-fold. The treadmill settings necessary for this level of exercise were approximately 2.0 mph and 10% elevation. During exposure, $\dot{V}_E$ was measured with a respiratory integrator (Hewlett-Packard, Palo Alto, CA), and end-tidal carbon dioxide tension was measured with a medical gas analyzer (Beckman Instruments, Palo Alto, CA). Peak flow measurements also were

Table 1. Protocol for Exposure to Oxidants Plus an Acid Aerosol

Days 1 and 2
Baseline measurements
Symptom rating form
Oral ammonia
Peak flow rate
Pulmonary function tests in plethysmograph: total respiratory resistance, FRC, flow-volume curves, FEV_1, FVC
Exposure (90 minutes) to one of four exposure atmospheres assigned randomly
Alternating 15-minute periods of rest and exercise (3 periods each)
Exercise: approximately 2 mph and 10% elevation on treadmill
Continuous measurement of $\dot{V}_E$
Continuous measurement of end-tidal CO_2 tension
Peak flow rate measured after each exercise period (2 measurements during exposure)
Postexposure measurements
Same pulmonary function tests in plethysmograph as at baseline (postexposure 1 measurements)
Oral ammonia
Same pulmonary function tests in plethysmograph as at baseline (postexposure 2 measurements)
Peak flow rate
Symptom rating form
At home in the evening
Symptom rating form
Peak flow rate
Day 3 (To detect delayed effects)
Baseline measurements as listed for Days 1 and 2
Methacholine challenge: saline (for baseline values), then methacholine at 0.006, 0.0125, and 0.025 mg/mL; during year 3, these concentrations ranged from 0.0125 to 0.31 mg/mL; FEV_1 and R_T were recorded after each concentration

recorded at the end of the first and second exercise periods (after 30 and 60 minutes of exposure). The peak flow measurements taken during exposure required less than one minute. It was necessary for the subject to remove the mouthpiece briefly so it could be raised or lowered at the end of each 15-minute period. At the beginning and end of each exposure, the subjects were asked to score a symptom-rating scale for cough, substernal pain, sore throat, wheezing, shortness of breath, unusual taste or smell, fatigue, headache, and nasal discharge (Figure 2). At the end of each exposure day, the subject took home (1) the symptom-rating scale and recorded scores later that day and on the following day; and (2) the peak flow meter and recorded measurements at approximately 6 and 9 p.m. to detect delayed effects of the exposure.

Day 3

The subjects returned to the laboratory on the third consecutive day. On that day, a pulmonary function baseline for that day was recorded. No oral ammonia measurements were made, and no exposure was administered. Subjects performed a modified methacholine challenge with three increasing concentrations to reveal enhanced bronchial hyperresponsiveness. The methacholine, in

University of Washington

SYMPTOM RATING SCALE

Phone: Jane Koenig 543-2026
HSB Room F551: 685-1596
Emergency 24 hour phone: 527-1200

HEI Combined Oxidants
Subject number _____

Exposure date _____ Do you regularly take vitamins? _____
Did you brush your teeth in the last 2 hrs.? When _____

Did you drink anything in the last 2 hrs.? When _____ What _____

How have you been feeling this week? _____
Better than average _____ Average _____ Worse than average _____

Have you had any illness or had an asthmatic attack during the last week? _____

When did you take your last medication? _____

Please rate the following symptoms dependent on severity:

0 None 1 2 3 4 5 Severe

	Before Exposure	During exposure	Following exposure (Remainder of same day) 8:30 pm	Vitalograph Reading
Cough	_____	_____	_____	Baseline
Chest pain or burning	_____	_____	_____	____/____/____
Dyspnea (shortness of breath)	_____	_____	_____	Post Exposure
Fatigue	_____	_____	_____	____/____/____
Headache	_____	_____	_____	
Unusual taste or smell	_____	_____	_____	Following exposure 6 pm
Sore throat	_____	_____	_____	____/____/____
Nasal discharge	_____	_____	_____	Following exposure Bedtime
Wheezing	_____	_____	_____	____/____/____
Dizziness	_____	_____	_____	

Figure 2. Symptom rating scale.

phosphate-buffered saline, was administered via a hand-held nebulizer containing 5.0 mL of solution, with an airflow rate of 6 L/min at 15 psi. The output of the nebulizer was 0.193 mL/min (SD ± 0.007) with an aerosol MMAD of 1.75 μm (GSD, 1.90). Low concentrations of methacholine were used to avoid eliciting bronchoconstriction of 20% or more. The concentrations in years 1 and 2 were 0.006, 0.0125, and 0.025 mg/mL. These values were chosen because they were all below the concentrations shown to elicit a 20% drop in FEV_1 in any subject. A concentration of 0.025 mg/mL is the lowest concentration of methacholine used in a standard challenge (Chai et al. 1975; Shapiro et al. 1982). However, an initial check of the methacholine challenge data for Day 3 indicated that little or no response occurred after any of the exposures during years 1 and 2. Therefore, in year 3, concentrations were adjusted to the individual so that the three concentrations used were closer to the PD_{20} measured for each subject during the screening methacholine challenge. These concentrations were 0.006, 0.0125, 0.025, 0.075, 0.15, and 0.31 mg/mL. Each subject was given three of these concentrations in increasing dosages.

STATISTICAL ANALYSIS

The study results were analyzed using several statistical techniques, including graphical and numerical methods. The data were first screened using histograms and boxplots to identify potential data entry errors and extreme values (outliers). (An example of the raw data boxplots is shown in Figure 8.) A few initial outliers were actually data entry errors, and after correcting these, no apparent outliers remained in the raw data; however, some extreme values do appear when we look at differences in lung function measurements or select subsets of the data set for analyses.

The study design is a repeated-measures design because multiple measurements were obtained on each study subject over time. Analysis of repeated measures requires complex statistical techniques that are difficult to perform, and the results are difficult to explain. We therefore chose to use simpler (but no less valid) techniques whenever possible to better communicate the study results. We broke the study down into its component parts, so that much simpler paired t tests could be performed to analyze the data statistically, and the results are much easier to explain and interpret.

Two factors are paramount in explaining the variation in the lung function measurements obtained: exercise and other protocol-related effects, and the combined oxidant mixtures. To measure the exercise and protocol-related effects on lung function, all subjects inhaled pure air in one of the exposure episodes. Any effect of exercise or other protocol factors on lung function was thus estimable for each subject. These effects then were removed from the changes in lung function recorded while the subject was breathing combined oxidant mixtures, or oxidants combined with acids, leaving an estimate of the effect of each oxidant mixture alone.

Mathematically, the numerical analyses can be described as follows. Let y_{ijklm} denote the measure of lung function (for example, FEV_1 in liters) for subject i (where i is subject number 1, . . . , 22), breathing atmosphere j (where j is exposure atmosphere 1, air; 2, $O_3 + NO_2$; 3, $O_3 + NO_2 + H_2SO_4$; or 4, $O_3 + NO_2 + HNO_3$), on day k (where k is 1, 2, or 3), at time l (where l is 1, baseline for that day; 2, postexposure 1; or 3, postexposure 2), and for the number of measurements m (where m is 1, . . . , M). When multiple measurements were obtained (for example, three measurements for FEV_1, FVC, and $\dot{V}_{max50}$ were recorded, so $M = 3$), the average was computed using the following formula and was used in all analyses:

$$y_{ijkl} = \sum_{m} = \frac{1 Y_{ijklm}}{M}$$

We can therefore simplify matters by dropping reference to the multiple measurements subscript m.

To examine the effect of the three combined oxidant-acid mixtures, we first calculated the change in lung function at recording time 1 from the baseline lung function measurement y_{ijkl}:

$$\delta_{ijkl} = y_{ijkl} - y_{ijk1},$$

where l is 2 or 3. Next we estimated the exercise effect for each subject as

$$\delta_{i1kl} = y_{i1kl} - y_{i1k1},$$

where l is 2 or 3. Then we removed any effect due to exercise or other protocol effects:

$$\delta_{ijkl} = \delta_{ijkl} - \delta_{i1kl}$$

where j is 2, 3, or 4.

The variables δ_{ijkl} are thus independent for fixed j, k, and l, and we can use a statistical test on these independent values of δ_{ijkl} to determine whether lung function values are significantly different from zero. If the pollutant mixtures had no effect, then the change in lung function δ_{ijkl} (j is 2, 3, or 4) would be the same as for pure air, δ_{i1kl}, so that the expected value $E(\delta_{ijkl})$ would be zero. We used both the t test and the signed-rank test to assess the effect of the combined oxidants.

Complete data were available for nearly all of the 22 subjects who completed the study. However, occasionally we had missing data; therefore, some of the t tests in Tables 9 through 12 show 20 degrees of freedom instead of 21. See Brown and Hollander (1977; pages 96 and 310) for a complete description of the t test and the signed-rank test.

Because we computed t tests for the three combined oxidant mixtures (j is 2, 3, or 4) on day 1 and day 2 (k is 1 or 2) at postexposure 1 and postexposure 2 (l is 2 or 3), we looked at $3 \times 2 \times 2 = 12$ t tests, and we guarded against the multiple comparisons effect. To maintain an experimental error rate of $\alpha = 0.05$, we declared a t test significant if its p value was smaller than $0.05/12 = 0.004$ (see Brown and Hollander 1977, pp. 231-239, for a discussion of multiple comparisons). If one or more of the t tests showed a p value less than 0.004, then we could be sure at the $\alpha = 0.05$ level that we had observed a statistically significant effect on lung function due to the combined oxidants or oxidant and acid mixtures.

We also evaluated the data graphically using boxplots (for a description of boxplots, see Tukey 1977, p. 39). The notches in the sides of the boxplots represent a nonparametric 95% confidence interval for the median value. Otherwise they are identical to more traditional boxplots. (For example, Figure 10 shows boxplots of the δ_{ijkl} values for FEV1.) Under the null hypothesis of no effect from combined oxidants or oxidant and acid mixtures, the data for δ_{ijkl} should have a mean equal to zero; therefore, we plotted a solid line at zero to guide the eye in assessing whether the data differ substantially from zero. In the study protocol, a 5% change in lung function was set as the level of change to be detected; therefore, we also plotted dashed lines at ±5% of the baseline mean lung function measurement. For FEV_1, the mean value was about 3 L; therefore, the dashed lines were drawn at ± 0.15 L. If the pollutants influenced a marked change in lung function, then the boxes in the boxplots should be centered about one of the dashed lines, or outside of the dashed lines. Also, if the pollutants had a marked effect on lung function, then some of the notches should be well above or below the solid zero line.

For plots of raw data values (e.g., see Figure 8), the solid line is the 80% trimmed mean of all Day 1 baseline data. To form a $100(1 - \alpha)\%$ trimmed mean, we sorted the data and discarded the largest and the smallest $100(\alpha/2)\%$ of the data, and computed the average of the remaining data. Because the estimated mean of a small data set is heavily influenced by large or small outlying observations, the trimmed mean yields a more stable estimate of the mean whenever extremely large or small data values are present.

All graphical and numerical statistics procedures were performed using version 3.0 of the New S Language (Becker et al. 1988).

QUALITY ASSURANCE

Standard operating procedures were written for all protocols and kept in a three-ring binder in the laboratory. Volume, flow, and pressure signals were calibrated before every subject visit, and calibrations were marked on the strip chart and the x-y recording paper. A chronological data book was used to record the time of each exposure listing the subject number and the sequence of events. This data book was initialed by the coach, the recorder, and the attending physician for that day. The monitoring equipment was calibrated every Friday afternoon. (During two periods, from 7/13/90 through 7/31/90 and from 7/30/91 through 8/19/91, the weekly ozone calibration was not done due to technical difficulties.)

Two subjects had less than a one-week separation between exposures due to scheduling difficulties. Subject 5 finished one exposure cycle on 7/6/89 and started the next one on 7/11/89; and subject 29 finished one exposure cycle on 8/8/91 and started the next one on 8/12/91. Because no

significant changes were found in this study, these deviations from the standard operating procedure cannot be said to bias the data. The randomized schedule for subject 24 was reordered due to difficulties in generating HNO_3.

An outside auditor, contracted by the Health Effects Institute provided further quality assurance. The external quality assurance report for the study is included in Appendix A.

RESULTS

The clinical characteristics of the 28 subjects who participated are listed in Table 2. The subjects who did not complete the study are indicated by an asterisk before their subject number. The subjects' ages ranged from 12 to 19 years. Nine of the 28 subjects were female. The male:female ratio for subjects who completed the study was 15:7, which is very close to the 2:1 ratio of asthma prevalence in males and females for this age group of the general population. The average FEV_1 was 3.07. The percent of predicted FEV_1 ranged from 65% to 119%; the average was 86.5%. Only one subject (26) did not use regular medication for her asthma. She would be categorized as a subject with mild asthma, although she complained of regular shortness of breath during ice skating. All subjects had positive responses to the methacholine challenge test. The exercise tolerance test data for subjects 1 and 4 were lost; however, their medical history charts indicate that they both have exercise-induced bronchospasm. Recent guidelines from the American Academy of Allergy and Immunology for methacholine responsiveness list a response below 0.25 mg/mL as severe asthma, a response at 0.25 to 2.5 mg/mL as moderate asthma, and a response at 10 to 25 mg/mL as mild asthma (Shapiro and Sinon 1992). According to these guidelines, 6 subjects in the present study had severe asthma, 13 had moderate asthma, and 8 had mild asthma (including 2 who responded at 5 mg/mL).

Table 2 also shows the results of the screening exercise tolerance test. Subjects 3, 7, 14, 20, 21, and 26 had less than a 15% decrease in FEV_1 after the exercise tolerance test. However, they all complained of shortness of breath upon exertion. Subjects 3, 7, 14, 21, and 26 (subject 20 left the study) do not appear as outliers on boxplots of the pulmonary function values. In a previous study, the magnitude of the drop in FEV_1 after an exercise tolerance test was shown to be a significant predictor of decreases in maximal flow induced by H_2SO_4 (Hanley et al. 1992), although this was not the case in the present study.

Six of the twenty-eight subjects enrolled in the study left before completion. Five of these subjects complained of unpleasant symptoms, although the fifth subject would not be specific as to why he quit. Subject 7 quit after one day because she said the exposure made her nauseous. She had been exposed to oxidants + HNO_3. Subject 15 quit after Week 1. He stated that he had to start using an inhaler in the evening, which he had not needed to use for more than a year. He had been exposed to oxidants alone. Subject 17 developed pneumonia after Week 3 of the study. His exposures had been oxidants + H_2SO_4 in Week 1, air in Week 2, and oxidants + HNO_3 in Week 3. He did not want to come in for the fourth week after he recovered from pneumonia; by then the study season (April through August) was over. Subject 19 developed a sinus infection after Week 2 of exposure. At first, she planned to continue, but decided not to resume the study. She complained of being very tired while participating. She had been exposed to air in Week 1, and oxidants + HNO_3 in Week 2. Subject 20 did not reveal why he quit. He had been exposed to air for Week 1, and to oxidants for Day 1 of Week 2; he failed to come in on Day 2 of Week 2. Subject 25 also dropped out in the middle of Week 2. He said that since starting the study he was too tired to play baseball in the evenings, and complained of bad headaches. He had been exposed to oxidants + H_2SO_4 in Week 1, and oxidants in Week 2. (These data are summarized in Table 15.)

Table 3 lists the time-weighted average concentration of each test atmosphere for individual subjects. The oxidant gases showed very low variability and stayed within our stipulated percentage of variation. The acids both had higher variability. The averages stayed within the percentage of variation stipulated in our standard operating procedures, but on seven days the time-weighted average concentration of H_2SO_4 was higher than our range, and on one day the time-weighted average concentration for HNO_3 was higher than our expected range. The trace concentrations of the test atmospheres during clean-air exposure were 0.003 ppm HNO_3 and H_2SO_4 at 2 $\mu g/m^3$. These values probably originated from trace amounts on the filters rather than in the air stream used for exposures. Although initial tests indicated that the O_3 + NO_2 atmosphere would not undergo reactions to form HNO_3, 0.005 to 0.010 ppm of HNO_3 was measured in this atmosphere. We believe this concentration did originate in the exposure atmosphere.

Table 4 lists the individual oral ammonia concentrations for each subject before and after exposure. It should be noted that oral ammonia levels after exposure are approximately 40% to 60% greater than the baseline ammonia measurements. This increase in oral ammonia concentration has been noted consistently in experiments in our laboratory (Hanley et al. 1992; Stevens et al. 1992); the reason for this increase is not readily apparent. Correlations

Table 2. Clinical Characteristics of Subjects

Subject[a]	Gender	Age	Height (cm)	Baseline FEV_1 (L/min)	Predicted FEV_1 (L/min)	% Predicted FEV_1	Response to Exercise Tolerance Test[b]	PD_{20}[c] (mg/mL)	Medications[d]
1	M	19	184.30	4.16	4.15	100	–[e]	27% @ 0	I
2	F	17	160.50	2.96	3.05	97	55% @ 3′	42% @ 2.5	None
3	M	15	167.50	2.90	3.50	83	13% @ 0	11% @ 25	I, H
4	F	18	167.25	2.56	3.45	74	–[e]	28% @ 0.25	H
5	F	18	170.50	3.24	3.40	95	23% @ 5′	29%@ 25	None
6	M	15	172.50	3.22	3.75	86	15% @ 3′	21% @ 2.5	I, H
7*	F	16	172.00	2.80	3.30	85	13% @ 3′		
8	M	15	176.50	3.24	4.00	81	15% @ 5′	50% @ 2.5	I, C, H, S
9	M	16	169.50	3.72	3.70	100	50% @ 3′	37% @ 10	O, C, S
10	M	12	162.00	2.36	3.10	76	18% @ 0	25% @ 2.5	I, H
11	M	14	173.00	2.93	3.70	79	38% @ 3′	26% @ 25	I, C
12	M	15	181.50	3.51	4.15	84	27% @ 15′	36% @ 0.25	C, H, S, N
13	M	15	178.00	3.33	4.00	83	17% @ 5′	33% @ 5	400
14	F	14	163.50	3.45	2.90	119	11% @ 5′	54% @ 2.5	O, I
15*	M	14	159.00	2.93	3.10	95	61% @ 5′	20% @ 0.25	O, C, H
16	M	12	164.50	2.33	3.20	73	25% @ 15′	32% @ 2.5	I
17*	M	17	180.00	4.86	4.25	114	21% @ 10′	26% @ 0.62[f]	I
18	M	14	188.50	4.01	4.60	87	24% @ 10′	31% @ 5	20
19*	F	13	166.50	2.57	2.90	89	23% @ 3′	25% @ 0.15[f]	I, C
20*	M	17	190.50	3.55	4.70	76	8% @ 3′	31% @ 0.15[f]	I
21	M	13	153.50	2.72	2.85	95	7% @ 5′	21% @ 25	C
23	M	16	168.00	2.34	3.60	65	60% @ 3′	28% @ 0.15[f]	I
24	M	14	162.00	2.13	3.20	67	66% @ 3′	35% @ 0.25	I, O
25*	M	16	190.50	3.72	4.60	81	37% @ 3′	29% @ 0.15[f]	I
26	F	16	154.50	3.18	3.00	106	2% @ 3′	21% @ 10	None
28	F	18	160.00	2.72	3.20	85	54% @ 3′	30% @ 0.075[f]	I
29	F	14	166.00	2.22	3.00	74	60% @ 3′	30% @ .15[f]	I, S
30	M	13	159.00	2.19	3.05	72	16% @ 10′	48% @ 2.5	I
Mean				3.07	3.55	86.46			

[a] An * indicates that the subject left the study.

[b] The value given is the percentage of decrease in FEV_1 at the time point (in minutes [′]) when the decrease occurred.

[c] PD_{20} is the provocative dose of methacholine at which FEV_1 decreases by 20% or more. The value given is the percentage of drop in FEV_1 and the concentration of methacholine (in mg/mL of phoshate-buffered saline) at which the drop occurred.

[d] C = cromolyn; H = antihistamine; I = inhaled bronchodilator; N = nasal corticosteroid; O = oral bronchodilator; S = inhaled corticosteroid.

[e] Data for these two subjects were lost.

[f] These subjects were tested for inclusion in other studies at the concentrations given. Because a value for PD_{20} had already been established, we used it for this study as well.

Table 3. Test Atmosphere Time-Weighted Averages

		Test Atmosphere[a]							
		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$			$O_3 + NO_2 + HNO_3$		
Subject Number[b]	Day	O_3 (ppm)	NO_2 (ppm)	O_3 (ppm)	NO_2 (ppm)	H_2SO_4 ($\mu g/m^3$)	O_3 (ppm)	NO_2 (ppm)	HNO_3 (ppm)
1	1	0.122	0.302	0.122	0.303	84.5	0.122	0.304	59.6
	2	0.121	0.302	0.119	0.300	74.8	0.119	0.298	54.8
2	1	0.121	0.297	0.120	0.299	87.4	0.117	0.304	46.1
	2	0.120	0.299	0.120	0.302	77.2	0.118	0.302	48.3
3	1	0.120	0.302	0.121	0.297	87.3	0.122	0.304	44.1
	2	0.120	0.320	0.121	0.301	80.3	0.121	0.301	47.5
4	1	0.120	0.305	0.120	0.300	84.5	0.120	0.302	51.2
	2	0.124	0.302	0.119	0.300	84.4	0.120	0.304	66.8
5	1	0.120	0.300	0.120	0.302	85.9	0.120	0.301	48.4
	2	0.119	0.297	0.120	0.300	91.8	0.122	0.301	56.9
6	1	0.120	0.300	0.120	0.298	64.1	0.120	0.303	44.2
	2	0.121	0.302	0.120	0.298	90.5	0.117	0.301	45.7
8	1	0.119	0.301	0.119	0.302	75.3	0.121	0.303	55.5
	2	0.120	0.300	0.118	0.300	68.6	0.119	0.303	46.1
9	1	0.119	0.296	0.119	0.299	64.5	0.119	0.298	44.5
	2	0.122	0.300	0.120	0.301	76.3	0.120	0.302	51.7
10	1	0.118	0.303	0.120	0.297	68.1	0.120	0.304	43.3
	2	0.121	0.301	0.120	0.302	67.8	0.118	0.301	43.1
11	1	0.121	0.302	0.121	0.301	65.3	0.120	0.300	48.5
	2	0.120	0.301	0.121	0.302	78.9	0.119	0.298	41.5
12	1	0.120	0.300	0.121	0.300	67.1	0.120	0.302	51.9
	2	0.121	0.299	0.122	0.302	60.7	0.120	0.299	53.3
13	1	0.121	0.302	0.121	0.300	77.5	0.120	0.300	45.5
	2	0.121	0.298	0.121	0.298	71.4	0.121	0.298	50.5
14	1	0.119	0.301	0.121	0.302	79.4	0.120	0.300	42.5
	2	0.119	0.303	0.120	0.301	66.1	0.119	0.300	52.7
15*	1	0.119	0.301						
	2	0.120	0.301						
16	1	0.123	0.300	0.121	0.300	99.6	0.122	0.298	59.6
	2	0.123	0.302	0.120	0.296	55.8	0.119	0.299	41.7
17*	1			0.119	0.298	69.1	0.122	0.298	47.6
	2			0.119	0.301	78.6	0.122	0.302	46.3
18	1	0.120	0.300	0.120	0.304	90.2	0.119	0.299	44.9
	2	0.121	0.296	0.119	0.291	68.8	0.119	0.303	41.6
19*	1						0.120	0.301	93.4
	2						0.120	0.303	35.1
20*	1	0.120	0.300				0.124	0.302	
	2						0.122	0.300	
21	1	0.121	0.299	0.120	0.297	74.1	0.122	0.300	45.8
	2	0.123	0.301	0.122	0.299	60.7	0.123	0.210	39.7

(Table continues next page.)

Table 3. Test Atmosphere Time-Weighted Averages *(continued)*

Subject Number[b]	Day	Test Atmosphere[a]							
		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$			$O_3 + NO_2 + HNO_3$		
		O_3 (ppm)	NO_2 (ppm)	O_3 (ppm)	NO_2 (ppm)	H_2SO_4 ($\mu g/m^3$)	O_3 (ppm)	NO_2 (ppm)	HNO_3 (ppm)
23	1	0.118	0.298	0.122	0.298	69.9	0.124	0.291	48.7
	2	0.121	0.294	0.117	0.294	65.5	0.118	0.300	49.9
24	1	0.122	0.301	0.124	0.301	63.6	0.122	0.300	47.1
	2	0.119	0.290	0.120	0.302	68.3	0.122	0.290	41.1
25*	1	0.118	0.300	0.119	0.302	66.8			
	2			0.118	0.303	69.6			
26	1	0.117	0.296	0.121	0.294	61.7	0.121	0.296	41.5
	2	0.121	0.304	0.126	0.294	64.6	0.121	0.304	39.6
28	1	0.125	0.301	0.120	0.302	73.2	0.118	0.300	56.9
	2	0.119	0.303	0.122	0.294	61.3	0.120	0.303	63.3
29	1	0.120	0.300	0.124	0.298	69.1	0.118	0.302	49.5
	2	0.121	0.299	0.119	0.297	67.3	0.122	0.303	44.3
30	1	0.120	0.301	0.123	0.303	73.2	0.127	0.299	41.4
	2	0.120	0.292	0.122	0.298	68.1	0.123	0.300	55.6
Mean		0.1204	0.3005	0.1203	0.2995	73.3	0.1203	0.2985	49.1
SD		1.6	4.1	1.5	2.9	10.1	1.7	13.7	9.2

[a] Clean air exposure was also included as a control test atmosphere.

[b] An * indicates that the subject left the study.

Table 4. Oral Ammonia Concentrations[a] for Individual Subjects by Test Atmosphere

Subject Number[b]	Test Atmosphere							
	Air		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$		$O3 + NO_2 + HNO_3$	
	Before	After	Before	After	Before	After	Before	After
1	542	564	361	571	699	851	624	633
	532	278	791	511	978	717	924	896
2	559	531	207	534	721	790	217	673
	137	485	554	469	414	649	120	554
3	198	293	162	525	139	236	91	541
	123	370	572	646	170	289	181	423
4	403	404	184	288	275	490	372	454
	384	403	481	352	163	208	383	665
5	372	868	504	860	530	682	186	451
	481	596	234	713	1,087	931	1,130	867
6	559	606	466	470	550	499	624	836
	112	502	525	739	461	854	546	1,060
7*							712	624
8	280	427	292	475	1,010	No data	74	389
	346	288	270	544	140	455	112	396

(Table continues next page.)

Table 4. Oral Ammonia Concentrations[a] for Individual Subjects by Test Atmosphere *(continued)*

	Test Atmosphere							
	Air		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$		$O3 + NO_2 + HNO_3$	
Subject Number[b]	Before	After	Before	After	Before	After	Before	After
9	134	174	139	327	140	393	87	553
	131	818	274	518	161	463	339	552
10	506	1,230	433	108	559	522	507	
	90	812	798	1,010	226	1,040	337	562
11	417	517	648	979	461	495	558	589
	1,780	1,440	378	471	255	650	275	788
12	112	544	644	716	933	827	540	670
	364	591	323	743	1,040	479	336	967
13	113	296	653	282	94	319	558	316
	399	496	137	377	79	214	177	319
14	121	391	377	432	397	321	140	287
	470	590	1,090	1,090	406	338	218	346
15*			136	297				
			241	259				
16	187	304	366	671	141	515	331	628
	90	530	187	557	298	639	219	889
17*	617	993			1,080	1,390	1,030	1,150
	792	985			1,020	1,010	378	1,060
18	410	962	72	396	303	583	121	303
	207	394	279	862	160	582	131	270
19*	202	578					141	616
	225	299					30	694
20*							217	935
							508	1,030
21	70	442	295	836	851	812	1,102	912
	610	851	399	641	611	676	728	1,072
23	159	223	74	210	50	72	148	231
	241	575	863	224	82	146	422	251
24	656	598	424	252	153	480	426	605
	471	678	195	306	243	272	1,080	No data
25*					493	932	260	611
					588	1,240		
26	55	145	303	531	180	510	330	599
	282	370	87	318	270	284	312	634
28	166	192	107	368	392	540	215	439
	125	232	128	369	83	266	41	78
29	157	559	111	160	155	292	225	253
	288	453	235	318	82	141	104	312
30	63	102	192	388	45	206	468	346
	134	105	372	321	95	161	152	286
Mean	330.7	522.6	360.1	500.7	405.5	541.7	374.8	592.3
SD	285.5	283.5	231.4	233.6	323.1	299.8	286.4	265.4

[a] Values are given in ppb.

[b] An * indicates that the subject left the study.

between individual oral ammonia levels and pulmonary function changes were tested, and no interesting comparisons were found. A significant relation was noted between increased ammonia concentrations and decreased FEV_1 values after exposure to oxidants + HNO_3 on Day 1 (p = 0.004).

Subjects' individual $\dot{V}_E$ values for each test atmosphere are given in Table 5. Minute ventilation rates during HNO_3 exposures in year 1 could not be measured because the stainless-steel pneumotachograph normally used for this measurement would have absorbed the gas. Before the second and third years of the study, a polyethylene pneu-

Table 5. Individual Subject Minute Ventilation Rates[a] by Test Atmosphere

	Test Atmosphere							
	Air		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$		$O_3 + NO_2 + HNO_3$	
Subject Number[b]	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
1	10.6	33.0	9.0	28.9	11.3	28.6	No Data	No Data
	10.6	33.7	10.3	26.9	10.9	32.8	No Data	No Data
2	8.9	26.1	7.7	21.5	10.1	26.9	No Data	No Data
	9.7	24.8	9.0	24.4	9.0	27.0	No Data	No Data
3	7.5	17.4	6.8	18.5	7.6	20.7	No Data	No Data
	7.7	20.0	6.3	18.1	7.4	18.5	No Data	No Data
4	7.5	21.0	7.7	18.4	8.2	19.9	No Data	No Data
	7.6	21.0	7.7	18.8	7.2	18.7	No Data	No Data
5	10.7	22.4	7.5	23.4	8.5	24.6	No Data	No Data
	9.9	25.9	11.9	24.2	9.9	23.8	No Data	No Data
6	12.2	30.6	10.7	32.5	10.7	30.1	No Data	No Data
	10.3	32.4	8.3	31.4	9.9	30.0	No Data	No Data
8	10.0	23.7	10.5	24.8	11.5	27.6	No Data	No Data
	11.8	30.5	11.6	25.6	10.5	25.7	No Data	No Data
9	9.8	24.5	10.5	24.7	10.5	24.5	No Data	No Data
	10.4	24.2	10.8	25.4	10.6	26.9	No Data	No Data
10	9.1	25.3	8.4	27.9	9.5	26.7	No Data	No Data
	8.2	22.5	9.7	29.3	7.3	28.4	No Data	No Data
11	6.6	20.6	8.3	24.6	8.6	23.2	6.7	19.0
	7.0	20.8	8.8	22.1	7.5	21.1	7.7	20.5
12	10.2	29.5	9.5	24.2	9.5	28.7	9.6	23.5
	10.4	27.6	8.8	22.8	10.3	29.0	9.9	22.9
13	8.1	19.1	8.9	20.8	7.4	20.1	6.8	18.6
	7.3	15.5	8.9	18.8	6.6	19.7	7.6	19.0
14	6.7	16.0	6.3	14.8	6.2	15.2	7.2	16.8
	6.7	15.2	6.0	14.4	6.2	14.9	7.2	16.1
16	7.3	20.9	6.9	20.4	7.1	19.5	6.8	19.0
	8.4	21.4	6.5	19.3	7.0	18.3	6.9	17.3
17*	8.7	24.6			7.3	33.4	6.2	25.1
	7.7	22.4			8.2	24.9	9.2	27.2
18	8.8	22.8	10.5	24.7	9.4	25.6	8.7	22.3
	8.4	22.4	9.8	23.7	10.0	24.6	9.4	24.6
21	7.5	20.6	8.2	25.4	7.5	20.0	6.6	20.1
	8.0	22.7	8.5	25.2	6.4	20.2	7.5	19.1

(Table continues next page.)

Table 5. Individual Subject Minute Ventilation Rates[a] by Test Atmosphere *(continued)*

	Test Atmosphere							
	Air		$O_3 + NO_2$		$O_3 + NO_2 + H_2SO_4$		$O_3 + NO_2 + HNO_3$	
Subject Number[b]	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
23	9.5	19.1	10.2	25.3	9.0	23.1	8.1	22.3
	8.6	20.9	8.5	20.2	9.0	22.2		
24	8.3	26.8	7.2	24.1	8.0	22.0	8.3	26.8
	8.2	25.5	8.6	25.6	8.0	20.5	8.8	28.0
26	5.6	20.1	7.0	23.0	7.3	19.9	7.0	19.5
	6.4	21.6	7.6	20.0	6.5	18.4	5.9	18.9
28	6.0	16.0	6.7	19.5	7.1	17.0	5.7	16.2
	6.2	15.8	7.1	16.6	6.6	17.0	6.5	16.0
29	8.5	21.4	8.4	21.1	7.4	23.6	7.2	15.7
	8.8	23.1	7.3	19.3	7.6	25.7	7.8	16.8
30	6.6	14.3	6.5	13.0	6.9	16.1	7.1	16.5
	6.6	14.9	6.3	13.5	6.1	13.5	9.6	17.7
Mean	8.5	23.0	8.6	23.0	8.52	3.3	7.6	20.8
SD	1.6	4.7	1.5	4.1	1.6	4.7	1.2	3.7

[a] Values are given in L/min.

[b] An * indicates that the subject left the study.

motachograph was designed and built in our laboratory, which allowed us to measure $\dot{V}_E$ during the oxidants + HNO_3 exposures. (HNO_3 does not adsorb onto polyethylene.) The missing data for HNO_3 exposures in year 1 are responsible for the low mean $\dot{V}_E$ associated with HNO_3 exposure (20.8 L/min) when compared with the means for $\dot{V}_E$ for the other test atmosphere exposures (23.0, 23.0, and 23.3 L/min). Furthermore, subjects 1 through 10 (for whom HNO_3 data are missing) tended to have higher $\dot{V}_E$ values for all test atmospheres than subjects 11 through 30. As a consequence of both of these factors, the mean $\dot{V}_E$ value for HNO_3 exposures is artificially low.

PULMONARY FUNCTION

Complete tables of individual values for FEV_1, FVC, R_T, $\dot{V}_{max50}$, $\dot{V}_{max75}$, and peak flow are included in Appendix B. Table 6 is a summary of the means ± SD for FEV_1, FVC, $\dot{V}_{max50}$, $\dot{V}_{max75}$, and R_T. Table 7 summarizes these values for peak expiratory flow rate. Figures 3 through 7 show the changes in the values of these functions from Day 1 through Day 3 for the four test atmospheres via line graphs. Boxplots were constructed for each pulmonary function parameter showing changes for each of the seven recording periods: Day 1 baseline, postexposure 1, and postexposure 2; Day 2 baseline, postexposure 1, and postexposure 2; and Day 3 baseline. The boxplots for changes in FEV_1 values are shown in Figure 8; others are available upon request from the authors.

FORCED EXPIRATORY VOLUME

Graphical Results

Boxplots of the raw FEV_1 values are shown in Figure 8. The solid line represents the 80% trimmed mean of all Day 1 baseline data. (To calculate the 80% trimmed mean, we ignored the highest 10% and the lowest 10% of values, and averaged the remaining 80%. This procedure protects, to some degree, against outliers.) The dotted lines in the figure represent the size of effects that were expected to be detected in this study (about a 5% change in function); the dotted lines are drawn at 150 mL above and below 80% trimmed mean. As can be seen in the boxplots, the data values didn't stray much outside of these limits of clinical interest. The shifts in lung function measurement were small.

Effects Within Days 1 and 2

Figure 9 shows boxplots of FEV_1 data as they changed from baseline. For each subject, we calculated the values [postexposure 1 average – baseline average] and [postexpo-

Table 6. A Summary of Pulmonary Function Values for 22 Subjects with Asthma After Exposure to Oxidants Combined with Acidic Compounds[a]

PFT[b]	Atmosphere	Day 1 Baseline	Day 1 Post-exposure 1	Day 1 Post-exposure 2	Day 2 Baseline	Day 2 Post-exposure 1	Day 2 Post-exposure 2	Day 3 Baseline
FEV_1	Air	2.94 ± 0.13	2.87 ± 0.14	2.94 ± 0.13	2.97 ± 0.12	2.91 ± 0.12	3.01 ± 0.12	2.99 ± 0.13
FEV_1	$O_3 + NO_2$	2.95 ± 0.13	2.86 ± 0.14	3.01 ± 0.12	2.96 ± 0.13	2.90 ± 0.13	2.96 ± 0.12	3.01 ± 0.12
FEV_1	$O_3 + NO_2 + H_2SO_4$	3.01 ± 0.12	2.91 ± 0.13	2.99 ± 0.12	2.98 ± 0.12	2.89 ± 0.13	2.98 ± 0.11	2.98 ± 0.12
FEV_1	$O_3 + NO_2 + HNO_3$	3.00 ± 0.12	2.93 ± 0.12	3.03 ± 0.11	2.98 ± 0.14	2.90 ± 0.14	3.02 ± 0.13	3.00 ± 0.13
FVC	Air	3.94 ± 0.12	3.93 ± 0.14	3.94 ± 0.13	3.98 ± 0.12	3.95 ± 0.12	3.99 ± 0.13	3.98 ± 0.13
FVC	$O_3 + NO_2$	3.91 ± 0.12	3.83 ± 0.13	3.98 ± 0.13	3.92 ± 0.13	3.85 ± 0.13	3.92 ± 0.12	3.96 ± 0.12
FVC	$O_3 + NO_2 + H_2SO_4$	3.94 ± 0.12	3.87 ± 0.12	3.95 ± 0.12	3.96 ± 0.13	3.87 ± 0.14	3.94 ± 0.13	3.93 ± 0.13
FVC	$O_3 + NO_2 + HNO_3$	3.94 ± 0.13	3.90 ± 0.13	3.98 ± 0.12	3.89 ± 0.15	3.85 ± 0.15	3.94 ± 0.13	3.94 ± 0.14
$\dot{V}_{max50}$	Air	2.71 ± 0.25	2.66 ± 0.27	2.74 ± 0.27	2.71 ± 0.21	2.65 ± 0.21	2.78 ± 0.24	2.78 ± 0.26
$\dot{V}_{max50}$	$O_3 + NO_2$	2.81 ± 0.27	2.68 ± 0.28	2.82 ± 0.27	2.74 ± 0.24	2.80 ± 0.26	2.77 ± 0.25	2.87 ± 0.23
$\dot{V}_{max50}$	$O_3 + NO_2 + H_2SO_4$	2.90 ± 0.28	2.79 ± 0.26	2.85 ± 0.24	2.76 ± 0.24	2.68 ± 0.24	2.79 ± 0.23	2.77 ± 0.22
$\dot{V}_{max50}$	$O_3 + NO_2 + HNO_3$	2.85 ± 0.24	2.78 ± 0.25	2.86 ± 0.23	2.87 ± 0.26	2.77 ± 0.25	2.92 ± 0.24	2.87 ± 0.23
$\dot{V}_{max75}$	Air	1.28 ± 0.15	1.24 ± 0.14	1.28 ± 0.15	1.22 ± 0.13	1.18 ± 0.11	1.25 ± 0.14	1.29 ± 0.15
$\dot{V}_{max75}$	$O_3 + NO_2$	1.31 ± 0.16	1.30 ± 0.15	1.28 ± 0.14	1.25 ± 0.14	1.30 ± 0.14	1.31 ± 0.13	1.36 ± 0.15
$\dot{V}_{max75}$	$O_3 + NO_2 + H_2SO_4$	1.42 ± 0.18	1.37 ± 0.16	1.40 ± 0.16	1.15 ± 0.11	1.15 ± 0.11	1.17 ± 0.11	1.28 ± 0.14
$\dot{V}_{max75}$	$O_3 + NO_2 + HNO_3$	1.34 ± 0.16	1.35 ± 0.16	1.42 ± 0.15	1.18 ± 0.13	1.15 ± 0.12	1.23 ± 0.14	1.38 ± 0.19
R_T	Air	4.03 ± 0.26	4.37 ± 0.26	3.82 ± 0.22	3.97 ± 0.27	4.07 ± 0.28	3.82 ± 0.24	3.96 ± 0.25
R_T	$O_3 + NO_2$	4.14 ± 0.30	4.15 ± 0.30	3.80 ± 0.24	3.96 ± 0.28	3.85 ± 0.25	3.99 ± 0.28	3.89 ± 0.29
R_T	$O_3 + NO_2 + H_2SO_4$	3.99 ± 0.29	3.97 ± 0.27	3.91 ± 0.28	4.10 ± 0.33	4.19 ± 0.29	3.93 ± 0.24	3.92 ± 0.24
R_T	$O_3 + NO_2 + HNO_3$	3.80 ± 0.28	3.98 ± 0.21	3.54 ± 0.22	3.80 ± 0.26	4.31 ± 0.27	3.92 ± 0.27	3.75 ± 0.26

[a] All values are given as means ± SE in L/min.

[b] Pulmonary fuction test.

Table 7. A Summary of Peak Flow Values[a] for 22 Subjects with Asthma Before and After Exposure to Four Test Atmospheres

Day	Atmosphere	Baseline	Exercise 1[b]	Exercise 2[c]	Postexposure[d]	Home 1[e]	Home 2[f]
1	Air	376 ± 20.0	367 ± 22.1	367 ± 22.0	376 ± 23.8	375 ± 21.2	380 ± 21.4
1	$O_3 + NO_2$	372 ± 26.0	348 ± 31.0	365 ± 25.3	377 ± 27.3	373 ± 15.5	372 ± 16.2
1	$O_3 + NO_2 + H_2SO_4$	376 ± 21.9	371 ± 22.6	354 ± 17.9	357 ± 16.5	368 ± 14.6	363 ± 16.3
1	$O_3 + NO_2 + HNO_3$	379 ± 25.0	367 ± 25.7	364 ± 26.8	378 ± 25.3	368 ± 16.6	368 ± 16.4
2	Air	368 ± 20.9	363 ± 20.5	368 ± 22.2	368 ± 21.3	356 ± 14.9	362 ± 14.8
2	$O_3 + NO_2$	378 ± 26.1	371 ± 27.1	367 ± 26.0	376 ± 25.5	380 ± 22.4	362 ± 17.6
2	$O_3 + NO_2 + H_2SO_4$	381 ± 25.3	369 ± 26.4	370 ± 25.8	358 ± 14.4	355 ± 15.2	351 ± 16.0
2	$O_3 + NO_2 + HNO_3$	374 ± 25.7	372 ± 28.8	377 ± 26.4	388 ± 26.3	356 ± 17.3	356 ± 19.1
3	Air	377 ± 21.0					
3	$O_3 + NO_2$	380 ± 26.9					
3	$O_3 + NO_2 + H_2SO_4$	375 ± 22.3					
3	$O_3 + NO_2 + HNO_3$	384 ± 26.6					

[a] Values are given as means ± SE in L/min.

[b] Exercise 1 = measurement made after first 15-minute exercise period.

[c] Exercise 2 = measurement made after second 15-minute exercise period.

[d] Postexposure = measurement made at the end of the postexposure recording session in the laboratory.

[e] Home 1 = Measurement made at home in the evening of the first day of exposure, at approximately 6 pm.

[f] Home 2 = measurement made at home in the evening of the second day of exposure, at approximately 9 pm.

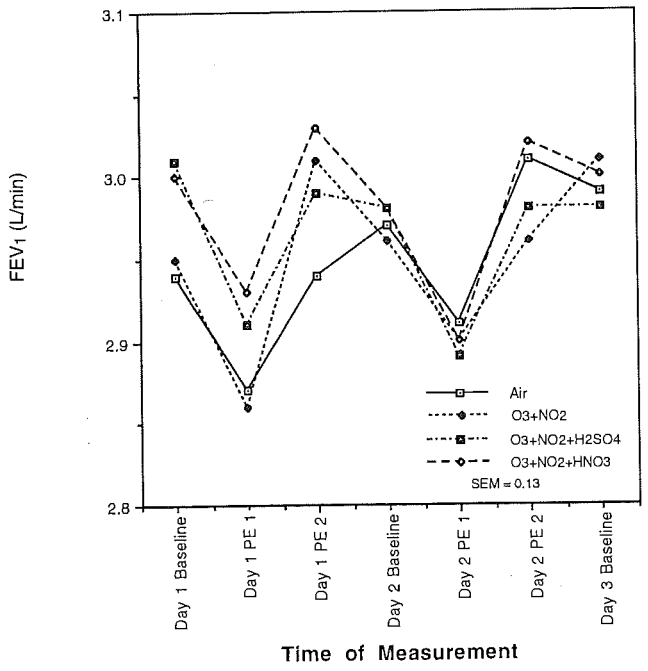


Figure 3. FEV_1 group means for each test atmosphere.

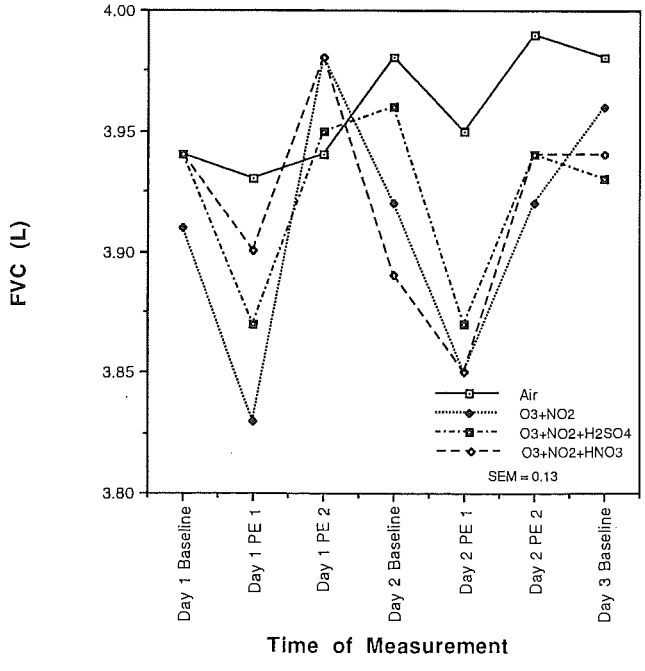


Figure 4. FVC group means for each test atmosphere.

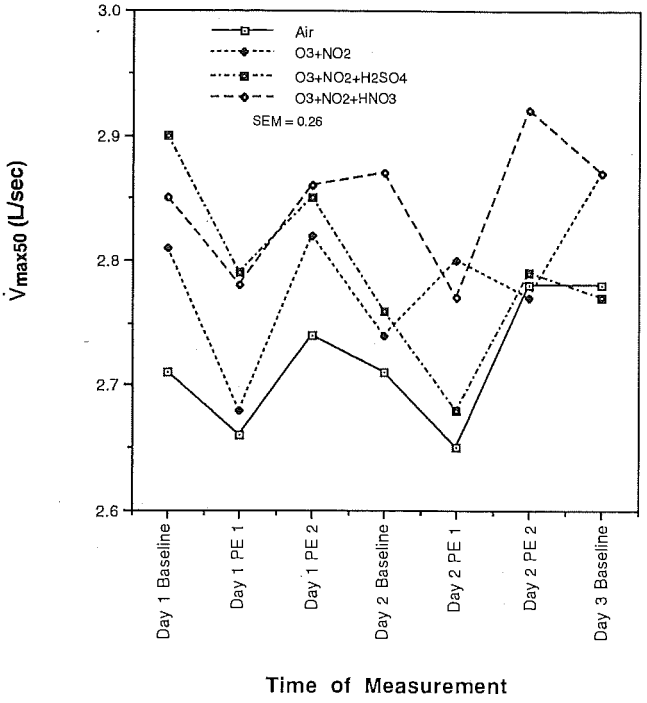


Figure 5. $\dot{V}_{max50}$ group means for each test atmosphere.

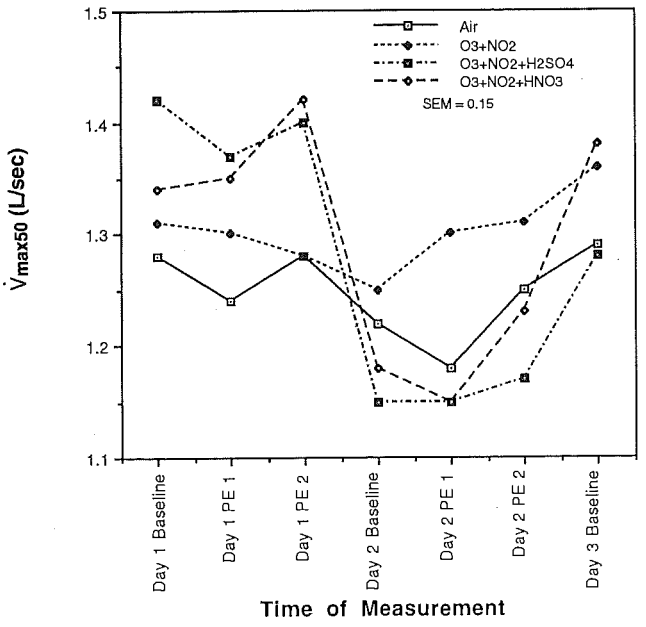


Figure 6. $\dot{V}_{max75}$ group means for each test atmosphere.

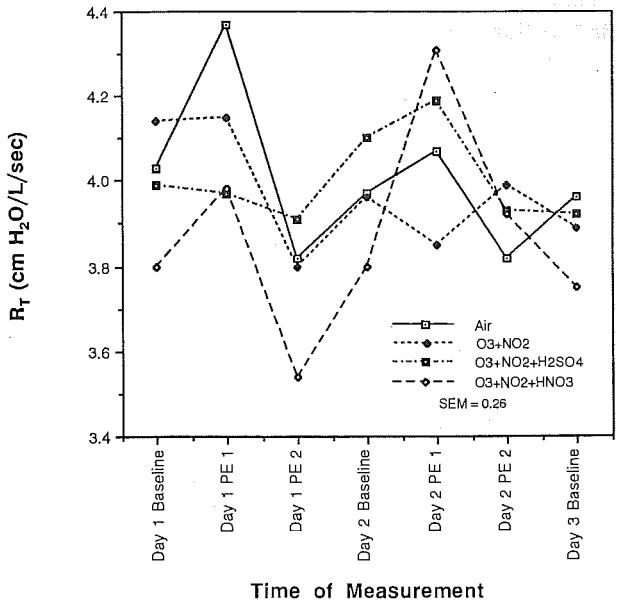


Figure 7. R_T group means for each test atmosphere.

sure 2 average – baseline average] for Days 1 and 2. Also shown are boxplots of the values [Day 2 postexposure 1 average – Day 1 baseline average] to evaluate persistent or delayed effects. The plots of values for [Day 1 postexposure 1 – baseline] and [Day 2 postexposure 1 – baseline] typically show a decrease in lung function after exposure to oxidants, H_2SO_4, and HNO_3, but the decrease is small (1%, 2%, or 3% respectively), and the boxplots show no large shift in lung function outside the 150-mL range. Some of this decrease may be due to the stress of the treadmill exercise or some other constant factor in the protocol, and some may be due to the atmospheres inhaled. Some decrease in lung function was evident even after exposure to the clean air atmosphere, which indicates the effects of the protocol itself. The data for the other three atmospheres did not show markedly different distributions, so visually it appears that most of the apparent effect was due to the treadmill stress or other protocol conditions (e.g., effects of forced expiratory maneuvers). The plots of values for [Day 1 postexposure 2 – baseline] and [Day 2 postexposure 2 – baseline] typically show a return to near-baseline values for all atmospheres.

Each test atmosphere was compared statistically to the other atmospheres. No significant differences were seen.

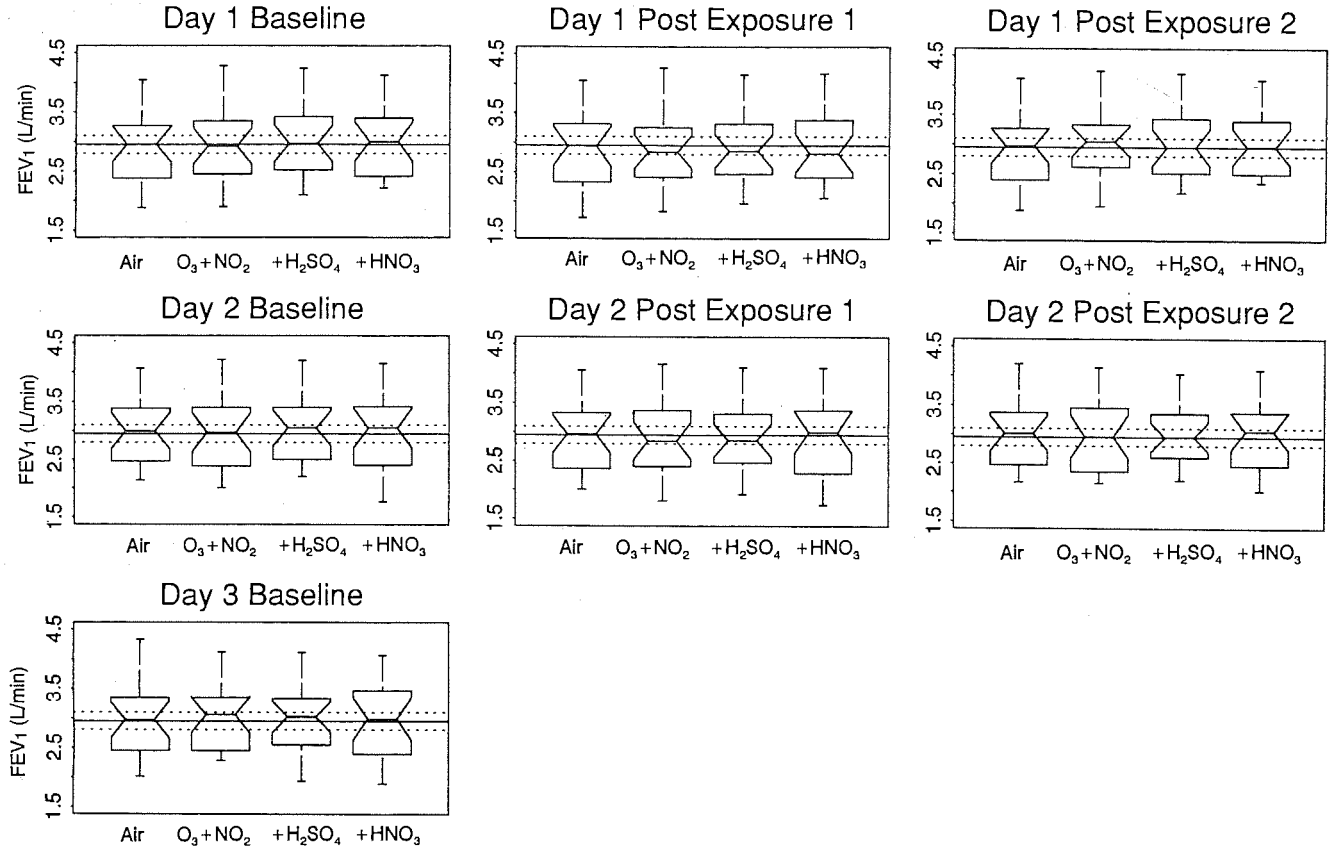


Figure 8. Boxplots of FEV_1 raw data for each recording period and test atmosphere. The solid line represents the 80% trimmed mean of all Day-1 baseline data. The dotted lines represent the 5% change in mean FEV_1 that this study was designed to detect.

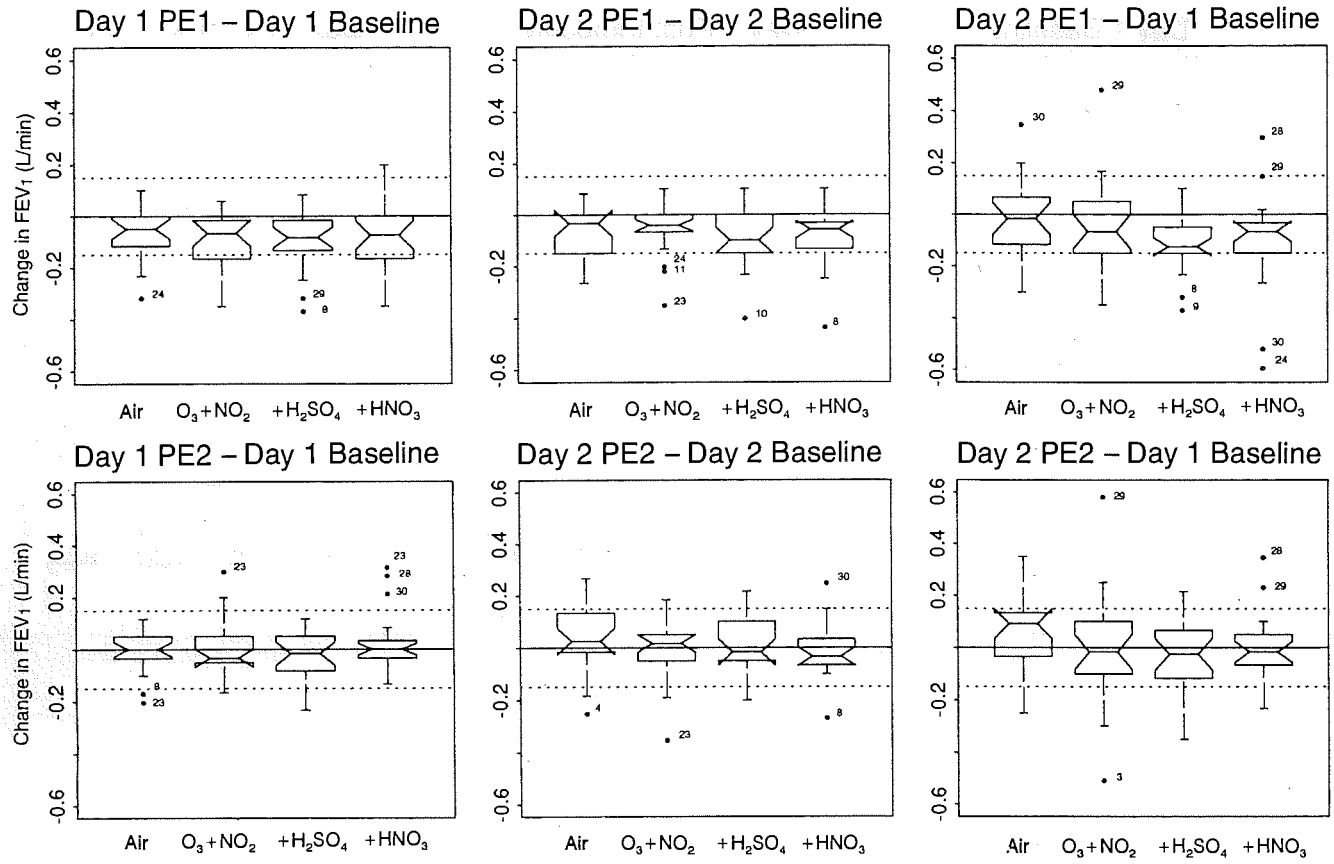


Figure 9. Boxplots of changes in FEV_1 data for each recording period and test atmosphere. The solid line represents 0% change. The dashed lines represent the 5% change in mean FEV_1 that this study was designed to detect.

Baseline and Air-Corrected Data

Figure 10 shows boxplots of FEV_1 data corrected for clean air as they changed from baseline. For each subject, we calculated [(postexposure 1 average – baseline average for the oxidant mixture) – (postexposure 1 average – baseline average for clean air)] and [(postexposure 2 average – baseline average for the oxidant mixture) – (postexposure 2 average – baseline average for clean air)] so that we removed the effects due solely to the protocol, and revealed the effects due to the oxidants alone or combined with acids. The effects were not marked, and never strayed far outside the ± 150-mL range of interest. Thus, graphically, the data showed no striking evidence to suggest that the combined oxidants alone or with acidic compounds impaired lung function 5% or more as measured by FEV_1.

Effects Between Days 1, 2, and 3

Figure 11 shows boxplots of the baseline FEV_1 data for each test atmosphere. No significant differences were apparent within each test atmosphere among the 3 days. The solid line is the 80% trimmed mean of the Day 1 baseline values for each of the four atmospheres. The dashed lines are ± 150 mL from the solid average line. The data did not show marked shifts from day to day.

Figure 12 shows box plots that compare baseline FEV_1 values on Days 2 and 3 minus the baseline values recorded on Day 1, and the Day 3 baseline values minus the Day 2 values for each test atmosphere.

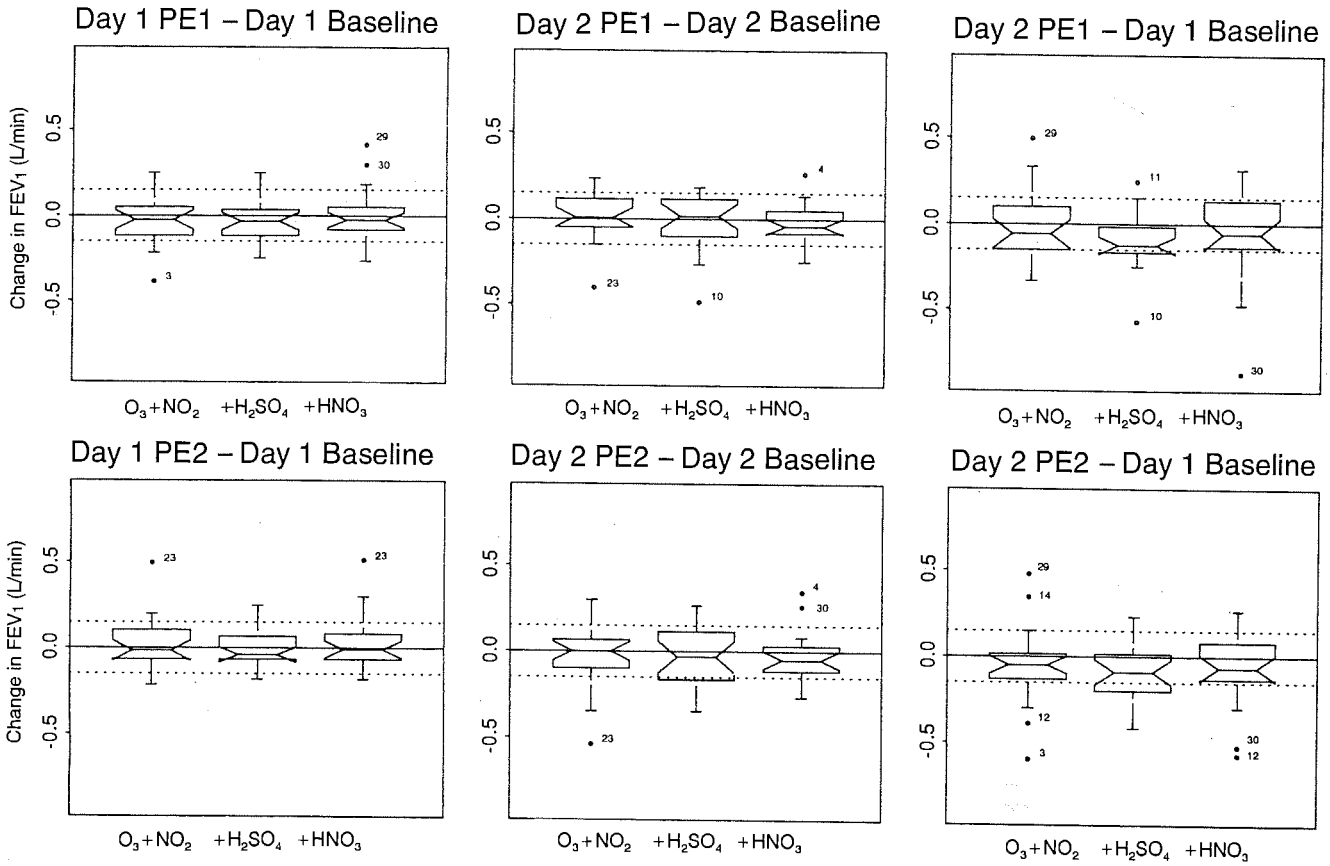


Figure 10. Boxplots of changes in FEV_1 data for each recording period and test atmosphere adjusted for air exposure. The solid line represents 0% change. The dashed lines represent the 5% change in mean FEV_1 that this study was designed to detect. Subject numbers are noted on outlying values.

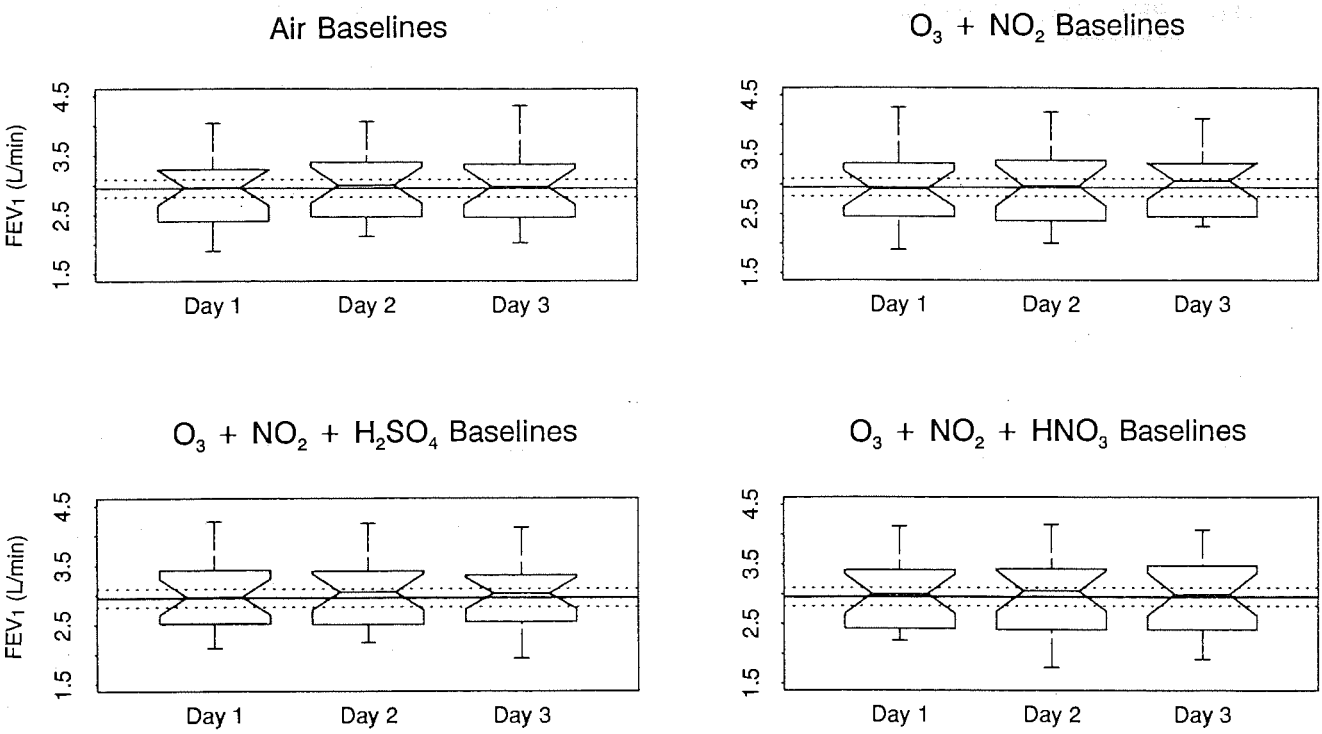


Figure 11. Boxplots of daily baseline FEV_1 data by day for each test atmosphere. The solid line represents the 80% trimmed mean of all Day-1 baseline data. The dotted lines represent the 5% change in mean FEV_1 that this study was designed to detect. Subject numbers are noted on outlying values.

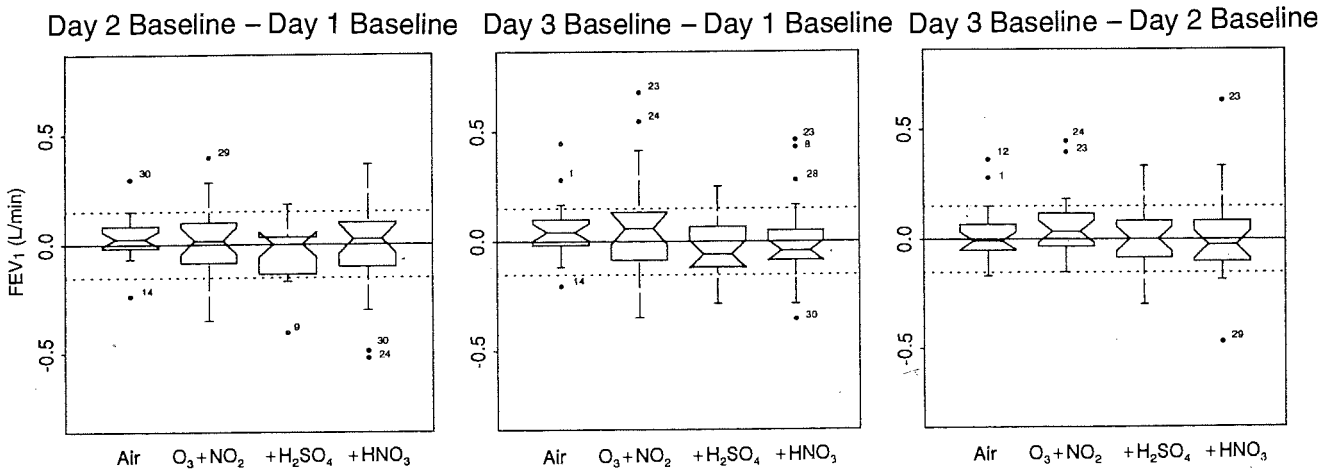


Figure 12. Boxplots of changes in daily baseline FEV_1 data for each test atmosphere. The solid line represents 0% change. The dashed lines represent the 5% change in mean FEV_1 that this study was designed to detect. Subject numbers are noted on outlying values.

Pairwise *t* and Nonparametric Signed-Rank Tests

For each pulmonary function test, Tables 8 through 12 show the results of pairwise *t* and signed-rank tests that compare combined $O_3 + NO_2$ to clean air, $O_3 + NO_2 + H_2SO_4$ to clean air, and $O_3 + NO_2 + HNO_3$ to clean air, at postexposure 1 and postexposure 2 measurement periods for Days 1 and 2 (the data for each exposure day were adjusted for that day's baseline values on Day 1 before exposure to each atmosphere). Because we were looking at approximately a dozen tests, we adjusted the *p* values for the multiple comparisons effect, so all *p* values need to be multiplied by 12. This is a rough Bonferroni adjustment; alternatively, one needs to look for *p* values less than 0.05/12 ($p = 0.004$) before declaring a result significant.

The changes in the lung function measurement were all substantially below the ± 150-mL level of interest for the study. The standard deviation for FEV_1 was estimated to be about 150 mL in the protocol, and the actual data showed FEV_1 SDs in the range of 101.22 to 185.49, so the protocol estimates were accurate.

This study therefore did generate sufficient data to detect differences in the 150-mL range for FEV_1. Thus, the lack of statistically significant results in *t* tests indicates that differences induced by the combined oxidants and acidic mixtures were well below the 150-mL level of interest that this study was designed to detect. Even when we adjusted the Day 2 postexposure 1 data for Day 1 baseline, the *t* tests did not reveal any statistically significant differences at the 150-mL level.

Symptom Data

No consistent patterns of symptom reporting by atmosphere were observed.

Individual Responses

Table 13 shows the subjects whose FEV_1 value decreased by more than 10% from the baseline.

Several biomarkers of susceptibility (response to exercise, response to methacholine challenge, gender, and age) were evaluated to determine whether these were correlated

Table 8. Signed-Rank and *t* Test Results for FEV_1

	Exposure Atmosphere	Sample Size	Mean Change in FEV_1 (L)	SEM (L)	SD (L)	*t*	Degrees of Freedom	*t* Test *p* Value	Signed-Rank *p* Value
Day 1 Postexposure Measurement Minus Day 1 Baseline									
1[a]	$O_3 + NO_2$	22	−0.025	0.030	0.139	−0.84	21	0.413	0.355
2	$O_3 + NO_2$	21	+0.015	0.035	0.159	+0.44	20	0.662	1.000
1	$O_3 + NO_2 + H_2SO_4$	22	−0.027	0.026	0.121	−1.05	21	0.304	0.108
2	$O_3 + NO_2 + H_2SO_4$	22	−0.013	0.022	0.104	−0.58	21	0.566	0.426
1	$O_3 + NO_2 + HNO_3$	22	−0.002	0.033	0.153	−0.05	21	0.963	0.475
2	$O_3 + NO_2 + HNO_3$	22	+0.037	0.034	0.159	+1.09	21	0.286	0.697
Day 2 Postexposure Measurement Minus Day 2 Baseline									
1	$O_3 + NO_2$	22	+0.005	0.028	0.130	+0.16	21	0.871	0.603
2	$O_3 + NO_2$	22	−0.032	0.040	0.186	−0.81	21	0.426	0.570
1	$O_3 + NO_2 + H_2SO_4$	22	−0.023	0.036	0.169	−0.65	21	0.522	0.745
2	$O_3 + NO_2 + H_2SO_4$	22	−0.028	0.037	0.176	−0.75	21	0.463	0.615
1	$O_3 + NO_2 + HNO_3$	22	−0.025	0.024	0.112	−1.06	21	0.302	0.211
2	$O_3 + NO_2 + HNO_3$	21	−0.035	0.032	0.148	−1.08	20	0.293	0.110
Day 2 Postexposure Measurement Minus Day 1 Baseline									
1	$O_3 + NO_2$	22	−0.022	0.044	0.204	−0.52	21	0.612	0.346
2	$O_3 + NO_2$	22	−0.059	0.048	0.244	−1.24	21	0.229	0.080
1	$O_3 + NO_2 + H_2SO_4$	22	−0.091	0.037	0.171	−2.49	21	0.021	0.015
2	$O_3 + NO_2 + H_2SO_4$	22	−0.095	0.039	0.185	−2.42	21	0.025	0.030
1	$O_3 + NO_2 + HNO_3$	22	−0.074	0.057	0.268	−1.29	21	0.210	0.277
2	$O_3 + NO_2 + HNO_3$	21	−0.060	0.048	0.220	−1.25	20	0.224	0.330

[a] From this postexposure measurement, the baseline value for the indicated day was subtracted.

Table 9. Signed-Rank and *t* Test Results for FVC

	Exposure Atmosphere	Sample Size	Mean Change in FVC (L)	SEM (L)	SD (L)	*t*	Degrees of Freedom	*t* Test *p* Value	Signed-Rank *p* Value
Day 1 Postexposure Measurement Minus Day 1 Baseline									
1[a]	$O_3 + NO_2$	22	−0.067	0.049	0.231	+1.37	21	0.187	0.031
2	$O_3 + NO_2$	21	+0.033	0.045	0.207	+0.72	20	0.480	0.614
1	$O_3 + NO_2 + H_2SO_4$	22	−0.057	0.040	0.187	−1.42	21	0.169	0.064
2	$O_3 + NO_2 + H_2SO_4$	22	+0.008	0.030	0.142	+0.25	21	0.804	0.570
1	$O_3 + NO_2 + HNO_3$	22	−0.032	0.042	0.198	−0.76	21	0.458	0.355
2	$O_3 + NO_2 + HNO_3$	22	+0.035	0.041	0.192	+0.85	21	0.405	0.314
Day 2 Postexposure Measurement Minus Day 2 Baseline									
1	$O_3 + NO_2$	22	−0.040	0.025	0.119	−1.57	21	0.130	0.119
2	$O_3 + NO_2$	22	−0.011	0.033	0.155	−0.34	21	0.741	0.709
1	$O_3 + NO_2 + H_2SO_4$	22	−0.056	0.035	0.164	−0.61	21	0.123	0.206
2	$O_3 + NO_2 + H_2SO_4$	22	−0.034	0.029	0.138	−1.17	21	0.256	0.178
1	$O_3 + NO_2 + HNO_3$	22	−0.003	0.024	0.114	−0.11	21	0.917	0.795
2	$O_3 + NO_2 + HNO_3$	21	+0.008	0.042	0.194	+0.18	20	0.859	0.889
Day 2 Postexposure Measurement Minus Day 1 Baseline									
1	$O_3 + NO_2$	22	−0.072	0.041	0.194	−1.74	21	0.097	0.189
2	$O_3 + NO_2$	22	−0.043	0.050	0.237	−0.86	21	0.402	0.372
1	$O_3 + NO_2 + H_2SO_4$	22	−0.078	0.046	0.218	−1.68	21	0.108	0.173
2	$O_3 + NO_2 + H_2SO_4$	22	−0.056	0.040	0.190	−1.39	21	0.178	0.211
1	$O_3 + NO_2 + HNO_3$	22	−0.094	0.055	0.259	−1.71	21	0.102	0.144
2	$O_3 + NO_2 + HNO_3$	21	−0.055	0.045	0.208	−1.22	20	0.238	0.322

[a] From this postexposure measurement, the baseline value for the indicated day was subtracted.

Table 10. Signed-Rank and *t* Test Results for $\dot{V}_{max50}$

	Exposure Atmosphere	Sample Size	Mean Change in $\dot{V}_{max50}$ (L/sec)	SEM (L/sec)	SD (L/sec)	*t*	Degrees of Freedom	*t* Test *p* Value	Signed-Rank *p* Value
Day 1 Postexposure Measurement Minus Day 1 Baseline									
1[a]	$O_3 + NO_2$	21	−0.066	0.088	0.406	−0.74	20	0.467	0.848
2	$O_3 + NO_2$	19	−0.099	0.121	0.528	−0.82	18	0.422	0.778
1	$O_3 + NO_2 + H_2SO_4$	22	−0.059	0.053	0.248	−1.13	21	0.273	0.446
2	$O_3 + NO_2 + H_2SO_4$	21	−0.069	0.090	0.414	−0.76	20	0.454	0.876
1	$O_3 + NO_2 + HNO_3$	22	−0.018	0.058	0.273	−0.32	21	0.756	0.465
2	$O_3 + NO_2 + HNO_3$	21	−0.011	0.078	0.356	−0.14	20	0.888	0.958
Day 2 Postexposure Measurement Minus Day 2 Baseline									
1	$O_3 + NO_2$	22	+0.118	0.091	0.429	+1.29	21	0.212	0.381
2	$O_3 + NO_2$	22	−0.045	0.073	0.341	−0.62	21	0.539	0.673
1	$O_3 + NO_2 + H_2SO_4$	22	−0.012	0.074	0.345	−0.16	21	0.877	0.961
2	$O_3 + NO_2 + H_2SO_4$	22	−0.040	0.098	0.459	−0.41	21	0.686	0.733
1	$O_3 + NO_2 + HNO_3$	22	−0.039	0.053	0.248	−0.75	21	0.464	0.721
2	$O_3 + NO_2 + HNO_3$	21	−0.095	0.079	0.363	−1.20	20	0.244	0.566
Day 2 Postexposure Measurement Minus Day 1 Baseline									
1	$O_3 + NO_2$	21	+0.034	0.140	0.644	+0.24	20	0.809	0.794
2	$O_3 + NO_2$	21	−0.148	0.139	0.636	−1.06	20	0.300	0.289
1	$O_3 + NO_2 + H_2SO_4$	22	−0.154	0.096	0.452	−1.60	21	0.125	0.211
2	$O_3 + NO_2 + H_2SO_4$	22	−0.182	0.095	0.445	−1.92	21	0.068	0.249
1	$O_3 + NO_2 + HNO_3$	22	−0.023	0.121	0.569	−0.19	21	0.850	0.948
2	$O_3 + NO_2 + HNO_3$	21	−0.035	0.090	0.412	−0.38	20	0.704	0.876

[a] From this postexposure measurement, the baseline value for the indicated day was subtracted.

Table 11. Signed-Rank and *t* Test Results for $\dot{V}_{max75}$

	Exposure Atmosphere	Sample Size	Mean Change in $\dot{V}_{max75}$ (L/sec)	SEM (L/sec)	SD (L/sec)	*t*	Degrees of Freedom	*t* Test *p* Value	Signed-Rank *p* Value
Day 1 Postexposure Measurement Minus Day 1 Baseline									
1[a]	$O_3 + NO_2$	21	+0.035	0.036	0.167	+0.95	20	0.352	0.498
2	$O_3 + NO_2$	21	−0.067	0.064	0.295	−1.05	20	0.308	0.476
1	$O_3 + NO_2 + H_2SO_4$	21	−0.016	0.044	0.202	−0.35	20	0.727	0.889
2	$O_3 + NO_2 + H_2SO_4$	21	−0.030	0.045	0.206	−0.66	20	0.514	0.794
1	$O_3 + NO_2 + HNO_3$	21	+0.058	0.048	0.218	+1.22	20	0.239	0.244
2	$O_3 + NO_2 + HNO_3$	21	+0.085	0.081	0.371	+1.05	20	0.304	0.274
Day 2 Postexposure Measurement Minus Day 2 Baseline									
1	$O_3 + NO_2$	22	+0.090	0.065	0.307	+1.37	21	0.185	0.149
2	$O_3 + NO_2$	22	+0.024	0.070	0.329	+0.34	21	0.740	0.758
1	$O_3 + NO_2 + H_2SO_4$	22	+0.049	0.058	0.270	+0.84	21	0.408	0.338
2	$O_3 + NO_2 + H_2SO_4$	22	−0.007	0.054	0.252	−0.13	21	0.900	0.961
1	$O_3 + NO_2 + HNO_3$	22	−0.009	0.059	0.277	+0.16	21	0.875	0.961
2	$O_3 + NO_2 + HNO_3$	21	−0.020	0.075	0.345	−1.26	20	0.796	0.251
Day 2 Postexposure Measurement Minus Day 1 Baseline									
1	$O_3 + NO_2$	21	+0.054	0.072	0.330	+0.75	20	0.464	0.509
2	$O_3 + NO_2$	21	−0.047	0.072	0.332	−0.66	20	0.520	0.590
1	$O_3 + NO_2 + H_2SO_4$	21	−0.222	0.153	0.700	−1.45	20	0.162	0.289
2	$O_3 + NO_2 + H_2SO_4$	21	−0.272	0.184	0.842	−1.48	20	0.154	0.230
1	$O_3 + NO_2 + HNO_3$	21	−0.128	0.165	0.755	−0.78	20	0.447	0.821
2	$O_3 + NO_2 + HNO_3$	21	−0.145	0.194	0.889	−0.75	20	0.463	0.794

[a] From this postexposure measurement, the baseline value for the indicated day was subtracted.

Table 12. Pairwise Signed-Rank and *t* Test Results for R_T[a]

	Exposure Atmosphere	Sample Size	Mean Change in RT (cm H_2O/L/sec)	SEM (cm H_2O/L/sec)	SD (cm H_2O/L/sec)	*t*	Degrees of Freedom	*t Test p Value*	Signed-Rank p Value
Day 1 Postexposure Measurement Minus Day 1 Baseline									
1[b]	$O_3 + NO_2$	22	−0.326	0.300	1.407	−1.09	21	0.289	0.223
2	$O_3 + NO_2$	22	−0.136	0.160	0.753	−0.85	21	0.407	0.426
1	$O_3 + NO_2 + H_2SO_4$	22	−0.352	0.260	1.220	−1.35	21	0.190	0.200
2	$O_3 + NO_2 + H_2SO_4$	22	+0.134	0.213	0.998	+0.63	21	0.537	0.615
1	$O_3 + NO_2 + HNO_3$	22	−0.153	0.263	1.233	−0.58	21	0.567	0.581
2	$O_3 + NO_2 + HNO_3$	21	+0.020	0.170	0.778	+0.12	20	0.907	0.808
Day 2 Postexposure Measurement Minus Day 2 Baseline									
1	$O_3 + NO_2$	22	−0.209	0.206	0.964	−1.02	21	0.320	0.263
2	$O_3 + NO_2$	21	+0.214	0.200	0.918	+1.07	20	0.297	0.434
1	$O_3 + NO_2 + H_2SO_4$	21	−0.034	0.194	0.891	−0.18	20	0.862	0.986
2	$O_3 + NO_2 + H_2SO_4$	22	−0.013	0.209	0.979	−0.06	21	0.950	0.808
1	$O_3 + NO_2 + HNO_3$	22	+0.404	0.202	0.950	+2.00	21	0.059	0.053
2	$O_3 + NO_2 + HNO_3$	20	+0.251	0.239	1.069	+1.05	19	0.306	0.204
Day 2 Postexposure Measurement Minus Day 1 Baseline									
1	$O_3 + NO_2$	22	−0.331	0.254	1.192	−1.30	21	0.207	0.338
2	$O_3 + NO_2$	21	+0.090	0.191	0.876	+0.47	20	0.642	0.715
1	$O_3 + NO_2 + H_2SO_4$	21	+0.105	0.198	0.907	+0.53	20	0.601	0.741
2	$O_3 + NO_2 + H_2SO_4$	22	+0.159	0.218	1.023	+0.73	21	0.475	0.408
1	$O_3 + NO_2 + HNO_3$	22	+0.465	0.273	1.280	+1.70	21	0.103	0.123
2	$O_3 + NO_2 + HNO_3$	20	+0.210	0.156	0.698	+1.35	19	0.194	0.145

[a] R_T is averaged over 30 seconds of breathing.

[b] From this postexposure measurement, the baseline value for the indicated day was subtracted.

Table 13. Percentage of Change in Lung Function as Measured by FEV_1 Values that Changed by 10% or More

Atmosphere	Subject	Percent Change	Actual Change (L)
Day 1 Postexposure 1 Measurement Minus Day 1 Baseline			
Air	24	−15	−0.317
$O_3 + NO_2$	3	−12	−0.350
$O_3 + NO_2$	30	−12	−0.267
$O_3 + NO_2 + H_2SO_4$	29	−14	−0.317
$O_3 + NO_2 + H_2SO_4$	8	−11	−0.367
$O_3 + NO_2 + HNO_3$	24	−14	−0.350
$O_3 + NO_2 + HNO_3$	8	−10	−0.317
Day 1 Postexposure 2 Measurement Minus Day 1 Baseline			
$O_3 + NO_2$	23	+13	+0.300
$O_3 + NO_2 + HNO_3$	23	+13	+0.317
$O_3 + NO_2 + HNO_3$	29	+13	+0.283
$O_3 + NO_2 + HNO_3$	28	+10	− 0.283
Day 2 Postexposure 1 Measurement Minus Day 2 Baseline			
$O_3 + NO_2$	23	−13	−0.350
$O_3 + NO_2 + H_2SO_4$	10	−17	−0.400
$O_3 + NO_2 + HNO_3$	8	−13	−0.433
Day 2 Postexposure 2 Measurement Minus Day 2 Baseline			
Air	24	+12	+0.250
$O_3 + NO_2$	23	−13	−0.350
$O_3 + NO_2 + HNO_3$	30	+14	+0.250

with the magnitude of pollutant-induced effects. The most sensitive pulmonary function test was FEV_1, and peak flow values were used as the only indication of changes during exposure. Neither response to exercise nor to methacholine correlated with any pollutant-induced changes in FEV_1. Age did show a relation with peak flow values that were measured after 30 and 60 minutes of exposure to oxidants + H_2SO_4 ($r = 0.54$, $p = 0.01$). Younger ages were associated with larger decrements in peak flow.

Postexposure Methacholine Responses

For all four of the test atmospheres, FEV_1 values measured on Day 3 after the methacholine challenge to detect delayed effects were not significantly different from baseline FEV_1 values on that day. Table 14 gives the percentage of change from the FEV_1 value at baseline methacholine concentration (phosphate-buffered saline) to the FEV_1 value after the highest methacholine concentration given to each subject. The largest average decrease in FEV_1 was seen after air exposure.

DISCUSSION

The concentration of air pollutants in urban areas varies widely over time. The concentrations and time scale of this variability are controlled by meteorological forces, and the sources and chemistry of the pollutants are obviously complex. However, two main scales of variability associated with high concentrations of pollutants can be generalized, and are important to consider in developing protocols for health effects studies such as ours.

First is the longer time scale associated with synoptic scale (1000 km) meteorological stagnation. High pollutant levels associated with these conditions develop and persist for a period of several days. They tend to occur randomly in time, although they are more prevalent in some seasons depending on climate and geography. Among the pollutants that are present at high concentrations throughout such a period are particulate matter (including sulfate compounds), SO_2, and hydrocarbon compounds.

Second is the shorter time scale associated with photochemical reactions, variable sources of pollutants, and micro- to mesoscale meteorology. High pollutant levels associated with these conditions persist for hours rather than days, and have diurnal cycles that occur during the longer-term stagnation periods. Pollutants associated with these cycles are oxidants and chemically reactive species, such as O_3 and the oxidized nitrogen compounds NO_2, peroxyacetyl nitrate, and HNO_3.

Detailed statistics of the time variability of our four target compounds during air pollution episodes are not available; therefore, we cannot generalize the levels, duration of elevated concentrations, or frequency of occurrence on successive days. Of the four test atmospheres, only O_3 and NO_2 are criteria pollutants for which data from intensive air chemistry studies are available. Nitric acid and O_3 are secondary pollutants that result from photochemical oxidation of nitric oxide and hydrocarbon compounds. Some concentrations of NO_2 and HNO_3 are emitted directly from diesel combustion (Finlayson-Pitts and Pitts 1986; Harris et al. 1987).

In our efforts to determine relations between the doses of air pollutants (or combination of air pollutants) and respiratory effects, we exposed a selected population of test subjects to each of four specific combinations of pollutants on two successive days. The protocol simulated the exposure that persons receive from the ambient air. The pollut-

Table 14. Percentage of Change in FEV_1 After Day 3 Methacholine Challenge[a]

	Exposure Atmosphere on Days 1 and 2			
Subject Number	Air	$O_3 + NO_2$	$O_3 + NO_2 + H_2SO_4$	$O_3 + NO_2 + HNO_3$
1	−2	1	0	3
2	2	0	3	5
3	2	15	4	3
4	14	−14	3	−25
5	−19	3	−2	−2
6	0	3	0	3
8	3	0	−6	2
9	−14	0	1	0
10	6	0	−9	0
11	−2	5	−2	2
12	−4	−1	−1	4
13	1	5	3	2
14	3	−1	0	2
16	2	−6	2	-4
17	3	–[b]	1	0
18	−1	1	−2	−1
19	−17	–	–	−19
20	−5	–	–	–
21	0	2	−2	−7
23	−11	−14	−18	−11
24	−15	2	−21	−11
25	–	−4	–	–
26	3	3	0	2
28	−3	−2	0	−2
29	−16	6	−15	−8
30	−18	−8	−13	−5
Mean	−3.5	−0.2	−3.2	−2.8
SD	−8.7	−6.3	−7.2	−7.5

[a] Each value is the percentage of change from the Day 3 baseline FEV_1 value to the FEV_1 value after the highest methacholine concentration given that day to each subject.

[b] Dashes indicate that the subject did not complete the exposure and no postexposure methacholine challenge was performed.

ants studied were H_2SO_4 (particulate phase), HNO_3, NO_2, and O_3 (gas phase). Of these, the latter three vary widely in concentration on a diurnal cycle. Peak one-hour daily values of O_3 and HNO_3 generally occur during midday, and are often 5 to 10 times higher than the night time values (Finlayson-Pitts and Pitts 1986). Nitrogen dioxide levels follow a predictable cycle, with peak values occurring before and after O_3 and HNO_3 peaks. Although the peak one-hour values for these three compounds do not necessarily overlap in time, the periods of elevated levels are usually several hours long and do overlap. Furthermore, the high daytime values usually occur repetitively on successive days. Although the concentration of sulfate compounds tends to be high throughout the period of stagnation, the chemical composition or acidity of the sulfate does vary diurnally. The sulfate is most acidic during the daytime hours as a result of photochemical and mixing processes (Weiss et al. 1983).

This summary of atmospheric chemistry provided the basis for our study protocol of exposure to multiple pollutants on successive days. The duration of exposure in our protocol was 90 minutes. This was chosen not to match the duration of elevated levels, which is variable and can be several hours, but to match the length of time that subjects at risk of complications in pulmonary function might spend exercising outdoors.

The levels of exposure were chosen to provide a maximum dose consistent with ethical and medical limits, and our ability to detect changes based on past experiments involving single pollutant exposures. Thus, our HNO_3 exposure level (0.05 ppm) was high compared with known levels of HNO_3 in the ambient air (0.005 to 0.010 ppm), but has not shown adverse effects in our previous studies (Koenig et al. 1989). The ozone concentration in our exposure protocol (0.12 ppm) was at or below the peak levels observed in the atmosphere (0.12 to 0.18 ppm), and is the NAAQS (0.12 ppm). Our sulfuric acid exposure concentration was 70 $\mu g/m^3$, which is close to the maximum four- to eight-hour values observed in the atmosphere (Spengler et al. 1989). The concentration of NO_2 (0.30 ppm) was chosen to provide more data at a concentration that has elicited small respiratory changes in some studies (Roger et al. 1985; Bauer et al. 1986) but not in others (Koenig et al. 1988). This NO_2 concentration is two to three times higher than the peak one-hour outdoor values, but lower than peak one-hour indoor values (Environmental Protection Agency 1991).

As mentioned earlier in this report, we hypothesized that two 90-minute exposures to $O_3 + NO_2$ on two consecutive days would enhance the effects of a one-day exposure. This hypothesis was based on traditional toxicology principles that state that "the dose makes the poison," and on data from studies of O_3 exposure that show that, with consecutive five-day exposure sequences, the response on Day 2 is more pronounced than that on Day 1 (Hackney et al. 1977; Folinsbee et al. 1980; Horvath et al. 1981). Because we saw no effects on Day 1, this hypothesis was not tested even though we administered exposures on two consecutive days.

When this study was initiated, we had conducted several studies investigating the acute effects of low concentrations of H_2SO_4 on adolescent subjects with asthma (Koenig et al. 1983a, 1989). The duration of exposures in those studies was 30 to 45 minutes. We therefore assumed that a 90-minute exposure would increase the dose and the effect. However, we recently completed a dose-response study of H_2SO_4 effects in adolescents with asthma that yielded a very surprising result; 45-minute exposures were associated with effects, but 90-minute exposures were not (Koenig et al. 1992). A somewhat similar result was presented by Utell and coworkers (1989) who found that changes in pulmonary function did not progress over the course of a four-hour exposure. Thus, although it is surprising that the study reported here using pollutant exposure atmospheres to mimic summer "acid haze" did not replicate the findings in field studies (Lioy et al. 1985; Bates and Sizto 1987; Spektor et al. 1988), the findings reported here are not inconsistent with recent results from our research group. Whether significant changes in pulmonary function would have been seen at the end of 45 minutes of exposure in the present study is not known. Peak expiratory flow rate was recorded during the exposures at the end of the first and second exercise periods, which were at 30 and 60 minutes of exposure. Although the values decreased during all test atmosphere exposures, no significant decrements in these measurements were seen. The largest decrease (24 L/min) was seen during $O_3 + NO_2$ exposure.

The reason for the disappearance of H_2SO_4-induced pulmonary function changes is unknown. Several possibilities have been suggested. In the study by Utell and associates (1989) of H_2SO_4 exposure, they depleted oral ammonia with an acidic gargle and found larger changes in pulmonary function with lower ammonia levels. These authors suggested that increasing oral ammonia concentrations may have been responsible for the plateau seen in pulmonary function in that study. However in the dose-response study in our laboratory (Koenig et al. 1992), oral ammonia also was depleted (with a lemonade drink), but lemonade was taken ad libitum throughout the exposure. Thus, increasing oral ammonia levels seems a less likely explanation for our results. On the other hand, as mentioned earlier and as seen in Table 5, oral ammonia concentrations consistently increase in studies in our laboratory. This increase was sig-

nificant in a recent study of the inhaled effects of hydrochloric acid in adult subjects with asthma (Stevens et al. 1992). Therefore, even with continuing lemonade ingestion, oral ammonia levels may have increased in the dose-response study, and are known to have increased in the present study; this increase in oral ammonia levels could neutralize all the HNO_3 and most of the H_2SO_4 at the concentrations used (Hanley et al. 1992).

Other possible mechanisms for the disappearance of pollutant-induced effects with continued duration of exposure are (1) the adaptive changes in the fluid in the epithelial lining of the airways, and (2) competitive bronchodilatory mediators elicited by exercise. Evidence against a diminished effect with continued exposure comes from studies of O_3 combined with exercise. Horstman and Folinsbee (1990) have shown progressive decrements in FEV_1 with 6.6-hour exposures to concentrations as low as 0.08 ppm ozone. However, pulmonary function changes were not seen in that study after only 90 minutes of exposure.

Of the 28 subjects enrolled in the study, 6 subjects left before completion (Table 15), as discussed in the Results section. The effect of the loss of these subjects on the statistical outcome of the study cannot be known. On the other hand, it also cannot be known what would be the effect of conducting a similar study in a group of subjects with more severe asthma (if, in fact, such a group would agree to undergo such a study). Parents of subjects with more severe asthma usually decline invitations to participate in our laboratory studies. With respect to this issue, we believe that the fact that six subjects left after pollutant exposure but not after air exposure is a piece of clinical data that should be included in a summary of the outcomes of this study.

Thus, even though no significant group changes were seen after this three-year study, several suggestions of effects are important to note. First, all six of the subjects who left did so after exposure to pollutants rather than after exposure to clean air. Although these are anecdotal data, they may not be trivial. If, in fact, these symptoms were related to the pollutant exposures, they are considerably more important than the 5% decrement in pulmonary function that our study was designed to detect.

Second, the magnitude of the exercise-induced bronchospasm and the PD_{20} for methacholine did not correlate with pulmonary function changes after pollutants. However it is interesting to note that peak flow values measured during oxidants + HNO_3 exposure were significantly related to younger age.

Finally, it must be emphasized that changes may have occurred in physiological or biochemical variables that were not measured. A recent study from our laboratory has shown a significant increase in white blood cell counts in adult subjects with asthma who were exposed to 0.24 ppm ozone for 90 minutes (McBride et al. 1994). The increase in cells was significant immediately after exposure, remitted at 6 hours after exposure, and was significant again at 24 hours after exposure. Although almost 80% of the cells were neutrophils, neutrophil influx alone after exposure was not significant when compared with air exposure. No significant changes in the white blood cell count in the healthy subjects were noted. Furthermore, no significant changes in any pulmonary function parameter were seen at any time in that study, which emphasizes the lack of coherence between biochemical and pulmonary function tests.

The greatest difficulty in interpreting the data from the present study is the lack of effects after oxidants + H_2SO_4 exposures. As discussed earlier, the effect of increasing the length of exposure has been shown to be attenuation, rather than exaggeration, of the decrease in pulmonary function

Table 15. Circumstances Of Subjects Who Left the Study

Subject	Time of Departure	Reason
7	After Day 1 (O_3 + NO_2 + HNO_3).	Felt ill.
15	After Week 1 (O_3 + NO_2).	Experienced aggravated asthma.
17	After Week 3 (O_3 + NO_2 + H_2SO_4; Air; O_3 + NO_2 + HNO_3).	Developed pneumonia.
19	After two weeks (Air; O_3 + NO_2 + HNO_3).	Developed a sinus infection and tiredness.
20	After Week 2, Day 1 (Air; O_3 + NO_2).	Reason unknown.
25	After Week 2, Day 1 (O_3 + NO_2 + H_2SO_4; O_3 + NO_2).	Developed severe headache and tiredness.

associated with exposure to H_2SO_4. Until this dilemma is understood, one must be very cautious in making conclusions from this study.

Although some range in response to the screening methacholine challenges was evident across the subjects in this study, the adolescents responded as a homogeneous group when compared with subjects with asthma in most controlled studies of air pollutant effects. Obviously, unless identical twins are studied, some heterogeneity will always be present in studies of humans. However a group of subjects with asthma who range in age from 12 to 19 years, who all have allergic asthma (immediate Type I hypersensitivity), and who all are recruited from the same clinic (and thus are receiving comparable medical treatment) is homogeneous by most standards. These subjects were recruited using criteria identical to those used in other studies in our laboratory where significant effects have been seen. To interpret these data, comparing them with the results of two relevant studies may be helpful: (1) a study by Hanley and associates (1992) in which significant decrements in FEV_1 and FVC were recorded for 22 adolescents with asthma who had been exposed to H_2SO_4 at 70 μg or 135 $\mu g/m^3$ for 40 or 45 minutes; and (2) Koenig and associates (1992), in which significant decrements in FEV_1 are described after 45-minute exposures, but not after 90-minute exposures for 14 adolescents with asthma who had been exposed to H_2SO_4 at 35 μg or 70 $\mu g/m^3$. In the present study, none of the variables that might be considered risk factors for pollutant-induced effects, such as exercise-induced bronchospasm, PD_{20}, or medication use showed any correlation with pulmonary function changes. Of course, the range of these pulmonary function changes was small.

With respect to public health significance, it is important to stress that adolescent subjects with asthma are representative of a large portion of the general population. Approximately 15% of the population has atopy or allergic hypersensitivity. Of the general population, 3% to 4% have asthma (Gregg 1983); approximately 8% of children have asthma. Of nonasthmatic atopic individuals (3% of the population) one-third have exercise-induced bronchospasm. Also, some studies indicate that 4% to 5% of people who have no allergies have exercise-induced bronchospasm (Kawabori et al. 1976). Altogether it is estimated that at least 10% of the general population has asthma or exercise-induced bronchospasm as a manifestation of bronchial hyperresponsiveness. Thus, in the United States, at least 25 million people are likely to have bronchial hyperresponsiveness to inhaled irritants or provocative challenges. The experience of the U.S. Olympic Committee confirms this estimate. Of 597 members of the Olympic team, 67 (11%) had documented exercise-induced bronchospasm when given an exercise treadmill test (Pierson and Voy 1988).

These data also have significance for the regulatory process. Little is known about pulmonary responses to combined pollutants and this study has begun to answer those questions. Furthermore, this study investigated acidic compounds which are of special interest to the EPA in light of the current discussions of the need to set an ambient air quality standard for acid aerosols. This study extended previous studies from our laboratory that investigated 45-minute exposures to SO_2, H_2SO_4, and HNO_3 (funded by the National Institute of Environmental Health Sciences) (Koenig et al. 1989). We also investigated 90-minute exposures to O_3, HNO_3, and H_2SO_4 (but not NO_2) in 13 young subjects with asthma recruited using the same criteria as in this Health Effects Institute study (unpublished data). No significant changes in pulmonary function were detected after those exposures either. The two data sets should provide important conclusions about the pulmonary effects of combined pollutant exposures in adolescent subjects with asthma.

Several protocols would be useful in further evaluations of controlled exposures to summer acid haze conditions. First, obviously, would be to repeat this protocol and add pulmonary function testing during the exposures to determine whether decrements occurred and then subsided. Another would be to repeat this protocol but to add measurements of upper or lower airway inflammation, or both, using lavage techniques. Many potential changes in the present protocol could reflect different exposure durations, peak versus constant exposure concentrations, and different exposure-recording intervals. We hope that future studies will explain the attenuation of H_2SO_4 effects with increased exposure duration, and that the effects seen in summer camp studies that were greater than the effects in this controlled laboratory study will be investigated. These results are necessary to have sufficient data to protect the public health.

ACKNOWLEDGMENTS

The authors acknowledge the guidance and enthusiasm for this study by our colleague William E. Pierson. The authors thank Drs. David Thom and David Atkins for medical assistance, Theodore Anderson for his technical assis-

tance with the nitric acid generation, and Carol Malte and Ann Dannhauer for their technical assistance with data collection and calculations. Finally we thank the subjects and their parents for the long hours required by participation in the study.

REFERENCES

Bates DV, Sizto R. 1983. Relationship between air pollution levels and hospital admissions in Southern Ontario. Can J Public Health 74:117–122.

Bates DV, Sizto R. 1987. Air pollution and hospital admissions in Southern Ontario: The acid summer haze effect. Environ Res 434:317–331.

Bauer MA, Utell MJ, Morrow PE, Speers DM. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. Am Rev Respir Dis 134:1203–1208.

Becker R, Chambers J, Wilks A. 1988. The New S Language. Brooks/Cole Publishing Co., Pacific Grove, CA.

Brown B, Hollander M. 1977. Statistics: A biomedical introduction. Wiley & Sons, New York, NY.

Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG. 1975. Standardization of bronchial inhalation challenge procedures. J Allergy Clin Immunol 56:323–327.

DuBois AB, Botelho SY, Bedell CN, Marshall R, Comroe JH Jr. 1956. A rapid plethysmographic method for measuring thoracic gas volume: A comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. J Clin Invest 135:322–326.

Eggleston PA, Rosenthal RP, Anderson SA, Anderton R, Bierman CW, Bleecker ER, Chai H, Cropp GJ, Johnson JD, Konig P, Morse J, Smith LJ, Summers RJ, Trautlein JJ. 1979. Guidelines for the methodology of exercise challenge testing in asthmatics: Study group on exercise challenge, bronchoprovocation committee. J Allergy Clin Immunol 64:642–645.

Finlayson-Pitts BJ, Pitts JN. 1986. Atmospheric Chemistry, pp. 522–586. Wiley Interscience, New York, NY.

Folinsbee LJ, Bedi JF, Horvath SM. 1980. Respiratory responses in humans repeatedly exposed to low concentrations of ozone. Am Rev Respir Dis 121:431–439.

Goldman M, Knudson RJ, Mead J, Peterson N, Schwaber JR, Wohl ME. 1970. A simplified measurement of respiratory resistance by forced oscillation. J Appl Physiol 28:113–116.

Gregg I. 1983. Epidemiological aspects. In: Asthma (Clark TJH, Godfrey S, eds.) pp. 242–284. Chapman & Hall, London, England.

Hackney JD, Linn WS, Mohler JG, Collier CR. 1977. Adaptation to short term respiratory effects of ozone in men exposed repeatedly. J Appl Physiol 43:82–85.

Harris GW, Gervase IM, Iguchi T, Schiff HI, Scheutzle D. 1987. Measurement of NO_2 and HNO_3 diesel exhaust gas by tunable diode laser absorption spectra. Environ Sci Technol 21:299–304.

Hanley QS, Koenig JQ, Larson TV, Anderson TL, van Belle G, Rebolledo V, Covert DS, Pierson WE. 1992. Response of young asthmatics to inhaled sulfuric acid. Am Rev Respir Dis 145:326–331.

Horstman D, Roger LG, Kehrl H, Hazucha M. 1986. Airway sensitivity of asthmatics to sulfur dioxide. Toxicol Ind Health 2:289–298.

Horstman DH, Folinsbee LJ. 1990. Ozone concentration and pulmonary response relationships for 6.6 hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am Rev Respir Dis 142:1158–1163.

Horvath SM, Gliner JA, Folinsbee LJ. 1981. Adaptation to ozone: Duration of effect. Am Rev Respir Dis 123:496–499.

Joseph DW, Spicer CW. 1978. Chemiluminescence method for atmospheric monitoring of nitric acid and nitrogen oxides. Anal Chem 50:1400–1430.

Kagawa J. 1983. Respiratory effects of two-hour exposure with intermittent exercise to ozone, sulfur dioxide and nitrogen dioxide alone and in combination in normal subjects. Am Ind Hyg Assoc J 44:14–20.

Kawabori I, Pierson WE, Conquest LL, Bierman CW. 1976. Incidence of exercise-induced asthma in children. J Allergy Clin Immunol 58:447–455.

Kleinman MT, Bailey RM, Whynot JD, Anderson KR, Linn WS, Hackney JD. 1985. Controlled exposure to a mixture of SO_2, NO_2 and particulate air pollutants: Effects on human pulmonary function and respiratory symptoms. Arch Environ Health 40:197–201.

Koenig JQ, Covert DS, Hanley Q, van Belle G, Pierson WE. 1990. Prior exposure to ozone potentiates subsequent response to sulfur dioxide in adolescent asthmatic subjects. Am Rev Respir Dis 141:377–380.

Koenig JQ, Covert DS, Larson TV, Pierson WE. 1992. The effect of duration of exposure on sulfuric acid–induced pulmonary function changes in asthmatic adolescent subjects: A dose response study. Toxicol Ind Health 8:285–296.

Koenig JQ, Covert DS, Marshall SG, van Belle G, Pierson WE. 1987. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 136:1152–1157.

Koenig JQ, Covert DS, Morgan MS, Horike M, Horike N, Marshall SG, Pierson WE. 1985. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 132:648–561.

Koenig JQ, Covert DS, Pierson WE. 1989. Effects of inhalation of acidic compounds on pulmonary function in allergic adolescent subjects. Environ Health Perspect 79:173–178.

Koenig JQ, Covert DS, Smith MS, van Belle G, Pierson WE. 1988. The pulmonary effects of ozone and nitrogen dioxide alone and combined in healthy and asthmatic adolescent subjects. Toxicol Ind Health 4:521–532.

Koenig JQ, Pierson WE, Frank R. 1980. Acute effects of inhaled SO_2 plus NaCl droplet aerosol on pulmonary function in asthmatic adolescents. Environ Res 22:145–153.

Koenig JQ, Pierson WE, Horike M, Frank R. 1981. Effects of SO_2 plus NaCl aerosol combined with moderate exercise on pulmonary function in asthmatic adolescents. Environ Health 25:340–348.

Koenig JQ, Pierson WE, Horike M. 1983a. The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 128:221–225.

Koenig JQ, Pierson WE, Horike M, Frank R. 1983b. A comparison of the pulmonary effects of 0.5 ppm versus 1.0 ppm sulfur dioxide plus sodium chloride droplets in asthmatic adolescents. J Toxicol Environ Health 11:129–139.

Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, McDonnell WF, Bromberg PA. 1989. Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis 139:407–415.

Kulle TJ, Kerr HD, Farrell BP, Sauder LR, Bermel MS. 1982. Pulmonary function and bronchial reactivity in human subjects with exposure to ozone and respirable sulfuric acid aerosol. Am Rev Respir Dis 126:996–1000.

Kulle TJ, Sauder LR, Shanty F, Kerr HD, Farrell BP, Miller WR, Milham JH. 1984. Sulfur dioxide and ammonium sulfate effects of pulmonary function and bronchial reactivity in human subjects. Am Ind Hyg Assoc J 45:156–161.

Larson TV, Covert DS, Frank R. 1979. A method for continuous measurement of ammonia in respiratory airways. J Appl Physiol 46:603–607.

Linn WS, Jones MP, Bailey RM, Kleinman MT, Spier CE, Fischer DA, Hackney JD. 1980. Respiratory effects of mixed nitrogen dioxide and sulfur dioxide in human volunteers under simulated ambient exposure conditions. Environ Res 22:431–438.

Linn WS, Venet TG, Shamoo DA, Valencia LM, Anzar VT, Spier CE, Hackney JD. 1983. Respiratory effects of sulfur dioxide in heavily exercising asthmatics: A dose response study. Am Rev Respir Dis 127:278–283.

Lioy PJ, Vollmuth TA, Lippmann M. 1985. Persistence of peak flow decrement in children following ozone exposures exceeding the National Ambient Air Quality Standard. J Air Pollut Control Assoc 35:1069–1071.

McBride DE, Koenig JQ, Luchtel DL, Williams PV, Henderson WR Jr. 1994. Inflammatory effects of ozone in the upper airways of subjects with asthma. Am J Respir Crit Care Med 149:1192–1197.

Pierson WE, Voy RO. 1988. Exercise-induced bronchospasm in the XXIII Summer Olympic games. N Engl Reg Allergy Proc 9:209–213.

Roger LJ, Horstman DH, McDonnell WF, Kehrl H, Seal E, Chapman RS, Massaro EJ. 1985. Pulmonary effects in asthmatics exposed to 0.3 ppm NO_2 during repeated exercise. Toxicologist 5:70.

Shapiro GG, Furukawa CT, Pierson WE, Bierman CW. 1982. Methacholine bronchial challenge in children. J Allergy Clin Immunol 69:365–369.

Shapiro GG, Sinon RA. 1992. Bronchoprovocation committee report. J Allergy Clin Immunol 89:775–778.

Sheppard D, Saisho A, Nadel JA, Boushey HA. 1981. Exercise increases sulfur dioxide–induced bronchoconstriction in asthmatic subjects. Am Rev Respir Dis 123:486–491.

Spektor DM, Lippmann M, Lioy PJ. 1988. Effects of ambient ozone on respiratory function in active, normal children. Am Rev Respir Dis 137:313–320.

Spengler JD, Keeler GJ, Koutrakis P, Ryan P, Raizenne M, Franklin CA. 1989. Exposures to acid aerosols. Environ Health Perspect 79:43–52.

Stacy RW, Seal E, House DE, Green J, Roger LG, Raggio L. 1983. A survey of effects of gaseous and aerosol pollutants on pulmonary function of normal males. Arch Environ Health 38:104–115.

Stevens B, Koenig JQ, Rebolledo V, Hanley QS, Covert DS. 1992. Respiratory effects from the inhalation of hydrogen chloride in young adult asthmatics. J Occup Med 34:923–929.

Tukey J. 1977. Exploratory Data Analysis. Addison-Wesley, Reading, MA.

U.S. Environmental Protection Agency. 1991. Air quality criteria for oxides of nitrogen. EPA/600/8–91/049. Office of Air Quality Planning and Standards, Research Triangle Park, NC.

Utell M, Morrow P, Bauer M, Frampton M. 1989. Effects of inhaled acidic aerosols on respiratory function: Controlled clinical studies. Presented at the 82nd Annual Meeting, Air and Waste Management Association, Anaheim, CA, June, 1989.

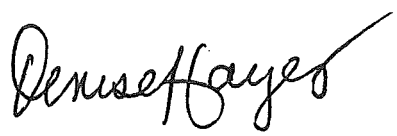
Warren DL, Last JA. 1987. Synergistic interaction of ozone and respirable aerosols on rat lungs: III. Ozone and sulfuric acid aerosol. Toxicol Appl Pharmacol 88:203–216.

Weiss RE, Larson TV, Waggoner AP. 1983. In situ rapid response measurement of $H_2SO_4/(NH_4)_2SO_4$ aerosols in rural Virginia. Atmos Environ 17:525–531.

APPENDIX A. External Quality Assurance Report

The conduct of this study has been subjected to periodic audits by the Quality Assurance Office from Arthur D. Little, Inc. The audits have included in-process monitoring of study activities and audits of the data. The dates of the audits, and the nature of the visits are listed in Table A.1. The results of the inspections were reported to the Director of Research at the Health Effects Institute, who was responsible for transmitting the reports to the Principal Investigator.

Observations made during these visits indicate that the study is adequately documented, and that the Investigators' Report describes the methods used and reflects the raw data. The effects of deviations from the protocol and standard operating procedures on the results of the study have been considered and addressed, as appropriate, in the analysis of the data and in the Investigators' Report.



Denise Hayes, M.S.
Quality Assurance Officer
Arthur D. Little

Table A.1 Audits by Quality Assurance Officer

Date of Audit	Focus of Audit
March 28, 1989	Pre-study review of protocol, procedures, and facilities
August 13-14, 1989	Observation of subject testing
February 23, 1990	Review of revised study procedures
June 20-21, 1990	Observation of subject testing
August 13-14, 1991	Audit of study data
March 2-3, 1992	Audit of study data
July, November, and December 1993	Audit of study data and review of final report

APPENDIX B. Individual Subject Values for Pulmonary Function Measurements

This appendix, which includes tables of individual subject values for FEV_1, FVC, R_T, $\dot{V}_{max50}$, $\dot{V}_{max75}$, and peak flow, is available upon request from the Health Effects Institute, 141 Portland Street, Suite 7300, Cambridge, MA 02139.

ABOUT THE AUTHORS

Jane Q. Koenig, Ph.D., is Professor in the Department of Environmental Health in the School of Public Health and Community Medicine at the University of Washington. She has been involved in research on the health effects of air pollutants for 15 years and has published widely in the field.

David S. Covert, Ph.D., is a Research Associate Professor in the Department of Environmental Health in the School of Public Health and Community Medicine at the University of Washington. He is an expert in atmospheric chemistry and cloud droplet formation and has published widely in these fields.

William E. Pierson, M.D., is a Clinical Professor in the Departments of Pediatrics in the School of Medicine and Environmental Health, and in the School of Public Health and Community Medicine at the University of Washington.

He is an international expert on asthma and other allergic diseases. He is a past president of the American Academy of Allergy and Immunology (1991-1992).

Quentin S. Hanley, B.A., was a Research Technician in the Department of Environmental Health in the School of Public Health and Community Medicine at the University of Washington for four years. He is currently in the Department of Chemistry at the University of Arizona, Tucson, AZ.

Viviana Rebolledo, B.S. in Biology, was a Research Technician in the Department of Environmental Health in the School of Public Health and Community Medicine at the University of Washington from 1988 to 1992. She has four years of experience measuring pulmonary function in children and adults.

Karen Dumler, B.A. in Zoology, was a Research Technician in the Department of Environmental Health in the School of Public Health and Community Medicine at the University of Washington. She has four years of experience measuring pulmonary function in children and adults.

Steve E. McKinney was a graduate student in the Department of Biostatistics in the School of Public Health and Community Medicine at the University of Washington from 1988 to 1992.

PUBLICATIONS RESULTING FROM THIS RESEARCH

Koenig JQ, Hanley QS, Rebolledo V, Dumler K, Covert DS, Pierson WE. 1991. Pulmonary effects of oxidants combined with sulfuric acid and nitric acid in asthmatic adolescents (abstract). Am Rev Respir Dis 143:A97.

ABBREVIATIONS

$C \times T$	concentration × time
FEV_1	forced expiratory volume in one second
FRC	functional residual capacity
FVC	forced vital capacity
H^+	hydrogen ion
H_2SO_4	sulfuric acid
HCl	hydrochloric acid
HNO_3	nitric acid
NAAQS	National Ambient Air Quality Standard
NO_2	nitrogen dioxide
O_3	ozone
PD_{20}	provocative dose at which FEV_1 decreases by 20% or more
ppm	parts per million
R_T	total respiratory resistance
SO_2	sulfur dioxide
$\dot{V}_E$	expired volume of ventilation per minute (minute ventilation)
$\dot{V}_{max}$	maximum flow
$\dot{V}_{max50}$	maximum flow @ 50% of expired vital capacity
$\dot{V}_{max75}$	maximum flow @ 75% of expired vital capacity

Oxidant and Acid Aerosol Exposure in Healthy Subjects and Subjects with Asthma

Part II: Effects of Sequential Sulfuric Acid and Ozone Exposures on the Pulmonary Function of Healthy Subjects and Subjects with Asthma

Mark J. Utell, Mark W. Frampton, Paul E. Morrow, Christopher Cox, Paul C. Levy, Donna M. Speers, and F. Raymond Gibb

ABSTRACT

These studies were undertaken to evaluate pulmonary responses of humans sequentially exposed to acidic aerosols and ozone at levels that could reasonably be encountered in actual environmental exposures. Subjects first were exposed to sulfuric acid (H_2SO_4) aerosol to sensitize the airways to ozone. The exposure protocols were designed to provide more quantitative information about the threshold levels of ozone that produce adverse biological effects and to provide exposure-response data on ozone.

Two groups of 30 nonsmoking volunteers of both sexes, between the ages of 18 and 45 years, were recruited. The healthy study population comprised 16 men and 14 women with an average age of 28 years and no airway hyperreactivity. The second group comprised 10 men and 20 women comparable in age to the control group, but with allergic asthma and positive skin tests.

The study examined an exposure-response relationship using three levels of ozone ranging from below the current standard to one and one-half times the ambient air quality standard (0.08, 0.12, and 0.18 ppm* [parts per million]) with preexposure 24 hours earlier to H_2SO_4 (100 $\mu g/m^3$) or sodium chloride (NaCl) (control) aerosol in a 45-m^3 environmental chamber. The study used an incomplete block design in which each subject was exposed to four of the six paired experimental atmospheres. Both the selection of paired exposures and the order in which they were presented were randomized. The exposure protocol required nine days: Day 1, training and baseline preexposure measurements; Day 2, the first of the three-hour particle (H_2SO_4 or NaCl) exposures; Day 3 (24 hours after Day 2), ozone exposure at 0.08, 0.12, or 0.18 ppm for three hours; Day 4 (two to four weeks later), exposure to the other test aerosol; Day 5 (24 hours after Day 4), exposure to the same ozone concentration as on Day 4. After at least another two weeks, Days 6, 7, 8, and 9 repeated Days 2, 3, 4, and 5 using a second ozone concentration. All three-hour exposures included several predetermined periods of exercise and pulmonary function measurements. To examine for delayed effects, pulmonary function tests were measured two and four hours after exposure on the ozone days.

Data were analyzed over the time course of exposure and by exposure level of ozone at each time point to reveal dose-response relationships more closely . The main findings of the study are as follows. No significant symptomatic or physiologic effects of exposure to either aerosol or ozone on lung function were found for the healthy group. No evidence for an effect of aerosol preexposure on the ozone response was found. For the asthmatic group, preexposure to H_2SO_4 had no direct effect on lung function but appeared to enhance the small mean decrements in forced vital capacity (FVC) that occurred in response to 0.18 ppm ozone (mean ± SE: –3.6% ± 1.5% with NaCl preexposure; –6.8% ± 1.7% with H_2SO_4 preexposure). Individual responses among subjects with asthma were quite variable, some demonstrating reductions of more than 35% in forced expiratory volume in one second (FEV_1) following ozone exposure. Analysis of variance of changes in FVC revealed evidence for interactions between aerosol and ozone exposure both immediately after ($p = 0.005$) and four hours after ($p = 0.030$) exposure. Similar effects were seen for FEV_1. When responses of healthy subjects and subjects with asthma were combined, four-way analysis of variance revealed an interaction between ozone and aerosol for the entire group ($p = 0.0022$) and a difference between healthy subjects and subjects with asthma ($p = 0.0048$).

In summary, no direct effects on lung function were observed in healthy subjects in response to H_2SO_4 aerosols

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

This Investigators' Report is Part II of Health Effects Institute Research Report Number 70, which also includes Part I: Effects of Oxidants, Combined with Sulfuric or Nitric Acid, on the Pulmonary Function of Adolescents with Asthma, by J.Q. Koenig and associates; a Commentary by the Health Review Committee on both Investigators' Reports; and an HEI Statement about the research projects. Correspondence concerning this Investigators' Report may be addressed to Dr. Mark J. Utell, Department of Medicine, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Box 692, Rochester, NY 14642.

Although this document was produced with partial funding by the United States Environmental Protection Agency under Assistance Agreement 816285 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 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 those parties, and no endorsement by them should be inferred.

or ozone, and no interaction between aerosol and ozone exposures was found. Our data, however, indicate that preexposure to H_2SO_4 aerosols may alter responses to ozone in exercising subjects with asthma. Thus, subjects with asthma differ from healthy volunteers in functional responses following sequential exposures to aerosols and ozone and appear to represent a susceptible population.

INTRODUCTION

Air in outdoor environments typically contains many gaseous and particulate pollutants that may adversely affect any individual at sufficiently high concentrations, and susceptible individuals at lower concentrations. Health effects believed to be associated with photochemical reaction products or components of acidic precipitation are of major concern. Ozone, the most important component of photochemical smog, results from a complex reaction of hydrocarbons and precursors of nitrogen oxides (NO_x) in the presence of sunlight. Once considered to be restricted to southern California, ozone pollution is now a common summertime problem in many central, northeastern, and southeastern cities and suburbs as well as rural regions downwind of these areas. In contrast, acidic aerosols result largely from the oxidation of sulfur oxide pollutants released above inversion layers from the tall stacks of utility plants and metal smelting operations during airborne transport. These aerosols, predominantly H_2SO_4, remain suspended and are carried for hundreds of miles, where they cause such environmental effects as acid rain in the northeastern United States and Canada (Dockery and Speizer 1989). Recent measurements have demonstrated 24-hour average H_2SO_4 concentrations exceeding 20 $\mu g/m^3$ and peak concentrations reaching 100 $\mu g/m^3$ (Spengler et al. 1989).

Pollutants in outdoor air are present in complex mixtures, although strategies for regulation and source control have tended to focus on single pollutants. The mixture of primary and secondary pollutants varies from urban to rural settings and across microenvironments. Although the complex nature of air pollution is well recognized, current estimates of acute risk from controlled inhalation studies of ozone or acidic aerosols are based largely on effects observed following single exposures to individual pollutants. Yet acidic aerosols and ozone occur together, especially during the summer, and could interact in producing health effects of greater significance than either pollutant alone.

Likewise, most epidemiologic studies of air pollution and health have focused on the effect of single pollutants or, at most, two specific pollutants such as total suspended particles and ozone. Interactions between ozone and total suspended particulate matter were reported to decrease expiratory flow in children (Lebowitz 1984), and in adults with obstructive airway disease (Lebowitz et al. 1982). Increased rates of hospital admissions for respiratory problems, including asthma, have been associated with summer haze events in southern Ontario, Canada, from 1974 through 1983 (Bates and Sizto 1987). During the summer, ambient levels of total sulfates and ozone in the previous 24 to 48 hours consistently were associated with admissions for acute respiratory problems. The measurement of total sulfates included strong acids (H_2SO_4) and partially or totally neutralized sulfate salts. In this region, sulfate concentrations were similar in winter (24-hour mean 12.4 $\mu g/m^3$) and summer (13.3 $\mu g/m^3$). However, significant associations with sulfate were observed only during the summer, suggesting that the excess admissions may be attributable to a sulfate-ozone interaction.

In several animal species, exposure to ozone and H_2SO_4 aerosols has been found to produce synergistic effects on host defenses including ciliary beating (Grose et al. 1980) and susceptibility to respiratory infections (Gardner et al. 1977). In rabbits, enhanced or antagonized secretory cell responses have been observed for 0.1 ppm ozone exposures in combination with 125 $\mu g/m^3$ H_2SO_4 aerosols (Schlesinger and Graham 1992). Studies of plasma protein leakage in rats by Last and coworkers (1984, 1986) demonstrated synergy of ozone with sulfate aerosols. Although the mechanisms of these interactions are not well defined, they have been postulated to involve the prolongation of free radical lifetimes in the atmospheric reaction mixture (Last et al. 1984).

Relatively few experimental exposures to mixtures of pollutants in healthy individuals or in those with acute and chronic respiratory diseases have been reported. In these studies, exposures to mixtures generally have been simultaneous, not sequential. Stacy and coworkers (1983) compared the effect of 0.40 ppm ozone exposure with that of the same ozone concentration plus H_2SO_4 or ammonium bisulfate (NH_4HSO_4) aerosols (100 $\mu g/m^3$) in different groups of healthy subjects. Kagawa (1986) reported the results of a series of exposure studies that included ozone plus H_2SO_4 aerosol combinations in healthy volunteers. In brief, both groups of investigators concluded that the simultaneous administration of 200 $\mu g/m^3$ H_2SO_4 aerosol and 0.15 ppm ozone produced no greater effect than ozone alone. More recently, Horvath and coworkers (1987) found no significant changes in pulmonary function in nine healthy men exposed to 1,500 $\mu g/m^3$ H_2SO_4 aerosols and 0.25 ppm ozone for two hours. The findings from these studies support our impression that, in general, controlled

human exposure studies in chambers have not demonstrated synergism in functional responses between ozone and H_2SO_4 in healthy volunteers.

Using a sequential exposure protocol, Kulle and colleagues (1982) studied the effects of a four-hour exposure to 100 $\mu g/m^3$ H_2SO_4 aerosol (0.13 μm mean size) preceded by a two-hour exposure to 0.30 ppm ozone. All pollutants were examined individually and then in combination with an identical exposure sequence: ozone, H_2SO_4 aerosol, and ozone plus H_2SO_4 aerosol, each exposure separated by one week. Although all exposures included one 15-minute period of moderate cycling exercise, none of the exposures produced significant changes in spirometry or plethysmography. It was suggested that the use of a larger particle-size H_2SO_4 aerosol might have produced different results because a large aerosol would tend to deposit by impaction to a greater extent in the larger airways, the apparent site of the symptomatic effects of ozone.

In contrast to healthy volunteers, subjects with asthma have not been exposed to mixtures of acid aerosols and ozone. However, two recent reports suggest interactions of simultaneous acid aerosol exposures with low levels of sulfur dioxide (SO_2) in subjects with asthma. Horstman and colleagues (1986) exposed mildly asthmatic subjects to a combination of 0.75 ppm SO_2 and 100 $\mu g/m^3$ H_2SO_4 aerosol to determine whether small amounts of the acid aerosol could influence the asthmatic response to SO_2. Preliminary analyses indicated increases in specific airway resistance, decreases in flow rates, and symptom responses after the combined exposure. These findings are similar to reports by Koenig and coworkers (1989) that decrements in lung function after exposure to H_2SO_4 at 68 $\mu g/m^3$ plus 0.1 ppm SO_2 were greater than those after exposure to either pollutant alone in exercising, adolescent subjects with asthma. To our knowledge, sequential pollutant exposures have not been performed in subjects with asthma.

Although the epidemiologic evidence does not establish the optimal time for studying interactive phenomena, available evidence tends to correlate many pollutant-induced effects with a delay between peak environmental levels and such criteria as hospital admissions (Bates and Sizto 1987). Controlled human studies with several air pollutants, especially the acidic sulfates, have demonstrated both immediate and delayed actions. In our laboratory, a four-hour exposure to 450 $\mu g/m^3$ H_2SO_4 aerosols in healthy subjects resulted in no immediate change in lung function or airway reactivity to carbachol (Utell et al. 1983a). However, 24 hours after exposure, carbachol reactivity was increased, and 60% of the subjects experienced throat irritation 12 to 24 hours after exposure. Linn and colleagues (1986) reported increasing symptom scores in subjects with asthma one week after controlled exposure to acid aerosols. Controlled clinical ozone studies have shown that ozone-induced pulmonary function decrements are progressive, unlike those induced by acidic sulfate exposures (Folinsbee et al. 1988). By using a sequential exposure protocol, the responses to these two important pollutants may be maximized. From the collective information available, the delayed or predisposing interaction of one pollutant on another is a highly significant part of health effects related to air pollution.

AIMS

The principal objective of this project was to examine pulmonary responses of humans to sequenced exposures of acidic aerosols and oxidants at levels that might be reasonably encountered and are, therefore, relevant to actual environmental exposures. Three specific goals for our studies were proposed. First, these studies were designed to evaluate the interactive effects of ozone with a common copollutant, namely, H_2SO_4 aerosols at near ambient concentrations. Second, we proposed that prior pollutant exposure to sensitize the airway to ozone would provide quantitative information about the threshold levels of ozone that produce adverse biological effects and also would provide exposure-response data on ozone. Third, these studies would permit additional investigation of responses to ozone in subjects with asthma. This remains an important issue because asthma incidence is increasing in the United States (Sheffer 1991). Moreover, recent epidemiologic studies suggest that ozone worsens asthma (Thurston et al. 1992) although clinical studies generally fail to show increased responses to ozone in subjects with asthma. Therefore, a potentially susceptible subgroup of subjects with allergic asthma was evaluated and compared with a control group of healthy subjects exposed to the same environmental conditions. Three levels of ozone were selected, ranging from below the present standard to one and one-half times the ozone standard (0.08, 0.12, and 0.18 ppm), with preexposure 24 hours earlier to 100 $\mu g/m^3$ H_2SO_4 or a NaCl aerosol of the same characteristics. Thus, all exposure levels were realistic and of clinical importance. An incomplete block exposure design was used such that each subject was exposed to ozone at two of the three exposure levels (see Methods section).

The foregoing objectives were expected to contribute at least partial answers to the following questions: Does previous exposure to an acidic aerosol sensitize the airway to ozone? How different are the dose-response characteristics to controlled pollutant exposure in healthy volunteers and

volunteers with asthma? How important is the activity state (exercise) of individuals to their susceptibility? Are subject selection criteria, which depend heavily on clinical evaluations, capable of providing sufficiently uniform groups in terms of responsiveness to pollutants? For example, do group data obscure the susceptibility of several individual responders within the group?

Providing partial answers to these questions would place the controlled human study in a clearer context and possibly improve the interpretability of findings. Clearly there was no expectation that this group of studies by their design and findings would directly affect regulatory decisions on ozone or acidic aerosols. However, we envisioned that these studies could constitute an important contribution to a developing database on human and experimental findings and epidemiologic studies. Furthermore, information obtained would provide ultimately unified concepts about human responses to oxidants and permit essential data for use in risk assessment.

METHODS

This section consists of a general overview of the study design, followed by a detailed description of the study protocol and specific research methods. The project consisted of separate studies in healthy volunteers and volunteers with asthma, but, in fact, subjects with and without asthma were paired experimentally.

STUDY DESIGN

The study examined an exposure-response relationship using three levels of ozone ranging from below the current standard to one and one-half times the ambient air quality standard (0.08, 0.12, and 0.18 ppm) with preexposure 24 hours earlier to H_2SO_4 or NaCl aerosol at a concentration of 100 $\mu g/m^3$ in a controlled human environmental chamber. The study, performed over three years, used an incomplete block design, such that each subject was exposed to two ozone concentrations. A potentially susceptible group of 30 extrinsic subjects with asthma was evaluated, and responses were compared with those of a control group of 30 healthy subjects. Ten subjects from each group received the same two of three ozone concentrations. This resulted in 20 subjects receiving each of the three individual ozone concentrations. Volunteers were assigned randomly to pairs of ozone concentrations; the randomization was blocked to control for changes over time. The term "blocked" refers to the fact that numbers of subjects receiving the various treatment combinations were periodically equalized over time, so that any given combination was not concentrated at the beginning or end of the study. In addition, the order of presentation of H_2SO_4 or NaCl aerosol and the two levels of ozone was randomized in a blocked fashion. Features of the exposure protocol for both healthy subjects and subjects with asthma are summarized in Table 1. Specifically, 12 possible treatment assignments were created, consisting of the following combinations (three choices for two ozone levels) × (two possible orders of administration of ozone) × (two orders of administration of aerosol for first ozone exposure, paired with the opposite order administration for the second ozone exposure). Two sets of these 12 were then combined with an additional subset of six, chosen so that each of the three possible choices of two ozone levels would be repeated a total of 10 times in 30 subjects. These 30 assignments were then blocked to provide balance in ozone assignment over time, and the order was randomly permuted within each block. Individuals under 18 years and over 45 years of age, members of the research team, and individuals from outpatient

Table 1. Protocol of Paired Exposures for Healthy Subjects and Subjects with Asthma

A. H_2SO_4 (100 $\mu g/m^3$) followed 24 hours later by 0.08 ppm ozone	Preceded or followed by[a]	D. NaCl (100 $\mu g/m^3$) followed 24 hours later by 0.08 ppm ozone
B. H_2SO_4 (100 $\mu g/m^3$) followed 24 hours later by 0.12 ppm ozone	Preceded or followed by[a]	E. NaCl (100 $\mu g/m^3$) followed 24 hours later by 0.12 ppm ozone
C. H_2SO_4 (100 $\mu g/m^3$) followed 24 hours later by 0.18 ppm ozone	Preceded or followed by[a]	F. NaCl (100 $\mu g/m^3$) followed 24 hours later by 0.18 ppm ozone

[a] A two- to four-week interval separated the exposure regimen.

clinics in the Department of Medicine's Pulmonary Disease Unit were excluded. On the exposure days, subjects exercised intermittently; underwent pulmonary function tests before and immediately after exposure with additional measurements two and four hours after exposure on the ozone day to assess delayed responses; and were questioned regarding symptoms.

Exposure Conditions

Both simultaneous and sequential exposures to combinations of particles and oxidants occur, and both need study particularly as the responses appear to have different time courses (Utell et al. 1983a; Folinsbee et al. 1988). The levels of H_2SO_4 aerosols and ozone exposures used in this study were selected on the basis of available data in animals and humans at higher and lower exposure concentrations. For example, findings from recent controlled exposure studies demonstrated that 68 $\mu g/m^3$ H_2SO_4 aerosols produced 7% decreases in FEV_1 in adolescent allergic subjects with asthma (Koenig et al. 1989), and a two-hour exposure to 75 $\mu g/m^3$ H_2SO_4 aerosols produced significant reductions in FEV_1 in exercising adult subjects with asthma (Morrow et al. 1994). In addition, inhalation of acidic sulfates provoked delayed effects (24 hours postexposure) even when their immediate effects were not evident. The principal delayed effect was enhancement of responsiveness to bronchial challenge and increased symptoms rather than a delayed alteration in baseline functional values (Utell et al. 1983a; Spektor et al. 1985; Bauer et al. 1988). Therefore, the acid aerosol exposure of 100 $\mu g/m^3$ was selected to provide a concentration and duration known to produce decrements in lung function in volunteers as well as allow for follow-up evaluations 24 hours after the acidic aerosol exposure with both pulmonary function and symptom analyses. In addition, the aerosol level was sufficiently low to be relevant to ambient particle exposures.

At the time this protocol was designed, Folinsbee and coworkers (1988) at the U.S. Environmental Protection Agency (EPA) had completed ozone exposures of 6.6 hours in adults incorporating moderate exercise. Progressive decrements in FVC and FEV_1 were observed with no residual functional decrements on the following day. Follow-up studies in the same laboratory were under way with 6.6-hour exposures at 0.08, 0.10, and 0.12 ppm. Although progressive decrements were observed in relation to the lower ozone levels, the available results did not demonstrate statistically significant effects. Based on the results of these studies, we proposed using ozone concentrations of 0.08 ppm, 0.12 ppm, and 0.18 ppm for three hours, to be administered 24 hours after the particle exposure.

Furthermore, based on a 1987 review of the literature, we proposed investigating the potentially synergistic actions of ozone and acidic sulfates from the prospective of physiologic interactions using the sequential exposure strategy. Substantial experimental data suggested that simply mixing pollutants would not be an informative method to pursue. First, studies of simultaneous exposures to two pollutants, especially those conducted in humans, had not revealed important synergisms between their pollutant-induced prompt effects but rather that the combined action was no greater than that of the more potent single pollutant (Stacy et al. 1983; Kagawa 1986). Second, simultaneous exposures to combined pollutants, for example, H_2SO_4 and ozone, also involved the possibility of airborne reactions between the pollutants. The speciation produced was very likely to change depending on chamber turnover characteristics, environmental factors such as relative humidity, and the pollutant concentrations chosen. While these may be important to investigate, the chemical processes involved and the development of optimal conditions for human studies were not well understood. Finally, the combined simultaneous exposure approach failed to take advantage of the observations with H_2SO_4 aerosols, which indicated a potentiation of effects in terms of symptoms and airway reactivity 24 hours postexposure.

Environmental Chamber

All exposures were conducted in an environmental chamber (Utell et al. 1984; Morrow and Utell 1986) located in the University of Rochester Medical Center's Clinical Research Center (CRC). The chamber facility consisted of a 45-m^3 exposure chamber with an attached instrument room, an adjacent environmental control area, an adjoining bathroom, and an airlock entry (Figure 1). The exposure chamber was a single-pass system of air flow operating at approximately 10 m^3/min. The air was first passed through a series of pollutant absorbers and high-efficiency filters and then conditioned as to temperature and humidity before it was admitted to the chamber through five ceiling diffusers. Before the air-intake system divided and went to the diffusers, ozone or particles (H_2SO_4 or NaCl) were dynamically added at a specific injection point and diluted. For these studies, our aerosol generator (Gibb et al. 1990) contained a 1% (w/v) H_2SO_4 solution and produced an aerosol that subsequently was diluted by the air supply at 10 m^3/min to produce a chamber concentration of 100 $\mu g/m^3$. For the ozone studies, a portion of the ozonator output of 800 to 1,800 ppm was introduced into the purified air supply of 10 m^3/min to achieve the desired chamber concentration. In all cases, the chamber air was removed through five floor-level exhaust outlets by an exhaust fan, thereby keeping the chamber at negative pressure of 0.01 to 0.02 in H_2O with respect to the CRC area.

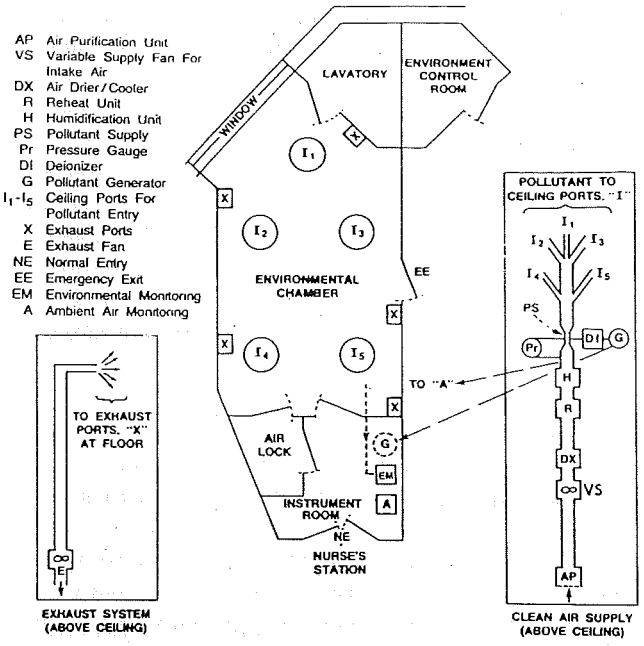


Figure 1. Schematic design of the 45-m^3 environmental chamber facility in the Clinical Research Center. The middle diagram indicates the major areas of the exposure facility: the environmental chamber, instrument room, environment control room, and lavatory. The independent ventilation system for the chamber is shown in two insets: the inset on the right depicts the air intake, purification, and conditioning system; the inset on the left depicts the exhaust portion of the system.

The mixing characteristics of the chamber under a constant airflow of 10 m^3/min were excellent, with 90% of the steady-state pollutant concentration achieved in approximately four minutes. Concentration differences in both ozone and aerosols throughout the chamber were within ±5% of the mean concentration.

Although the capability existed to set the relative humidity and temperature of the environmental chamber over a wide range, all studies were conducted in a comfort zone of 40% ± 5% humidity and 70° ± 2°F (21° ± 1°C) temperature.

Experimental Protocol

The study protocol was developed for two subjects per exposure period, but scheduling problems occasionally led to one-subject exposures. In all cases, the overall protocol required a minimum of nine days to complete (Appendix A). Day 1 was devoted to the preexposure baseline measurements. On Day 2, the first of the three-hour particle (H_2SO_4 or NaCl at 100 μg/m^3) exposures was conducted. On Day 3 (24 hours after Day 2), subjects were exposed to ozone for three hours at a concentration of either 0.080, 0.120, or 0.180 ppm. On Day 4 (two to four weeks later), subjects were exposed to the other test aerosol, and the protocol of Day 2 was repeated. And on Day 5 (24 hours after Day 4), subjects were exposed to the same concentration of ozone as on Day 4. After at least another two weeks, Days 6, 7, 8, and 9 repeated Days 2, 3, 4, and 5 using another ozone concentration. Studies were discontinued from mid-June through mid-September each year of the three-year study to avoid the periods of highest ambient ozone and acid aerosol concentrations.

The study used an incomplete block design in which each subject was exposed to four of the six paired-experimental atmospheres (Table 1). Both the selection of paired exposures and the order in which they were presented were randomized. A randomized complete block design in which each subject received every treatment would have been more efficient statistically; however, it would have required an additional four days per subject (total protocol would have been 13 days), which would have limited recruitment. The randomization scheme was devised by our statistician, Dr. C. Cox. The complete list of treatment assignments was prepared for each group of 30 subjects; each list was balanced with respect to the assignment of different levels of ozone through the course of the study. Given the complexity of pairs of assignments, we used a scheme in which both pairs of treatments were assigned, and then the order was randomized.

All studies were conducted under double-blind conditions: the subjects and the clinical investigation team responsible for the measurements and the interpretation of pulmonary function tests (M.J. Utell, M.W. Frampton, P.C. Levy, and D.M. Speers) were unaware of which aerosol was generated or which of the three concentrations of ozone was used. Only the chamber operations and analytical team (P.E. Morrow, F.R. Gibb, H.E. Beiter, and W. Kremer) were aware of the exposure conditions, and this codified information was not released until the entire experimental portion of each study was completed.

The criteria for determining responses in these studies were based primarily on pulmonary function testing in which individual responses were assessed before (baseline) and after each exposure, and comparisons were made between the sulfate and chloride preexposure conditions for each of the three levels of ozone. Analyses were made for each group of subjects and effects were compared in healthy subjects and subjects with asthma (see Data Handling and Statistical Methods section). The pulmonary function assessments included airway resistance, thoracic gas volume, and spirometric data. Similarly, symptom responses were assessed by questionnaire following exposures.

Subject Selection

Subject selection criteria were established for each group before volunteer solicitations were initiated in University

of Rochester publications. At initial interviews, each subject completed a standardized questionnaire (Appendix B) (Lebowitz et al. 1975). Each subject was assessed using a battery of pulmonary function tests, including spirometry, lung volumes, and specific airway conductance (s*G*aw). Additionally, a 12-lead electrocardiogram was recorded and interpreted for each subject. A thorough cardiac and respiratory history was taken and a general physical examination was performed. Information pertaining to lifestyle factors possibly relevant to oxidant-induced responses (for example, smoking habits of spouse or family, use of vitamin C and E, home cooking with gas, and kerosene space heating) was collected (Appendix B), but volunteers were not excluded on the basis of their answers.

The study protocols were approved by the Committee on Investigation Involving Human Subjects at the University of Rochester. Volunteers were informed of the study purposes, the experimental protocols and procedures, and the potential risk from participation in the study. Each subject signed an informed consent statement before participating in the study (see Appendix C). Each participant was informed of his or her right to withdraw from the study at any time. Of the 30 original participants, one asthmatic subject did not complete the assigned exposures, withdrew for reasons unrelated to the study, and is not included in the data presented. Another subject was recruited to provide a total of 30 subjects with asthma. Volunteers were compensated for the time they spent in the study.

Healthy Subject Group. Healthy, nonsmoking subjects, 18 to 45 years of age, were accepted if they fulfilled the following criteria: asymptomatic and no history of recurring respiratory disease; spirometry within the normal values based on the standards published by Morris and coworkers (1971); residual volume within 30% and total lung capacity within 15% of predicted values (Goldman and Becklake 1959); s*G*aw in the normal range published by Briscoe and DuBois (1958); lack of response to carbachol challenge at concentrations below 0.5% (Utell et al. 1979); and absence of an acute respiratory infection within the preceding six weeks.

From the healthy volunteers screened, we chose 30 subjects: 16 men and 14 women, with a mean age of 28 years. (Detailed characteristics of this group are given in Table 6 of the Results section.)

Asthmatic Subject Group. Subjects were considered to have bronchial asthma if they had two of the following: (1) a history of repetitive symptoms characteristic of intermittent bronchospasm (wheezing, shortness of breath); (2) abnormally low values for s*G*aw (Briscoe and DuBois 1958), or an FEV_1/FVC below age-predicted values, or both (Morris et al. 1971); and (3) an improvement of 15% or more in FEV_1 with the inhalation of isoproterenol, or a 40% or greater fall in s*G*aw or a 15% fall in FEV_1, or both, after a standardized carbachol aerosol challenge with 0.25% or less carbachol solution (Utell et al. 1979). Subjects with asthma did not require therapy with inhaled or systemic corticosteroids and were instructed to avoid using bronchodilators for six hours prior to each exposure. In addition, because allergic (extrinsic) subjects with asthma may comprise the most responsive group to inhaled pollutants (Koenig et al. 1983; Molfino et al. 1991), additional inclusion criteria included elevated serum immunoglobulin E (IgE) levels or wheal-and-flare responses to major aeroallergens present in Rochester including the house dust mite, grass and ragweed pollen, *Alternaria* and *Hormodendrum* mold spores, and cat and dog dander (obtained from Hollister-Stier, Rochester, NY).

Insofar as possible, selected subjects with asthma were age-matched with the healthy group. From the asthmatic volunteers screened, we chose 30 subjects: 10 men and 20 women, with a mean age of 28 years. (Detailed characteristics of this group are given in Table 7 of the Results section.)

EXPOSURE AND EVALUATIONS

Standard Three-Hour Protocol

Before the start of aerosol or ozone exposure, the desired values for relative humidity and temperature in the chamber were verified. All chamber instrumentation was calibrated, and the chamber air intake was adjusted to approximately 10,000 L/min by the variable-speed intake fan. The incoming purified air was then checked to verify that background levels of air pollutants were minimal, that is, with respect to particles, sulfur oxides, NO_x, and ozone at or below the following detection levels: less than 4 μg particles/m^3, approximately 0.01 ppm nitrogen dioxide (NO_2), and less than 0.005 ppm for ozone and sulfur dioxide.

Upon entering the chamber, subjects were provided with the abdominal and thoracic bands used with the inductive plethysmograph (Model 300 SC, Respitrace, Ardsley, NY, currently known as Noninvasive Monitoring Systems [NIMS], Miami Beach, FL). The terminals of each band were connected to oscillators, and each subject was initialized; that is, the bands were calibrated to conform to the individual. Elastic bandages and an elastic net shirt were then placed over the bands to stabilize their positions. With the subjects seated, the Respitrace calibration was continued, using a calibrated spirometer. Concurrently, all physiologic instrumentation was turned on, and calibration procedures were begun according to standard operating procedures for each instrument.

With these calibrations completed, a nurse from the CRC examined the subject and made brief ventilatory recordings to check Respitrace settings. To reduce oral ammonia (NH_3), which has been shown to alter respiratory responses to H_2SO_4 aerosols in subjects with asthma (Utell et al. 1989), the subjects gargled with a lemon-flavored mouthwash prior to each exposure. Preexposure baseline pulmonary function tests were then begun. Airway resistance, thoracic gas volume, and flow-volume curves were determined in the Emerson (Cambridge, MA) air-conditioned, integrated-flow, pressure-corrected, whole-body plethysmograph (Leith and Mead 1973). Multiple measurements of resistance or its reciprocal, conductance, and thoracic gas volume were performed rapidly during panting (DuBois et al. 1956; Leith and Mead 1973), and the average was recorded. Using the body plethysmograph in connection with a flow-meter at the mouth (pneumotachograph No. 7322, Fleisch, Lausanne, Switzerland) and an X-Y storage oscilloscope (Tektronix Inc., Portland, OR) display of flow versus volume, maximum flow-volume curves were constructed. A maximal expiratory flow-volume curve was obtained by recording as the subject inspired to total lung capacity and forcefully expired to residual volume. From the expiratory flow-volume curve, we measured expiratory reserve volume to obtain residual volume from functional residual capacity.

After these measurements were made in the body plethysmograph, spirometry was performed using a Medical Graphics Corporation (St. Paul, MN) Microloop System. This system incorporates a pneumotachograph interfaced with a microcomputer to measure flow rates and integrate signals to measure volumes. After tidal breathing, subjects inspired to total lung capacity and expired forcefully to residual volume to provide FVC and FEV_1 measurements, which were expressed in liters corrected to body temperature and pressure, saturated with water vapor (BTPS) conditions. Lung volumes, including vital capacity, residual volume, and total lung capacity, were readily determined from the foregoing maneuvers. All measurements were made in triplicate, and the mean value was calculated.

After the pulmonary function testing was completed, the H_2SO_4 or NaCl aerosol or ozone exposure was begun. The subjects began a period of exercise for 10 minutes, approximately every 30 minutes, at a workload designed to quadruple the expired volume of ventilation/min ($\dot{V}_E$) (the specific workload was determined on the screening evaluation) to simulate the increased pollutant dose associated with moderate outdoor activity; oxygen saturation (Nelcor N200 Pulse Oximeter, Haywood, CA) and $\dot{V}_E$ were monitored at baseline and throughout exercise periods. Pulmonary function tests were performed immediately after each of the aerosol exposures. On each ozone exposure day, pulmonary function tests also were measured during, immediately after, and two and four hours after exposure (Figure 2). Symptom reports were completed at termination of each exposure. The subject exited the chamber, and the pollutant supply was turned off. After approximately 10 minutes, background levels of pollutants in the chamber were rechecked and all recordings were collected and properly identified. The exposure protocols are described in detail in Appendix A.

Bronchial Challenges

Inhaled carbachol aerosol is a parasympathomimetic bronchoconstrictor agent used for airway reactivity testing. To assess inherent baseline reactivity, carbachol challenges were performed for all subjects during the initial screening session. For healthy subjects, aqueous propylene glycol alone and then two concentrations of carbachol in propylene glycol (02.5% and 0.5%) were given at 10-minute intervals. In preliminary testing, a fall of greater than 15% in FEV_1 or of greater than 40% in s*G*aw after carbachol resulted in exclusion of the healthy subject from the study on the basis of baseline airway hyperreactivity. In our laboratory, healthy subjects begin to demonstrate changes in lung function at concentrations of carbachol greater than 10 mg/mL. In subjects with asthma who demonstrated normal baseline spirometry, aqueous propylene glycol alone and then up to five concentrations of carbachol in propylene glycol (0.0125, 0.025, 0.05, 0.25, and 0.5%) were given at 10-minute intervals. A 40% or greater fall in s*G*aw or a 15% fall in FEV_1 was required to confirm the presence of baseline airway hyperreactivity and for inclusion in the study. For subjects with asthma with abnormal baseline spirometry, administration of isoproterenol aerosol was performed with a D-31 nebulizer that contained 0.5% isoproterenol with 0.5% phenylephrine in 80% (v/v) propylene glycol in water, using the same respiratory maneuvers

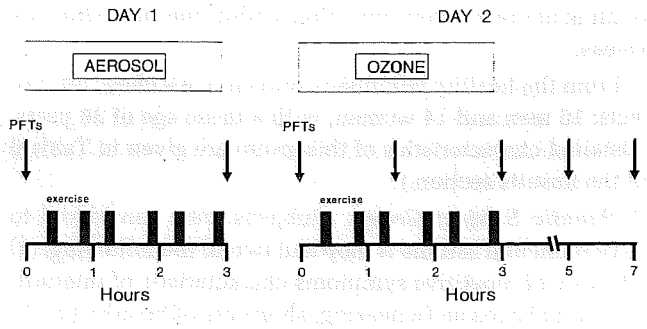


Figure 2. Protocol design. Day 1 indicates aerosol preexposure, Day 2 indicates ozone exposure 24 hours later. PFTs and arrows denote pulmonary function tests. Solid bars indicate exercise periods.

as in the carbachol administration. An increase in FEV_1 of at least 15% following inhalation of isoproterenol was considered evidence for airway hyperreactivity.

The particle size distribution and aerosol output of a Dautrebande D-31 nebulizer (R.E. Reynolds Co., Rochester, NY) were predetermined for the 80% (v/v) aqueous propylene glycol solutions of carbachol before their use with subjects. For the challenge, each subject took five deep breaths from functional residual capacity to total lung capacity with a five-second breath hold. Thereby, the entire aerosol output of the generator (1.5 L/min) was inhaled during each inspiratory maneuver; the supplemental air required by each individual was drawn through a side tube that continuously purged the aerosol delivery mouthpiece. In this manner, a 0.25% (w/v) carbachol solution, for example, was found to have a carbachol output of approximately 25 μg/min. The average mass median aerodynamic diameter (MMAD) of the droplet aerosol (determined by the aqueous propylene glycol) was 0.8 μm (σ_g = 2.5); subjects typically deposited 65% of the aerosol while breathing orally. Assuming 100% aerosol deposition, the maximum dose of carbachol delivered to the airways from the 0.25% carbachol solution was approximately 16 mg. Because aerosol output and droplet size were independent of the carbachol concentration, the carbachol output varied with the carbachol concentration in the generator; therefore, a challenge performed with a 1% carbachol solution produced a maximum deposited dose of 64 mg of carbachol. Measurements of airway resistance and spirometry were performed before testing and three minutes after each aerosol administration.

Pollutant Generation and Measurement

For the generation of H_2SO_4 aerosol, high purity H2SO4 (Baker's Certified High Purity-Ultrex 4802-1) was obtained; immediately before use it was diluted to an appropriate concentration (1% sulfate w/v) with 18 megohm water, transferred to a polyurethane container (previously conditioned by dilute H_2SO_4), and stored in a refrigerator. When used for aerosol generation, approximately 7 L of solution were transported to our all-plastic aerosol generator (Gibb et al. 1990), dispersed as an aerosol, brought to Boltzmann charge equilibrium (Aerosol Neutralizer, Model No. 3054, TSI Inc., St. Paul, MN), and admixed with flow of purified air at 10 m^3/min within a Venturi-injector mixer to produce a chamber concentration of 100 μg/m^3.

Aerosol atmospheres in the environmental chamber were characterized according to mass concentration and aerodynamic particle size. During the aerosol exposures, duplicate samples were collected for 60 minutes during two different periods. These samples were collected with 47-mm in-line stainless-steel filter holders (Gelman Sciences Inc., Ann Arbor, MI) on Fluoropore filters (Millipore Corporation, Bedford, MA). Sulfate and chloride measurements were made by ion chromatography (Dionex Corporation, Model 16, Sunnyvale, CA) and the determinations subsequently recorded. The monitored levels of concentration in the chamber were verified by mass concentration determinations (derived from the volume collected and the ion chromatograph analysis of mass on the filter). Using the four mass concentration determinations, a mean mass concentration for each exposure was calculated and recorded in the appropriate log book.

For particle sizing, a single one-hour sample was collected with the Mercer 7 Stage Cascade Impactor (In-Tox Products, Albuquerque, NM) on glass coverslips and an absolute filter (Fluoropore, pore size 1.0 μm). Impactor samples were collected carefully to avoid losses. The stage samples were analyzed by ion chromatography. Mass determinations for each stage were recorded in a raw data book. The MMAD was calculated by a BASIC computer program. When provided with the recorded data (masses, sampled volumes, and stage constants) the program calculated the MMAD and the σ_g for each sample distribution. Mass samples and particle size samples were collected for each aerosol exposure. Statistical analyses of the mean mass concentration, SD, SEM, and σ_g were performed for the mass and particle size distribution values using a BASIC 3.2 (Microsoft) program and the results recorded in the log books. A calibrated nephelometer (MIE, Model RAM-1) was used to monitor the acid aerosol within the chamber, along with a Meloy (Model SA285E) S_3* Analyzer (now manufactured by Columbia Scientific Instruments, Austin, TX) that had been adapted to measure the H_2SO_4 concentration continuously during exposures.

The single-pass ventilation system of the chamber gave the aerosol a residence half-time of approximately one minute (90% steady-state values were achieved in approximately four minutes). Ambient NH_3 has the potential to neutralize H_2SO_4 aerosols; however, because expired air NH_3 levels usually were less than 2 μg/L, with two subjects less than 50 μg/min was expired. The conversion of some fraction of the H_2SO_4 aerosol to NH_4HSO_4 was thereby possible, but it could neither accumulate nor reach important levels except transiently near the breathing zone. Because of the difficulty of modeling this phenomenon, which includes other factors such as the relatively slow reaction rate of NH_3 and H_2SO_4, we measured the NH_3 levels in the chamber without aerosol present and regularly determined that they were negligible. Concentrations of NH_3 in the occupied and the unoccupied chamber were less than 100 parts per billion (ppb). The partial conversion of

H_2SO_4 to NH_4HSO_4 is much more important in the airways, particularly during mouth breathing as we (Utell et al. 1989) and others have demonstrated (Larson et al. 1982).

The NaCl (control) aerosol was generated, electrically discharged, injected, and sampled by the same types of instruments used for H_2SO_4 aerosol. The concentration of the aerosolized NaCl solution was adjusted to provide virtually the same airborne particle size and mass concentration as the H_2SO_4 aerosol. The use of ion chromatography for sodium (or chloride) quantitation was accomplished with the ion chromatograph.

For the chamber operation, the ozone was produced by passing breathing-quality oxygen gas from a compressed tank through an adjustable flow meter into a water-cooled, high-voltage discharge ozonator (Model 03V5-0 Ozone Research and Equipment Corporation, Phoenix, AZ), thereby eliminating the concurrent production of NO_x. A portion of the resulting ozone output (for example, 800 to 1,800 ppm) was introduced into the purified air supply (10 m^3/min) to the chamber by means of the Venturi mixer. Real-time monitoring and measurement of the chamber ozone levels were performed with an ozone analyzer (Model 8810 Monitor Labs, Englewood, CO). The analyzer analog output signal controled the ozonator output by feedback circuitry. During exposures, ambient levels of ozone in the premixed dilution air were monitored with a second ozone analyzer (Model 1003 AH Dasibi, Glendale, CA) calibrated bimonthly with the ozone standard used by the local Air Quality Control Section, New York State Department of Environmental Conservation.

Data for the maximum hourly average ozone concentration in Rochester were received from the New York State Department of Environmental Conservation for the three-year period of the study. The National Ambient Air Quality Standard (NAAQS) was reached or slightly exceeded on four days in July and August but on no occasion for the period of September through June. Therefore, our policy of discontinuing exposures during July and August, when the ambient levels might exceed our chamber concentrations, was appropriate; likewise, our policy of reinitiating studies in mid-September was supported by the monitoring data indicating that the NAAQS was not exceeded in September or thereafter.

In addition, Dr. George Thurston of the New York University Institute of Environmental Medicine monitored H_2SO_4 aerosol concentrations in Rochester for the period August 20 through October 1, 1990. He compared the ambient concentrations with measurements from Buffalo, NY, where acid aerosols were monitored over the entire year (Thurston et al. 1992). Acid aerosol levels in the two cities were virtually identical. The peak H_2SO_4 aerosol concentration in Rochester was 22 $\mu g/m^3$, which is equivalent to 440 nmol of H^+/m^3 of air.

Data Handling and Statistical Methods

Data collection and storage were performed in accordance with guidelines established by a quality assurance (QA) plan and good laboratory practices (Salem 1987). For each study, all data books were custom-made, numbered, and bound. All entries were made in ink, dated, signed by the responsible person or persons, and verified periodically by the QA officer (H.E. Beiter). All digital and analog recordings were identified and filed similarly. Physiologic data entries and initial data processing were accomplished by a technician trained in biostatistics (D.M. Speers) using a Digital Pro 350 computer (Boston, MA) and RS1 data management, statistics, and graphics software (BBN, Cambridge, MA) under the direction of Dr. C. Cox, Associate Professor of Biostatistics. All records were identified by code numbers only and treated as confidential information.

Statistical analyses were performed by transferring data to the Department of Biostatistics. The primary statistical analyses were based on a three-way mixed model analysis of variance (ANOVA), followed by specific comparisons or contrasts. The two fixed (treatment) effects in the model were preexposure (sulfate/control) and ozone (three levels). A random subject effect also was included. All effects were crossed, although the data were incomplete because each subject received only two of the three levels of ozone. The analysis also included a test of interaction of the treatment effects (the effect of ozone depends on the preexposure). No interaction between subjects and the fixed effects was assumed. No terms were included in the model for order or carryover effects, because previous studies had indicated such effects were minimal, due to the low levels of the pollutants and the relatively long interval between repeated measurements on the same subject. Independent checks of this assumption also were carried out by adding a period effect for a limited number of analyses. These indicated that no period effects were present. In addition, each analysis included an examination of residuals as a check on the assumptions of normally distributed errors with constant variance. In addition to the overall test of interaction, two types of interaction contrasts were used. The first type made pairwise comparisons between the sulfate and chloride preexposure conditions for each of the three levels of ozone. The second type compared linear trends with increasing level of ozone (ozone trends) exposure between sulfate and chloride preexposure. Both types of contrasts measure specific kinds of interaction between the two treatment effects. A final set of analyses combined both groups of subjects and compared effects in healthy subjects and subjects with asthma using a four-way ANOVA.

One set of analyses was cross-sectional, using data only from a single point in time (for example, postexercise,

postexposure, after two hours, and after four hours). A second set was longitudinal and first computed linear, quadratic, and cubic orthogonal within-subject contrasts, using the four ozone postexposure time points. These contrasts measure linear, quadratic (second order), and cubic (third order) trends in the responses of individual subjects over time (time trends). For this analysis the observations were assumed to be equally spaced. The values of these trend contrasts were then used in the three-way ANOVA described previously.

The primary basis of analysis was percentage change in data. Except as indicated, separate analyses were carried out for healthy subjects and subjects with asthma. Data for FEV_1 and FVC were analyzed for each of the four time points mentioned, as well as for the preexposure condition. In addition, two *sG*aw values at two time points were analyzed. The results of these analyses are summarized in the ANOVA Tables for healthy subjects and subjects with asthma (Appendix D). The ANOVA tables address only questions of statistical significance. The direction and magnitude of the observed effects are illustrated in the figures (see Results section). Generally only graphs for statistically significant effects have been included.

A significance level of 5% was required for statistical significance. In addition, the overall *F* test for the ANOVA (for example, see Table 8) was required to be significant ($p < 0.05$) before any main effects or interactions were examined.

QUALITY ASSURANCE

The QA instituted for this study included the validation of aerosol and ozone exposure levels by using the EPA Reference Methods for ozone analysis, and adjunctive standardization procedures for ozone and particles using EPA-equivalent methods. We monitored the performance of the air purification system concurrently, which required EPA Reference Methods for NO_x, sulfur oxides, and ozone. We also established standardization and calibration methods for particulate matter in the unoccupied chamber. Therefore, the basic QA requirements, directed at achieving reliable, fully documented aerosol and ozone exposure conditions, were performed according to EPA-recommended procedures, but we extended our calibration and standardization procedures, for which there are no specific EPA guidelines, to include environmental instruments and all physiologic testing instrumentation.

The QA Program, under the direction of H.E. Beiter, includes provisions for spare-parts inventories, service records, preventative maintenance schedules, the design and use of data books, analog and digital printouts, data storage, and methods of calibration and standardization. Standard operating procedures are maintained in a manual. Appendix F describes the external QA procedures.

RESEARCH TEAM

The three-year study involved many individuals with complementary roles. The personnel and their designated responsibilities are given in Appendix E.

RESULTS

SUMMARY OF FINDINGS

In healthy subjects, no convincing evidence was seen for an effect of exposure to either aerosol or ozone on lung function, nor was there clear evidence for an effect of aerosol preexposure on the ozone response. The sequence of aerosol administration (H_2SO_4 or NaCl first) did not alter subsequent responses. Although isolated analyses reached statistical significance, the absence of an ozone exposure-response relationship following either aerosol preexposure suggested these were chance observations.

Lung function in subjects with asthma was affected by ozone exposure, and evidence for interaction between the aerosol and ozone exposures was found, both at the end of exposure and four hours after exposure. Preexposure to H_2SO_4 aerosols appeared to affect the pattern of response to and recovery from ozone exposure. Differences in ozone response between the two preexposure conditions persisted to four hours after exposure. Finally, responses of subjects with asthma to aerosol and ozone were significantly different from the responses of healthy subjects.

Tables 2 and 3 show absolute values for FVC, FEV_1, and *sG*aw (means ± SEM) for both healthy subjects and subjects with asthma following ozone exposures. Subsequent representative findings and statistical analyses refer to the percentage of change from baseline for each subject.

REPRESENTATIVE FINDINGS

Exposure Characterization

Table 4 shows the targeted and attained concentrations and MMAD for the NaCl and H_2SO_4 aerosols. Table 5 illustrates the targeted and achieved concentrations for the three ozone exposures.

Table 2. Pulmonary Function Responses After Aerosol and Ozone Exposure in Healthy Subjects[a]

Time of Measurement	FVC (L)		FEV_1 (L)		sGaw (cm H_2O/L/sec)	
	NaCl	H_2SO_4	NaCl	H_2SO_4	NaCl	H_2SO_4
0.08 ppm Ozone						
Baseline	4.72 ± 0.21	4.68 ± 0.21	3.80 ± 0.17	3.78 ± 0.16	0.375 ± 0.020	0.360 ± 0.020
After exercise	4.57 ± 0.21	4.61 ± 0.22	3.79 ± 0.17	3.81 ± 0.17	–	–
Immediately after exposure	4.62 ± 0.22	4.66 ± 0.23	3.84 ± 0.18	3.80 ± 0.18	0.364 ± 0.019	0.352 ± 0.020
2 Hours after exposure	4.61 ± 0.20	4.65 ± 0.22	3.86 ± 0.17	3.86 ± 0.18	–	–
4 Hours after exposure	4.61 ± 0.20	4.68 ± 0.22	3.85 ± 0.17	3.83 ± 0.17	–	–
0.12 ppm Ozone						
Baseline	4.60 ± 0.22	4.58 ± 0.24	3.67 ± 0.15	3.65 ± 0.16	0.334 ± 0.023	0.327 ± 0.017
After exercise	4.50 ± 0.22	4.53 ± 0.25	3.66 ± 0.15	3.65 ± 0.15	–	–
Immediately after exposure	4.57 ± 0.23	4.52 ± 0.22	3.66 ± 0.15	3.65 ± 0.15	0.319 ± 0.019	0.326 ± 0.020
2 Hours after exposure	4.54 ± 0.23	4.56 ± 0.24	3.72 ± 0.15	3.70 ± 0.15	–	–
4 Hours after exposure	4.58 ± 0.22	4.58 ± 0.25	3.73 ± 0.15	3.71 ± 0.16	–	–
0.18 ppm Ozone						
Baseline	4.71 ± 0.27	4.78 ± 0.27	3.79 ± 0.20	3.79 ± 0.19	0.316 ± 0.020	0.342 ± 0.019
After exercise	4.68 ± 0.26	4.69 ± 0.27	3.79 ± 0.20	3.82 ± 0.20	–	–
Immediately after exposure	4.68 ± 0.25	4.71 ± 0.26	3.82 ± 0.20	3.80 ± 0.19	0.310 ± 0.019	0.314 ± 0.016
2 Hours after exposure	4.73 ± 0.27	4.76 ± 0.27	3.89 ± 0.19	3.87 ± 0.20	–	–
4 Hours after exposure	4.70 ± 0.27	4.75 ± 0.26	3.83 ± 0.20	3.87 ± 0.20	–	–

[a] Values are expressed as means ± SEM.

Table 3. Pulmonary Function Responses After Aerosol and Ozone Exposures in Subjects with Asthma[a]

Time of Measurement	FVC (L)		FEV_1 (L)		sGaw (cm H_2O/L/sec)	
	NaCl	H_2SO_4	NaCl	H_2SO_4	NaCl	H_2SO_4
0.08 ppm Ozone						
Baseline	3.80 ± 0.17	3.73 ± 0.17	2.85 ± 0.11	2.79 ± 0.10	0.204 ± 0.021	0.209 ± 0.020
After exercise	3.64 ± 0.17	3.59 ± 0.18	2.84 ± 0.12	2.72 ± 0.12	-	-
Immediately after exposure	3.51 ± 0.18	3.64 ± 0.17	2.73 ± 0.12	2.79 ± 0.11	0.176 ± 0.024	0.177 ± 0.022
2 Hours after exposure	3.67 ± 0.17	3.70 ± 0.16	2.91 ± 0.12	2.89 ± 0.ll	–	–
4 Hours after exposure	3.67 ± 0.15	3.74 ± 0.18	2.92 ± 0.10	2.92 ± 0.13	–	–
0.12 ppm Ozone						
Baseline	3.97 ± 0.22	3.95 ± 0.22	2.98 ± 0.17	3.05 ± 0.17	0.220 ± 0.015	0.236 ± 0.020
After exercise	3.72 ± 0.20	3.76 ± 0.19	2.94 ± 0.17	3.01 ± 0.16	–	–
Immediately after exposure	3.72 ± 0.21	3.76 ± 0.20	2.90 ± 0.19	2.97 ± 0.18	0.186 ± 0.0l9	0.209 ± 0.025
2 Hours after exposure	3.91 ± 0.22	3.85 ± 0.21	3.10 ± 0.18	3.08 ± 0.17	–	–
4 Hours after exposure	3.87 ± 0.22	3.87 ± 0.21	3.07 ± 0.18	3.04 ± 0.18	–	–
0.18 ppm Ozone						
Baseline	3.89 ± 0.23	3.99 ± 0.22	2.92 ± 0.16	3.04 ± 0.17	0.183 ± 0.016	0.207 ± 0.016
After exercise	3.76 ± 0.23	3.71 ± 0.22	2.90 ± 0.19	2.99 ± 0.16	–	–
Immediately after exposure	3.76 ± 0.23	3.74 ± 0.24	2.90 ± 0.19	2.96 ± 0.18	0.170 ± 0.016	0.179 ± 0.018
2 Hours after exposure	3.81 ± 0.25	3.87 ± 0.23	3.03 ± 0.19	3.03 ± 0.17	–	–
4 Hours after exposure	3.90 ± 0.24	3.84 ± 0.25	3.06 ± 0.17	2.99 ± 0.18	–	–

[a] Values are expressed as means ± SEM.

Table 4. Aerosol Concentrations and Sizes[a]

	NaCl	H_2SO_4
Attained concentration	105.4 ± 13.8 (n = 66)	106.7 ± 14.7 (n = 63)
Particle size[b]	0.45 ± 0.13 (n = 65)	0.64 ± 0.06 (n = 65)
σ_g [c]	4.05 (n = 63)	2.50 (n = 63)

[a] Target concentration for both aerosols was 100 μg/m^3. Data are expressed as means ± SD; n = number of determinations.

[b] MMAD in μm.

[c] Averages.

Table 5. Average Ozone Concentrations

	Target Concentration		
	80 ppb	120 ppb	180 ppb
Attained concentration[a]	79.1 ± 3.6	118.6 ± 3.1	177.1 ± 4.1
Number of determinations	43	45	44

[a] Data are expressed as means ± SD.

Healthy Subjects

Table 6 summarizes the subjects' characteristics. All 30 healthy volunteers were nonsmokers, none required chronic medications and all those recruited for the study completed the protocol. Resting $\dot{V}_E$ ranged from 7.2 to 7.9 L/min at rest, and increased during exercise to between 33.3 and 40.4 L/min. During the exercise periods, workloads ranged from 60 to 150 watts (mean ± SD, 90 ± 29 watts). No correlations were found between aerosol preexposure or ozone concentration and $\dot{V}_E$ at rest or exercise. Oxygen saturation remained between 94% and 97% and was not affected by exposure conditions or exercise.

No effect of exposure to NaCl or H_2SO_4 aerosol on lung function was evident in these healthy subjects. Similarly, no convincing evidence for effects of ozone on lung function, or of a significant interaction between aerosol preexposure and ozone exposure was seen, although isolated analyses achieved statistical significance. A complete listing of pulmonary function data and analyses are included in Appendix G.

In Figures 3 and 4, the mean percentages of change from baseline for FVC during and after ozone exposure following the NaCl and H_2SO_4 preexposure are shown. After NaCl preexposure (Figure 3), a small (mean ± SEM, 3.3% ± 0.5%)

Table 6. Baseline Characteristics of Individual Healthy Subjects

Subject Number	Age (years)	Sex	FEV_1 (L)	FEV_1 (% predicted)	FEV_1/FVC (× 100)	Percentage of Change in FEV_1 After Carbachol Challenge[a]	Exercise Workload[b] (watts)
1	27	F	2.99	104	89	4	70
2	30	M	4.16	92	76	0	90
3	28	M	4.16	94	84	–5	100
4	25	M	4.10	92	75	12	90
5	26	M	4.73	123	81	0	90
6	22	F	3.36	103	79	1	80
7	21	M	4.51	92	77	–3	80
8	30	M	3.91	99	81	–4	100
9	29	M	3.92	89	80	2	100
10	34	F	3.50	112	83	1	70
11	41	M	3.78	90	80	–1	80
12	23	F	3.45	106	83	–1	80
13	24	M	3.89	82	82	3	100
14	29	F	2.82	91	81	–3	60
15	25	F	3.18	95	76	–2	80
16	25	F	3.72	107	92	–2	80
17	28	M	4.30	108	85	–1	90
18	25	M	5.28	113	72	–10	100
19	25	M	4.76	118	86	–5	100
20	23	F	2.91	91	81	2	60
21	32	F	2.53	86	70	–8	90
22	20	F	2.63	82	78	0	70
23	25	M	4.18	96	78	2	150
24	35	F	3.25	111	84	0	60
25	21	M	5.45	112	84	–3	200
26	29	F	3.25	110	89	–3	80
27	24	M	4.39	82	83	–8	130
28	32	M	5.13	121	80	–3	100
29	43	F	2.63	100	87	–1	60
30	28	F	3.28	103	78	–2	60
Mean ± SE	28 ± 1		3.8 ± 0.2	100 ± 2	81 ± 1	–1 ± 0.8	90 ± 5.3

[a] Subjects inhaled 0.5% carbachol.

[b] Load required to quadruple $\dot{V}_E$.

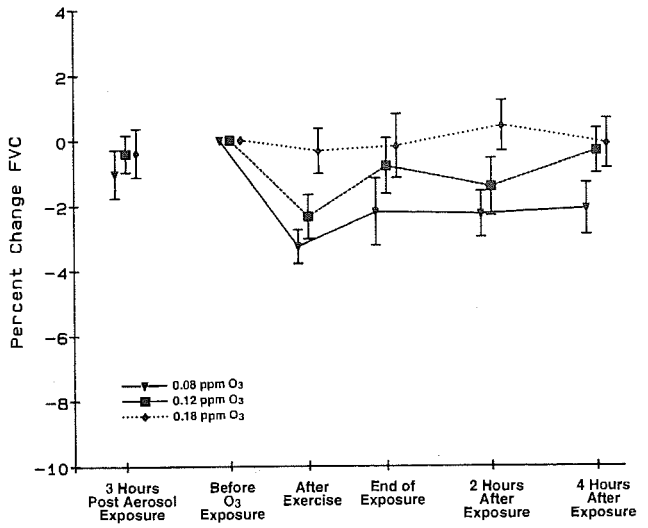


Figure 3. The percentage of change in FVC after NaCl aerosol exposure, and during and after ozone exposure 24 hours later for healthy subjects. Values are expressed as means ± SEM.

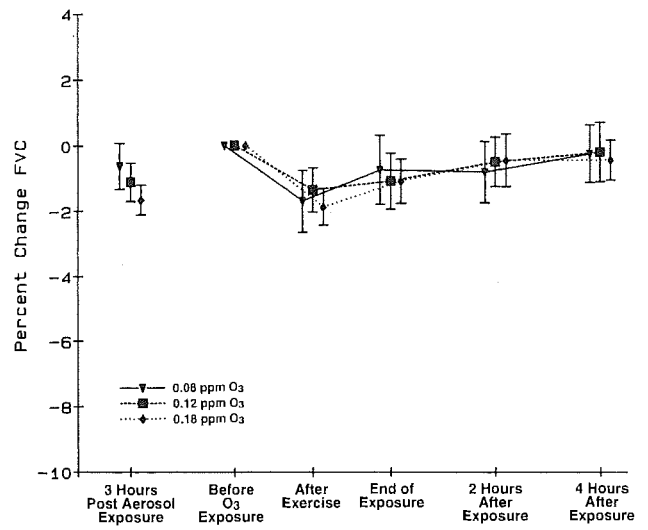


Figure 4. The percentage of change in FVC after H_2SO_4 aerosol exposure, and during and after ozone exposure 24 hours later for healthy subjects. Values are expressed as means ± SEM.

decrease in FVC was seen during exposure to the lowest concentration of ozone (0.08 ppm), which partially reversed by the end of exposure. No such change was seen with exposure to 0.18 ppm ozone, and no significant changes occurred with H_2SO_4 preexposure compared with NaCl preexposure (Figure 4). Figures 5 and 6 show the changes in FVC at the midexposure time point and at the end of exposure, respectively, in relation to ozone concentration.

As stated in the Methods section, dose-response relationships were examined by estimating linear trends with increasing ozone concentration. Examples of such trends can be seen in Figures 5 and 6, which show basically linear trends (increasing for NaCl preexposure and slightly de-

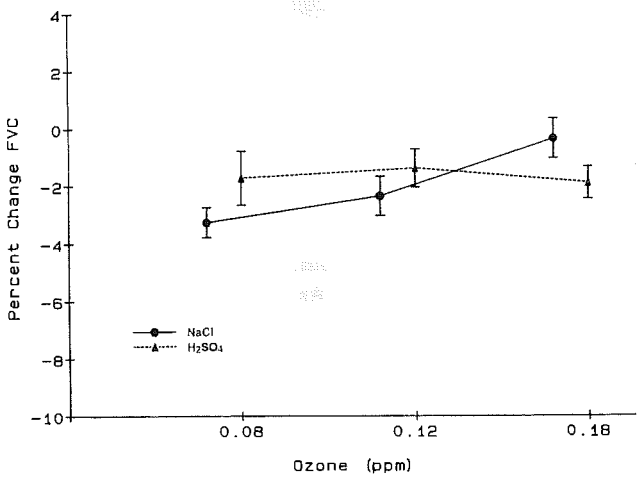


Figure 5. The percentage of change in FVC after exercise period 3 for each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexpo-

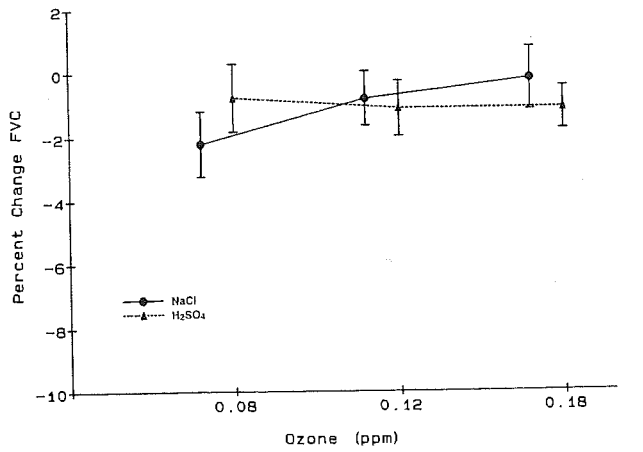


Figure 6. The percentage of change in FVC immediately after exposure for each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for healthy subjects. Values are expressed as means ± SEM.

creasing for H_2SO_4 preexposure). In addition to an overall test for interaction between preexposure and ozone, these linear trends for the two preexposures were compared using a single-degree-of-freedom contrast. Linear trend comparison of the ozone responses revealed a statistically significant difference between the two preexposure conditions (p = 0.013), as well as evidence for an overall interaction between aerosol and ozone (p = 0.039). However, the small changes in FVC occurred following NaCl (control) preexposure and not the H_2SO_4 aerosol preexposure. The analyses did not reveal an ozone exposure-response relationship and suggested that the statistical observations were probably due to chance. Linear trends for FVC immediately after exposure (Figure 6), and two and four hours after exposure (data not shown), were not significantly different.

In Figures 7 and 8, the percentages of change from baseline in FEV_1 are shown for each preexposure condition. Comparisons between the H_2SO_4 and NaCl preexposures for each of the three concentrations of ozone confirmed the absence of effects on FEV_1 as suggested by the figures. Linear trend analyses by ozone concentration also were uniformly negative and did not mirror the small reduction in FVC after NaCl preexposure.

Statistically significant changes in s*G*aw also were observed in healthy subjects. As shown in Figure 9, s*G*aw decreased 1.6% ± 1.1% (mean ± SEM) after 0.18 ppm ozone with NaCl preexposure, and 7.4% ± 1.7% after 0.18 ppm ozone with H_2SO_4 preexposure. Although these changes were small, the crossing pattern of the changes related to ozone exposure levels is similar to that seen with FVC during ozone exposure (Figure 6), and similar to that seen for FVC and FEV_1 after ozone exposure in the subjects with asthma (see below and Figures 12 and 13). Analysis of s*G*aw data suggested an overall interaction between aerosol and ozone (p = 0.023) and a significant difference in linear trends by ozone concentration (p = 0.048) between NaCl and H_2SO_4 preexposure.

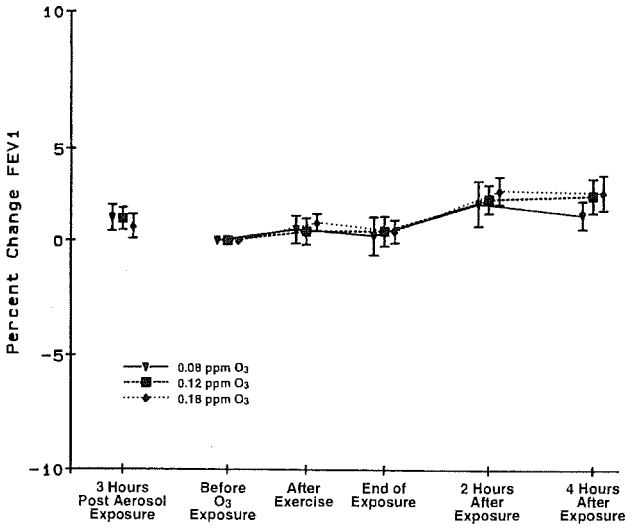


Figure 8. The percentage of change in FEV_1 after H_2SO_4 aerosol exposure, and during and after ozone exposure 24 hours later for healthy subjects. Values are expressed as means ± SEM.

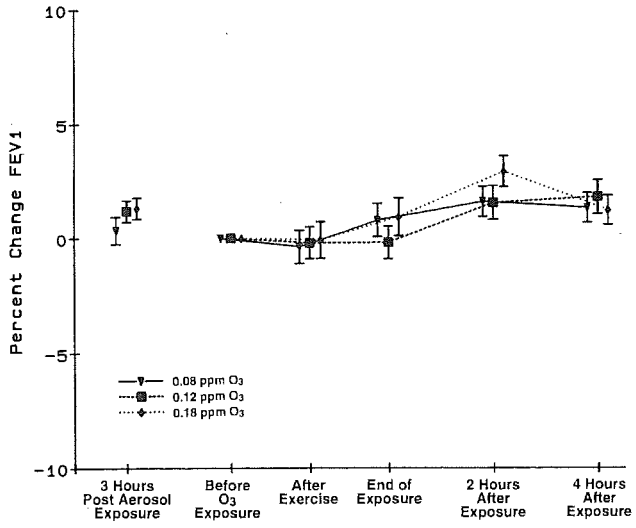


Figure 7. The percentage of change in FEV_1 after NaCl aerosol exposure, and during and after ozone exposure 24 hours later for healthy subjects. Values are expressed as means ± SEM.

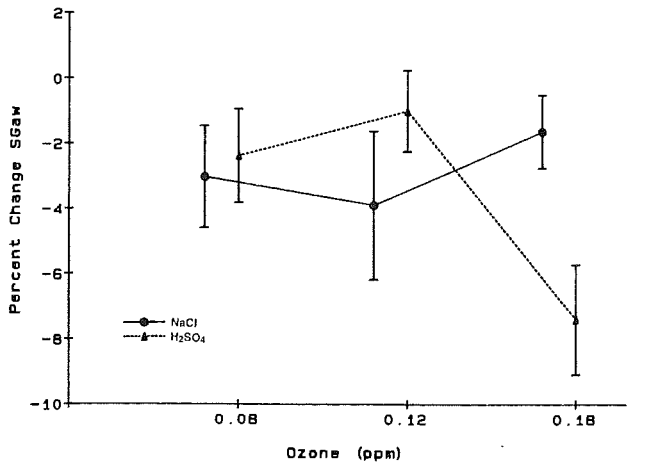


Figure 9. The percentage of change in s*G*aw immediately after exposure for each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for healthy subjects. Values are expressed as means ± SEM.

Subjects with Asthma

Table 7 summarizes the characteristics of the asthmatic group. The mean age (mean ± SD, 28 ± 7 years) was identical to the healthy group; 20 of the 30 subjects with asthma were female, whereas 14 of the 30 healthy volunteers were female. The degree of lung impairment for the subjects with asthma was generally mild, with the FEV_1 at 81% ± 21% of predicted (mean ± SD), although five subjects had FEV_1 less than 60% of predicted, and one subject had a baseline FEV_1 at 34% of predicted. By design, all subjects with asthma had positive skin tests to aeroallergens, and 10 subjects with asthma had elevated IgE levels (200 IU or greater). All subjects completed each exposure, although occasional exercise periods were missed because of asthmatic symptoms. For example, for subject 15 in Table 7, the workload was reduced markedly after the first exercise period due to symptoms of asthma. Subsequent exposures were performed at similar workloads. Bronchodilator therapy was not required during any exposure.

Resting $\dot{V}_E$ ranged from 7.0 to 7.8 L/min and increased during exercise to between 30.6 and 36.2 L/min. During the exercise period, workloads ranged from 50 to 100 watts (mean ± SD, 61 ± 15 watts). Oxygen saturation remained in the range of 94% to 97%. No effects of exposure conditions on $\dot{V}_E$ or oxygen saturation were apparent.

For the subjects with asthma, results suggest an effect of both the ozone exposure and the preexposure condition (main effects), as well as a differential effect of the preexposure condition on the ozone response (interaction) immediately after and four hours after exposure. Preexposure to H_2SO_4 aerosols appeared to affect the pattern of response to and recovery from ozone exposure. Data for FVC are shown in Figures 10 and 11. Small reductions in FVC occurred following both aerosol exposures, but no differences were seen between NaCl and H_2SO_4 exposure. During ozone exposure at each concentration, FVC declined and returned toward baseline during the four hours after exposure. Statistically significant differences between the preexposure conditions occurred at both the immediate postexposure and at the four-hour time points. With NaCl preexposure (Figure 10), the greatest decrements in FVC occurred following 0.08 ppm ozone, whereas with H_2SO_4 preexposure (Figure 11) the greatest decrements occurred following 0.18 ppm ozone. Analyses revealed evidence for interactions between aerosol and ozone exposures both immediately after ($p = 0.005$) and four hours after ($p = 0.030$) exposure. Figures 12 and 13 show the relationship between ozone exposure concentrations and percentage of change in FVC (mean ± SEM) at these two time points. A crossing pattern occurs similar to, but more pronounced than, that seen with the healthy subjects. Under the condition of H_2SO_4 preexposure, FVC clearly decreases in a manner consistent with an ozone exposure-response, with the greatest decrease (6.8% ± 1.7%) following exposure to 0.18 ppm ozone. With NaCl preexposure, a slight upward slope to the curve is seen, with the greatest decrement in FVC (7.3% ± 2.4%) occurring at 0.08 ppm ozone. Linear trend analysis revealed these curves to be different, both immediately after ($p = 0.001$) and four hours after ($p = 0.010$) exposure. Similar findings were observed when FVC was expressed in liters rather than percent change from baseline (Figure 14).

Polynomial (linear, quadratic, and cubic) trend analysis (see Discussion section) was used to analyze changes in lung function with time, that is, the four measurements made during and following exposure to ozone. Results for both the linear and quadratic time trend analysis were negative. In Figure 10, which shows changes in FVC for subjects with asthma with NaCl preexposure, the 0.08 ppm ozone exposure shows a negative cubic time trend, as compared with 0.18 ppm ozone, which is quadratic. As shown in Figure 15, the mean value for the cubic time trend at 0.08 ppm ozone is about −0.6, while for 0.18 ppm ozone the value is close to zero, indicating the absence of a third order trend. The remaining values in Figure 15 indicate group means for cubic time trends, which can be appreciated by examining the changes with time in Figures 10 and 11. In general, the shape of these higher order trends indicated acute decrements in lung function followed by recovery. A negative cubic trend is seen following NaCl exposure at 0.12 ppm ozone and following H_2SO_4 exposure at 0.18 ppm ozone. Evidence was seen for an overall interaction between the aerosol and ozone exposures ($p = 0.04$), and the linear trends in relation to ozone concentration were significantly different ($p = 0.017$). Of interest is the presence of an exposure-response relationship following H_2SO_4 preexposure but not following NaCl preexposure, where the greatest effect is seen at 0.08 ppm ozone.

Changes in FEV_1 are shown in Figures 16 and 17. As for FVC, no difference was found in the direct effect of NaCl and H_2SO_4 exposure on FEV_1. Unlike the findings for FVC, no significant effects were seen on FEV_1 during or immediately after ozone exposure. With NaCl preexposure (Figure 16), FEV_1 declined slightly and then increased slightly above baseline at two and four hours after exposure, with no differences among ozone exposure levels. With H_2SO_4 preexposure (Figure 17), a recovery above baseline was seen only for the 0.08 ppm ozone exposure; increasing levels of ozone were associated with less improvement in FEV_1 at two and four hours after exposure. Statistical analysis of

Table 7. Baseline Characteristics of Individual Subjects with Asthma

Subject Number	Age (years)	Sex	FEV_1 (L)	FEV_1 (% predicted)	FEV_1/FVC (× 100)	Skin Tests[a]	IgE Level[b]	Medications[c]	Airway Reactivity Testing[d]	Exercise Workload[e] (watts)
1	36	M	1.37	34	44	+	WNL	I,O	0.0125 C	50
2	40	F	1.82	61	79	+	WNL	I	BD	50
3	28	F	2.05	72	69	+	↑	I,O	BD	50
4	35	F	1.44	45	87	+	WNL	I,O	0.25 C	50
5	20	M	2.86	60	58	+	WNL	I,O	BD	50
6	37	F	2.50	90	86	+	↑	I	BD	50
7	24	F	1.72	54	52	+	WNL	I,O	BD	50
8	24	F	3.26	103	82	+	WNL	I	0.25 C	70
9	42	F	2.55	94	79	+	WNL	I	BD	70
10	35	F	2.97	109	89	+	WNL	I,O	0.25 C	50
11	20	F	2.65	86	83	+	WNL	I	BD	50
12	33	M	1.83	49	59	+	↑	I	0.25C	70
13	23	F	3.16	98	72	+	WNL	I	0.05 C	50
14	21	F	3.59	98	74	+	WNL	I	BD	100
15	25	F	2.20	70	73	+	WNL	I,O	0.05 C	50
16	21	M	2.98	97	78	+	WNL	I,O	0.25 C	70
17	42	F	2.16	81	81	+	↑	I,O	0.50 C	50
18	36	F	2.49	89	89	+	WNL	O	0.25 C	50
19	27	M	5.18	110	81	+	WNL	I	BD	100
20	23	M	2.83	67	73	+	↑	I	0.50 C	50
21	29	M	3.97	76	67	+	↑	I	0.25 C	90
22	29	F	2.73	87	80	+	WNL	I	BD	50
23	25	M	3.49	76	72	+	WNL	I	0.25 C	70
24	30	F	2.90	93	84	+	↑	I	0.25 C	60
25	27	F	2.40	73	55	+	↑	I,O	0.025 C	50
26	28	M	2.03	53	67	+	↑	I	BD	60
27	21	F	3.64	113	91	+	WNL	I	BD	70
28	22	F	3.34	103	88	+	WNL	I,O	BD	60
29	25	M	3.66	87	75	+	↑	N	0.50 C	80
30	24	F	2.81	89	69	+	WNL	N	0.05 C	70
Mean ± SE	28 ± 1		2.8 ± 0.2	81 ± 4	75 ± 2					61 ± 2.8

[a] Skin tests documented a positive wheal-and-flare response to the allergens tested.

[b] WNL = within normal limits; ↑ = high.

[c] I = inhaled beta agents; O = oral theophylline; N = none.

[d] C = the concentration of carbachol required to cause bronchoconstrictor response (see the Methods section); BD = bronchodilators.

[e] Load required to quadruple $\dot{V}_E$.

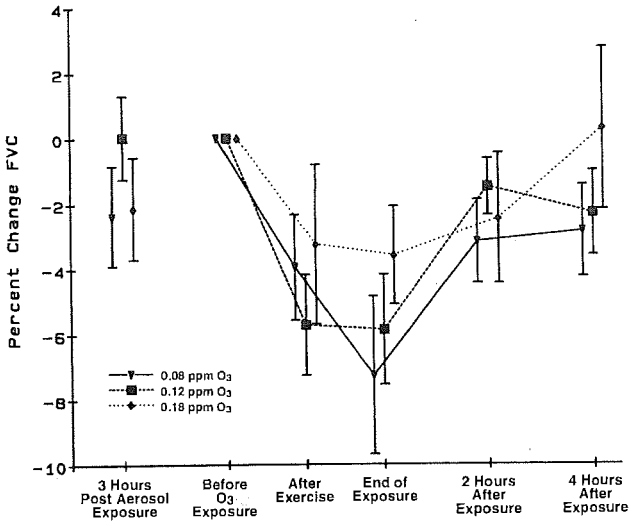


Figure 10. The percentage of change in FVC after NaCl aerosol exposure, and during and after ozone exposure 24 hours later for subjects with asthma. Values are expressed as means ± SEM.

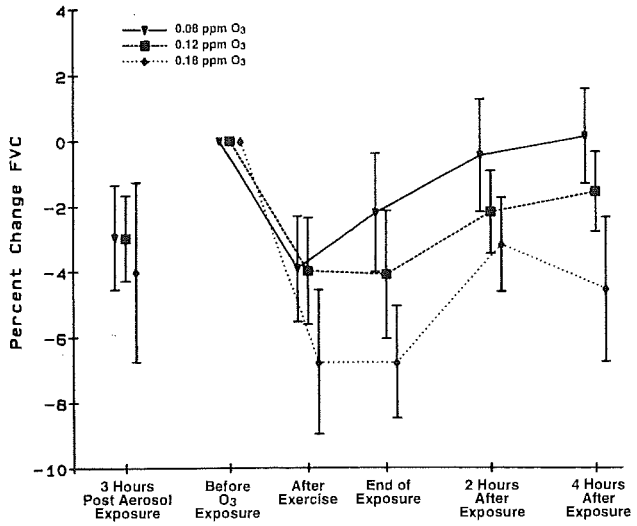


Figure 11. The percentage of change in FVC after H_2SO_4 aerosol exposure, and during and after ozone exposure 24 hours later for subjects with asthma. Values are expressed as means ± SEM.

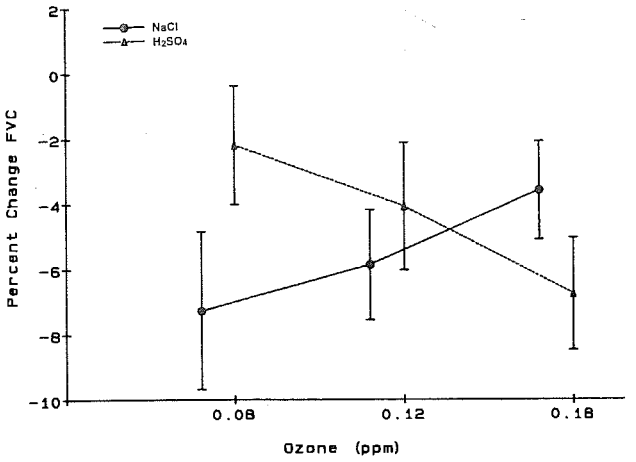


Figure 12. The percentage of change in FVC immediately after exposure for each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for subjects with asthma. Values are expressed as

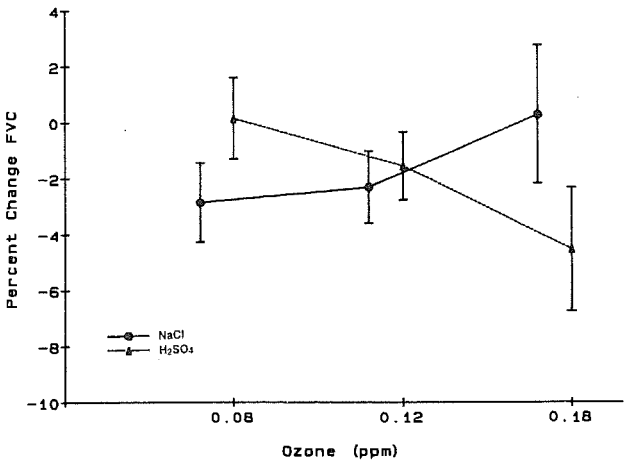


Figure 13. The percentage of change in FVC values measured four hours after exposure to each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for subjects with asthma. Values are

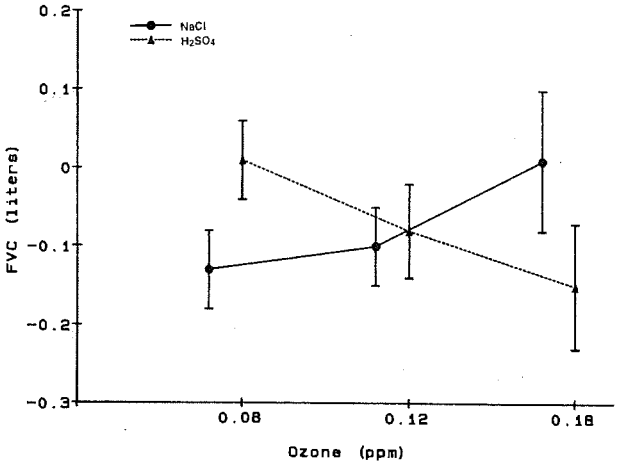


Figure 14. The absolute change in FVC (in liters) four hours after exposure to each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for subjects with asthma. Values are expressed as means ± SEM

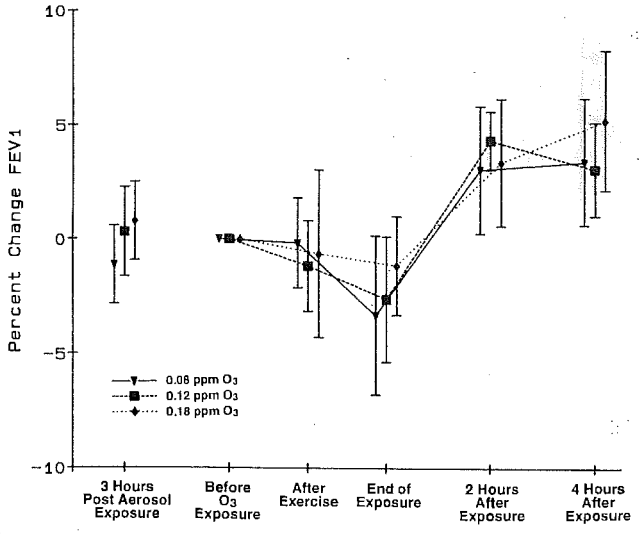


Figure 16. The percentage of change in FEV_1 after NaCl aerosol exposure, and during and after ozone exposure 24 hours later for subjects with asthma. Values are expressed as means ± SEM.

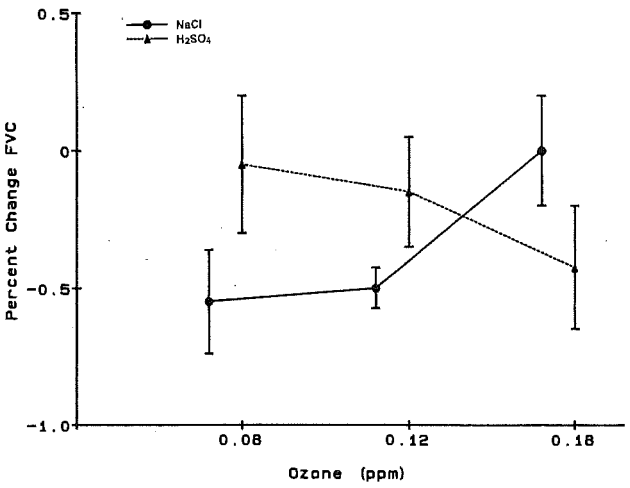


Figure 15. Cubic time trend analysis for FVC during and after ozone exposure for subjects with asthma. See Figures 10 and 11 for the trends being analyzed. The vertical axis indicates percentage of change in FVC per unit of time, approximately one hour. The analysis shows evidence for an interaction between the aerosol and ozone exposure ($p = 0.04$), and the linear trends in relation to ozone levels were significantly different ($p = 0.017$).

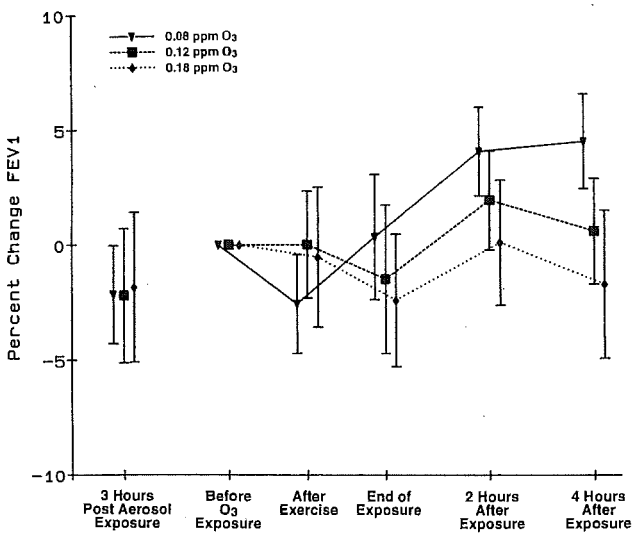


Figure 17. The percentage of change in FEV_1 after H_2SO_4 aerosol exposure, and during and after ozone exposure 24 hours later for subjects with asthma. Values are expressed as means ± SEM.

FEV_1 data four hours after exposure revealed evidence for a consistent effect of the preexposure condition across all three ozone levels (a main effect) ($p = 0.040$). A significant difference was found between the preexposure conditions at 0.18 ppm ozone ($p = 0.012$). In addition, a significant difference was seen between the linear trends relating ozone level to change in FEV_1 at four hours after exposure (Figure 18) ($p = 0.037$). These trends were similar when expressed as liters rather than percentage of baseline (Figure 19). Analysis of linear trends in FEV_1 over time again revealed a significant difference between the two aerosol exposures at 0.18 ppm ozone ($p = 0.023$) as well as a significant difference between ozone trends in relation to preexposure level ($p = 0.031$). The size of this difference is shown in Figure 20.

The variability in responses to ozone exposure among subjects with asthma is better understood by examining individual data. For example, Figure 21 shows the percentage change in FEV_1 for subjects with asthma following 0.18 ppm ozone with H_2SO_4 preexposure. Three subjects show an increase in FEV_1 by more than 10%. For these subjects, FEV_1/FVC also increased suggesting exercise-related bronchodilation. One subject, increased FEV_1 by more than

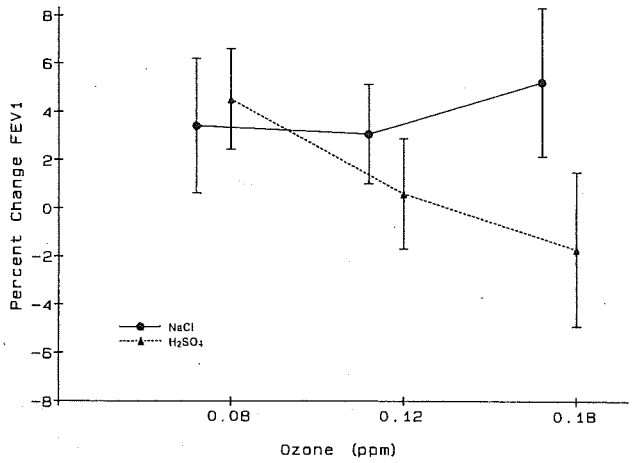


Figure 18. The percentage of change in FEV_1 four hours after exposure to each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for subjects with asthma. Values are expressed as means ± SEM.

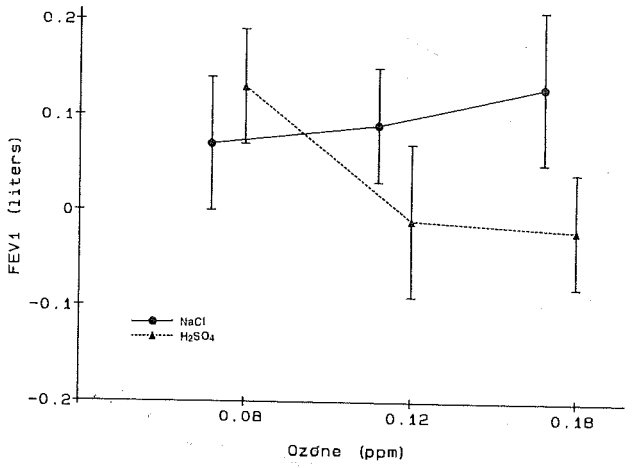


Figure 19. The absolute change in FEV_1 (L) four hours after exposure to each of the three ozone concentrations for the NaCl and H_2SO_4 aerosol preexposure conditions for subjects with asthma. Values are expressed as means ± SEM.

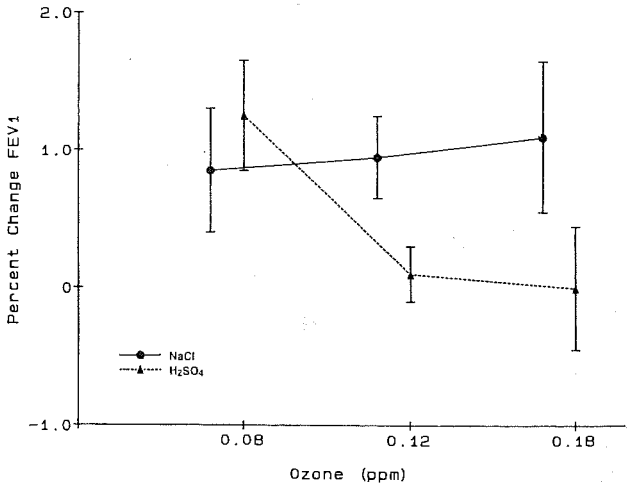


Figure 20. Linear trend analysis for FEV_1 using data from the four postexposure time points (after exercise period 3, at the end of exposure, and two and four hours after exposure) for subjects with asthma. The analysis indicates a significant difference for the two aerosol exposures at 0.18 ppm ozone ($p = 0.023$), and a significant difference between ozone trends in relation to exposure level ($p = 0.031$). Vertical axis indicates percentage of change in FEV_1 per unit of time, approximately one hour. Values are expressed as means ± SEM.

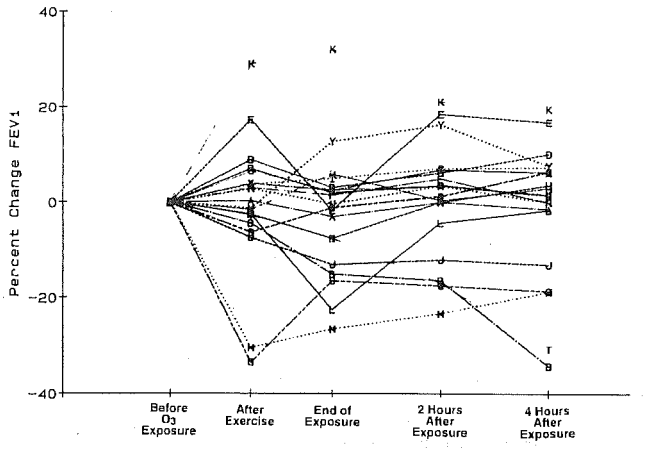


Figure 21. Individual changes in FEV_1 during and after exposure to 0.18 ppm ozone, with H_2SO_4 preexposure for subjects with asthma. Values are expressed as means ± SEM.

30% following 0.18 ppm ozone, and by more than 35% following 0.08 ppm ozone (data not shown). In contrast, six subjects manifested a decrease in FEV_1 of more than 10% following 0.18 ppm ozone exposure. These subjects demonstrated similar changes in FVC, with only minor reductions of FEV_1/FVC and no consistent changes in s*G*aw. This suggested that the effect was primarily a reduction in inspiratory capacity, a known response to ozone exposure, rather than bronchoconstriction. Two subjects had a continuous decrease in FEV_1 throughout the period of observation, reaching a maxima at four hours after exposure of 30% and 33%, respectively. One of the subjects in Figure 21 (subject 15, Table 7) was the individual for which the workload was reduced during each exposure because of asthmatic symptoms. This individual showed consistently larger decrements in lung function responses to ozone with H_2SO_4 aerosol preexposure compared with NaCl aerosol preexposure. Of the six subjects whose FEV_1 decreased more than 10% following exposure to 0.18 ppm ozone (Figure 21), three subjects also showed greater than 10% decreases at lower ozone concentrations. Three subjects and two subjects, respectively, showed a greater than 10% decline in FEV_1 following exposure to 0.12 ppm ozone and 0.08 ppm ozone. Changes in s*G*aw did not reach statistical significance for the subjects with asthma.

Specific subject characteristics did not appear to be predictive of responsiveness to ozone, regardless of the preexposure condition. Responses were not significantly different based on age, sex, serum IgE level, or baseline lung function in the subjects with asthma.

Comparison of Responses of Healthy Subjects and Subjects with Asthma

The responses of healthy subjects and subjects with asthma were compared by combining both groups of subjects and analyzing the data using a four-way ANOVA. Analyses were limited to data in which significant effects had been observed in the subjects with asthma. Analyses of changes in FVC immediately after exposure are shown in Table 8. A highly significant difference was found between healthy subjects and subjects with asthma (a main effect) ($p = 0.0048$), as well as a significant differential effect of the preexposure aerosol on the response to ozone (interaction) for the entire group ($p = 0.0022$). For FVC four hours after exposure, evidence was seen again for an interaction between aerosol and ozone exposure for the whole group of subjects ($p = 0.011$), but the difference between healthy subjects and subjects with asthma was no longer significant ($p = 0.24$).

Analysis of FEV_1 four hours after exposure and of linear time trends for changes in FEV_1 by four-way ANOVA provided similar results. A three-way trend comparison showed that the difference between ozone-related trends in FEV_1 at four hours ($p = 0.026$) and linear trends over time ($p = 0.024$) for the two aerosol preexposures was also different between healthy subjects and subjects with asthma. This suggests that the aerosol-ozone effect on FEV_1 may be qualitatively, rather than simply quantitatively, different for healthy subjects and subjects with asthma.

Symptoms

Symptoms were assessed by questionnaire after each exposure to both aerosol and ozone. Symptoms were graded as an intensity scale of 0 to 5, with 0 representing absence of the symptom in question. Both total symptom scores (sum of scores for all subjects undergoing a given exposure sequence) and number of subjects indicating any symptom greater than 0 were tallied. As expected, subjects with asthma had more respiratory symptoms, including cough, sputum production, shortness of breath, and wheezing, than healthy subjects. However, no significant differences in symptoms following NaCl or H_2SO_4 exposure were seen for either healthy subjects or subjects with asthma. Also no clear relationship was seen between ozone exposure level and intensity of symptoms for either healthy subjects or subjects with asthma.

Table 8. Four-Way ANOVA Comparing Forced Vital Capacity Immediately After Ozone Exposure in Healthy Subjects and Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	*p* Value
Ozone	2	6.03	3.01	0.14	0.87
Aerosol	1	25.42	25.42	1.14	0.29
Group[a]	1	922.15	922.15	8.59	0.0048
Ozone × Aerosol	2	281.68	140.84	6.33	0.0022
Ozone × Group	2	20.43	10.21	0.46	0.63
Aerosol × Group	1	20.16	20.16	0.91	0.34
Ozone × Aerosol × Group	2	93.06	46.53	2.09	0.13
Contrast Ozone × Aerosol × Group	1	86.53	86.53	3.89	0.050
Subject	58	6,140.34	105.87	4.76	0.0001
Error	170	3,782.87	22.25		
Corrected Total	239	11,283.91			
R^2	66.5%				

[a] Group indicates either subjects with asthma or healthy subjects.

Table 9. Selected Respiratory Symptoms in Subjects with Asthma After Exposure to 0.18 ppm Ozone

		H_2SO_4					
	Score →	0	1	2	3	4	5
NaCl ↓	**Cough**						
0		11	5	0	0	0	0
1		1	1	1	0	0	0
2		0	1	0	0	0	0
3		0	0	0	0	0	0
4		0	0	0	0	0	0
5		0	0	0	0	0	0
	Sputum production						
0		13	3	1	0	0	0
1		0	2	0	0	0	0
2		0	0	0	0	0	0
3		0	1	0	0	0	0
4		0	0	0	0	0	0
5		0	0	0	0	0	0
	Chest tightness						
0		5	5	2	0	0	0
1		0	2	1	1	0	0
2		0	2	1	0	0	0
3		1	0	0	0	0	0
4		0	0	0	0	0	0
5		0	0	0	0	0	0

Because a primary goal was to examine the effect of acid aerosol preexposure on the ozone response, 2 × 2 tables were constructed for symptom scores following each ozone exposure level, comparing the two preexposure conditions. Table 9 shows the results for three respiratory symptoms noted by subjects with asthma following exposure to 0.18 ppm ozone. Although a slightly larger number of subjects with asthma collectively reported a higher symptom score when ozone was preceded by H_2SO_4, no statistically significant difference was seen between NaCl and H_2SO_4 exposure by Wilcoxon's sum rank test (Lehmann 1975), and no clear relationship was seen between symptoms and ozone exposure concentration.

DISCUSSION

Despite years of laboratory, clinical, and epidemiologic research, the human health effects of ozone and acidic aerosol inhalation, alone or in combination, have not been fully characterized. Controlled clinical studies represent a unique approach to the assessment of health-related responses and effects of these air pollutants. Such studies complement epidemiologic and animal experimentation approaches and offer unique investigative capabilities and advantages. Controlled clinical studies are designed to investigate single air pollutants or simple air pollutant mixtures that are fully characterized and controlled. Ordinarily, they do not try to simulate air pollution as it occurs in the real world; consequently, interactive factors and pollutants may be missed. On the other hand, the conditions of air pollution in the real world are both highly complex and protean. This condition has created enormous difficulties in the epidemiologic evaluation of air pollution and especially in applying epidemiology to the task of setting standards for pollutants.

Another limitation of controlled clinical studies arises from ethical constraints. By design, controlled clinical studies should not produce responses in the subject that present appreciable risk; in fact, most clinical studies attempt to examine modifications of function without inducing more than mild symptoms. In addition, they fastidiously avoid including those subjects who have the greatest potential risk from air pollution according to epidemiologic studies, namely, subjects with severe respiratory disease, neonates and young children, and people over 70 years of age. Despite these constraints, controlled clinical studies provide a powerful alternative to epidemiologic and animal studies because their findings pertain directly to humans, the relationship between exposure and responses can be completely characterized, and the conditions of exposure can be controlled and systematically varied. Although it is probable that the control clinical study will make important contributions to the database for setting standards, we believe its main values are to obtain exposure-response data, investigate mechanisms of response, and examine pulmonary dysfunction in the context of enhanced susceptibility to air pollutants in relation to normal cohorts. In these studies, we have extended our approach to studying air pollutants by looking at sequential combinations and thus overcoming one of the limitations, namely, the failure to examine the biological interactions of pollutants.

The goal of this study was to determine whether preexposure to acidic aerosols enhances lung functional responses to ozone exposure in healthy subjects and subjects with asthma. Exposure conditions incorporated mild to moderate exercise periods to mimic outdoor activities and increase pollutant dose by increased ventilation. Ozone concentrations bracketed the current NAAQS of 0.12 ppm, an exposure range commonly experienced in many U.S. urban and suburban areas. Acidic aerosol preexposure concentrations (100 mg/m^3) were slightly above peak concentrations experienced in major urban areas in the northeastern United States in order to determine effects at the highest possible ambient exposure range (Spengler et al. 1989); this concentration is near the lower limit at which direct effects on lung function have been demonstrated in subjects with asthma (Koenig et al. 1989; Morrow et al. 1994). However, the potential for elevated outdoor levels to alter functional or symptomatic responses in our subjects was minimized by discontinuing the study for the summer months, the period of highest ozone and acidic aerosol concentrations in Rochester, NY. Indeed monitoring of outdoor ozone concentrations during this month of the study revealed peak one-hour levels of 0.05 ppm and average one-hour concentrations below 0.04 ppm. Even during the summer months, peak 24-hour H^+ levels did not exceed 10 $\mu g/m^3$ expressed as H_2SO_4.

In this study, we found no direct effect of either pollutant on lung function, and no evidence for an interaction between aerosol and ozone exposure was observed in healthy volunteers. Only changes in s*G*aw revealed a significant interaction between aerosol and ozone preexposure; the magnitude of changes was clinically insignificant. The absence of a response to ozone is consistent with other studies (Folinsbee et al. 1988), and suggests that healthy individuals without airway hyperreactivity will not experience adverse effects on lung function from short-term exposure (with mild exercise) to H_2SO_4 aerosols followed by ozone at levels near the NAAQS. Not excluded is the possibility that H_2SO_4 aerosols could enhance responses of healthy people to more intense, prolonged, or repeated exposures.

Similarly, subjects with asthma showed no direct response to aerosol exposure except for FEV_1 decrements at four hours. However, evidence was found for both a direct effect of ozone exposure and for differences in the responses to ozone depending on the preexposure aerosol. In particular, preexposure to H_2SO_4 aerosol, but not NaCl aerosol, resulted in a clear ozone concentration-response in both FVC and FEV_1 in subjects with asthma. This effect was seen both immediately (FVC changes) and four hours after exposure (both FVC and FEV_1 changes).

Polynomial trend analysis was used to examine changes in lung function over time, and significant differences between healthy subjects and subjects with asthma were found in their responses to ozone and in the effect of the preexposure aerosol on the ozone response. To understand the significance of these analyses, further consideration of trend analysis is warranted. A linear trend represents a straight line. A quadratic trend is the simplest kind of nonlinear trend, represented by a parabolic shape, which shows a decline followed by an increase (if the trend is positive) or an increase followed by a decrease (if the trend is negative) across the entire range of observation. A cubic trend is somewhat more complicated; it typically is represented as up-down-up (starting from an initial level) for a positive trend, and down-up-down for a negative trend. For the subjects with asthma, Figures 10 and 11 show ozone-related changes in FVC over time, and Figure 15 shows the results of the trend analyses. Of importance is the observation that cubic time trends differ between the two preexposure conditions. The figures show that these trends reflect an early decline followed by recovery. For NaCl preexposure, cubic trends are negative with 0.08 ppm ozone, but increase toward zero (absence of cubic trend) with increasing ozone concentrations. For H_2SO_4 preexposure, cubic trends become increasingly negative with increasing ozone concentrations. Negative cubic trends are consistent with pulmonary function decrements followed by recovery.

Changes in FEV_1 in subjects with asthma over time (Figures 16 and 17) also show differences between the preexposure conditions. Mean FEV_1 actually increases above baseline two hours and four hours following ozone exposure with NaCl preexposure, probably as a result of exercise-induced bronchodilation in a few subjects (Figure 21). However, with H_2SO_4 preexposure, this increase in FEV_1 is attenuated with increasing concentrations of ozone. These trend analyses therefore provide additional evidence that, for subjects with asthma, H_2SO_4 preexposure alters and enhances lung functional response to ozone when compared with NaCl preexposure. In addition, the responses of the asthmatic subject group were clearly distinct from the healthy subject group of volunteers.

Examination of individual data for the subjects with asthma (Figure 21) provides insight into the difficulties in interpreting group responses in clinical studies of subjects with asthma, but also suggests that some asthmatic individuals experience clinically important reductions in lung function in response to acidic aerosols and ozone at these concentrations. Several subjects responded to exercise with significant bronchodilation. Others experienced significant decrements in both FVC and FEV_1. Examination of mean response data therefore does not provide information about the most responsive individuals. For example, several asthmatics experienced decrements in FEV_1 in excess of 35% following ozone exposure. Two individuals demonstrated a gradual decline in lung function which extended throughout the four-hour follow-up period (Figure 21), approaching a 40% reduction in FEV_1. Despite these reductions in lung function, virtually no symptom changes were seen (Table 9) even in the most responsive subjects with asthma.

The surprising rise in asthma morbidity and mortality has been highlighted by the recent report of the National Asthma Education Program sponsored by the National Institutes of Health (Sheffer 1991). From 1980 to 1987, the prevalence rate of asthma in the United States increased 29%, death rates for asthma increased 31%, and hospital discharge rates for asthma increased 6% (Weiss and Wagener 1990). Typically, air pollution is included in the list of factors that precipitate or aggravate asthma, but little supporting evidence is available. However, the report by Bates and Sizto (1987) suggest a link between ozone and acidic aerosol levels in the atmosphere and hospital admissions for asthma. Furthermore, a more recent epidemiologic study (Thurston et al. 1992) concludes that both ozone and H^+ concentrations in the atmosphere are associated with hospital admissions for asthma. Cody and associates (1992) observed that, in New Jersey, emergency room visits for asthma correlated with ozone concentrations during the summer months, if temperature was controlled for in the analysis. Thurston and colleagues (1992) found an increased risk of hospital admissions for asthma associated with increased levels of H^+ and ozone. In New York City, the relative risk of hospital admissions for asthma attributable to ozone was 1.23 ± 0.10 (mean $\pm$ SE, and for H^+, 1.32 ± 0.11 ($p < 0.01$). These pollutants were estimated to account for between 6% and 24% of admissions for asthma in Buffalo and New York City.

Therefore, it is surprising that controlled clinical studies have yet to demonstrate that ozone dramatically affects lung function in subjects with asthma (Linn et al. 1978; Koenig

et al. 1985, 1987), atopic nonasthmatic subjects, or individuals with chronic obstructive pulmonary disease (Bromberg 1988). Several possible explanations exist. In contrast to studies of healthy volunteers, studies of subjects with asthma have not been performed using prolonged exposures or repeated daily exposures. Furthermore, few studies of subjects with asthma have incorporated multiple periods of exercise, an essential factor in provoking changes in airway function with low-level ozone exposure in healthy volunteers. More recent data (Kreit et al. 1989) suggest that if more intense exercise is included, subjects with asthma show increased airway resistance to a two-hour exposure to 0.4 ppm ozone.

Several clinical observations provide evidence that subjects with asthma ultimately may prove to be hyperresponsive to ozone. Aris and coworkers (1991) have observed a relationship between baseline airway hyperreactivity to methacholine and responsiveness to ozone, a finding of considerable interest but not in agreement with earlier studies. Their observations suggest that airway hyperresponsiveness may be a risk factor for ozone sensitivity even among healthy, asymptomatic athletes. Using a different approach to study environmental interactions, Molfino and colleagues (1991) investigated whether inhalation of 0.12 ppm ozone for one hour potentiated the airway allergic response in subjects with asthma with seasonal symptoms. Although ozone did not significantly alter baseline function, reactivity to inhaled allergen was enhanced significantly by prior ozone inhalation. Ozone has been shown to increase airway permeability using a variety of markers (Koren et al. 1989); it is conceivable that prior ozone exposure increase access of allergen to mediator-secreting cells in the subepithelium. These findings emphasize the potential for interaction between ozone and other relevant environmental pollutants.

Acidic aerosols could sensitize the airways to ozone by several possible mechanisms. Exposure to acidic aerosols could alter the alveolar microenvironment through direct epithelial injury, through alteration of alveolar inflammatory cell populations, or by altering specific functions of immunocompetent cells in the distal airways. These effects could be mediated through local changes in pH. Holma (1989) has shown that mucus viscosity is increased when pH decreases below 7.4, providing a possible mechanism for previously observed changes in mucociliary clearance following exposure to acidic aerosols (Lioy and Lippmann 1986). In addition, acid hydrolases generated by inflammatory cells, such as alveolar macrophages or polymorphonuclear leukocytes, may be activated at acidic pH (Schlesinger 1985). Acidic particles deposited at the alveolar level could cause altered epithelial permeability, activation of resident alveolar macrophages with release of toxic oxygen species, or recruitment of lymphocytes or polymorphonuclear leukocytes from the vascular compartment.

Using the technique of bronchoalveolar lavage to sample the deep lung 18 hours after exposure, Frampton and coworkers (1992) examined the effects of 1,000 $\mu g/m^3$ H_2SO_4 aerosol exposures for two hours in 12 healthy volunteers. No evidence of alveolar inflammation, influx of plasma proteins into the alveolar space, or alterations in selected antiviral functions of alveolar macrophages were detected. However, they could not exclude the possibility of an early, transient alteration in alveolar macrophage function that largely resolved by 18 hours after exposure. Because latent clinical effects are known to occur 18 to 24 hours after H_2SO_4 aerosol inhalation (Utell et al. 1983a; Spektor et al. 1985), it is possible that the acidic aerosols induced subclinical bronchospasm or altered the properties of airway mucus, in either case potentiating the effects of ozone. Studies in our laboratory (Utell et al. 1983b) and by others (Lippmann and Schlesinger 1984) have shown that at equal sulfate concentrations, aerosols of sodium sulfate, ammonium sulfate, NH_4HSO_4, and H_2SO_4 effect airway responsiveness in relation to their acidity (pH at the same sulfate concentration). The hydrogen ion level that occurs in the milieu of a deposited ammonium sulfate or H_2SO_4 aerosol droplet defies computation, because the H^+ level will be determined by the size and composition of the hygroscopic droplet and the composition (buffering capacity, proteins, etc.) of the epithelial lining layer. However, the fact that responses are related to an index of the actual H^+ concentration of the deposited aerosol is sufficient to deduce that the acute actions of acidic aerosols are based on the irritant properties of H^+ or titratable acidity (Fine et al. 1987), and not the sulfate moiety. If the generally accepted perception of acidity is correct, then the delayed actions of the acidic sulfates may be related to diverse mediator release, neutrophil activation, or related events developing from this initial irritant action.

In the United States, the Clean Air Act regulates particles, sulfur oxides, ozone, and NO_x as criteria pollutants for which NAAQS are established and periodically revised following a review of evidence assembled in a criteria document. The most recent revision of the NAAQS for particulate matter established the present standard as particulate matter equal to or less than 10 μm in aerodynamic diameter (the PM_{10} standard). This standard sets maximum concentrations of 150 $\mu g/m^3$ for 24 hours and of 50 $\mu g/m^3$ as the annual average for these particles. Since the promulgation of the PM_{10} standard, new epidemiologic findings suggest that the present NAAQS for particulate matter may

not protect against adverse health effects with an "adequate margin of safety", mandated by the Clean Air Act. Some of these new findings include positive associations between mortality rates and levels of particulate matter in a number of U.S. cities (Kinney and Özkaynak 1991; Schwartz and Dockery 1992) and between respiratory morbidity and PM_{10} pollution in the Utah Valley, the site of a steel mill (Pope and Kanner 1993). The toxicologic basis for these findings is perplexing. Perhaps inhalation of particles induces a low-grade irritant effect on the airway; then subsequent exposure to oxidants or perhaps reexposure to particles leads to an enhanced airway response, particularly in individuals with underlying respiratory disease.

In conclusion, our data indicate that preexposure to H_2SO_4 aerosols can alter responses to ozone in exercising subjects with asthma. Furthermore, our findings support the growing evidence from epidemiologic and clinical studies that subjects with asthma are more responsive to ozone than are healthy subjects. In 1989, 67 million people in the United States lived in counties that violated the NAAQS for ozone and another 27 million in communities that exceeded the NAAQS for particles. Thus, even transient impairments in host defense or lung function in response to mixtures of oxidants and particles, when applied across large populations of exposed individuals, could have significant public health implications. Given the recent epidemiologic finding linking mortality and respiratory morbidity with PM_{10}, new toxicologic studies on particulate matter alone and in combination with oxidants are required (Utell and Samet 1993). The toxicologic evidence of the health effects of particles may be strengthened by following leads identified from our current studies. Further exposure studies of the most susceptible subjects may help determine whether exposures to particles alone or in combination with oxidants are, in fact, responsible for the increased mortality of subjects, by showing effects on function of a clinically significant magnitude. Our understanding of the toxicity of urban particles needs to be increased by investigations directed at the combined effects of metal ions, which, in vitro, dramatically enhance the respiratory inflammation induced by sulfates (Amdur and Chen 1989) and particles. Our human experimental studies, which have attempted to more realistically replicate exposures to ambient atmospheres, have provided new data on a significant susceptible group in the general population. Although our studies were not conceived to fuel the regulatory process, it is evident that controlled clinical studies have been, and will continue to be, key elements in the process of setting standards for criteria pollutants. We believe, however, that efforts to characterize the influence of oxidant and particle inhalation—including repeated exposures with combinations of pollutants—on respiratory defense mechanisms and airway function are warranted if a more scientifically credible database is desired for the standard-setting process.

ACKNOWLEDGMENTS

Without the invaluable contributions of the members of the research team (Appendix E), these studies would not have been possible. The authors also appreciate the most capable assistance of Kim DeLong in preparing the many drafts of this report over an extended period of time.

REFERENCES

Amdur MO, Chen LC. 1989. Furnace-generated acid aerosols: Speciation and pulmonary effects. Environ Health Perspect 79:147–150.

Aris R, Christian C, Sheppard D, Balmes JR. 1991. The effects of sequential exposure to acidic fog and ozone on pulmonary function in exercising subjects. Am Rev Respir Dis 143:85–91.

Bates DV, Sizto R. 1987. Air pollution and hospital admissions in southern Ontario: The acid summer haze effect. Environ Res 43:317–331.

Bauer MA, Utell MJ, Speers DM, Gibb FR, Morrow PE. 1988. Effects of near ambient levels of H_2SO_4 aerosols on lung function in exercising subjects with asthma and COPD (abstract). Am Rev Respir Dis 137(4):167.

Briscoe WA, DuBois AB. 1958. The relationship between airway resistance, airway conductance, and lung volume in subjects of different age and body size. J Clin Invest 37:1279–1285.

Bromberg PA. 1988. Asthma and automotive emissions. In: Air Pollution, the Automobile, and Public Health (Watson AY, Bates RR, Kennedy D, eds.) pp. 465–498. National Academy Press, Washington, DC.

Cody RP, Weisel CP, Birnbaum G, Lioy PJ. 1992. The effect of ozone associated with summertime photochemical smog on the frequency of asthma visits to hospital emergency departments. Environ Res 58:184–194.

Dockery DW, Speizer FE. 1989. Epidemiological evidence for aggravation and promotion of COPD by acid air pollution. In: Lung Biology in Health and Disease, Vol. 43,

Clinical Epidemiology of Chronic Obstructive Pulmonary Disease (Hensley MJ, Saunders NA, eds.) pp. 201–225. Marcel Dekker, New York, NY.

DuBois AB, Botelho SY, Bedell GN, Marshall R, Comroe JH Jr. 1956. A rapid plethysmographic method for measuring thoracic gas volume: A comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. J Clin Invest 35:322–326.

Fine JM, Gordon T, Thompson JE, Sheppard D. 1987. The role of titratable acidity in acid aerosol-induced bronchoconstriction. Am Rev Respir Dis 135:826–830.

Folinsbee LJ, McDonnell WF, Horstman DH. 1988. Pulmonary function and symptom responses after 6.6 hour exposure to 0.12 ppm ozone with moderate exercise. J Air Pollut Control Assoc 38:28–35.

Frampton MW, Voter KZ, Morrow PE, Roberts NJ, Culp DJ, Cox C, Utell MJ. 1992. H_2SO_4 aerosol exposure in humans assessed by bronchoalveolar lavage. Am Rev Respir Dis 146:626–632.

Gardner DE, Miller FJ, Illing JW, Kirtz JM. 1977. Increased infectivity with exposure to ozone and H_2SO_4. Toxicol Lett 1:59–64.

Gibb FR, Utell MJ, Morrow PE. 1990. A multi-jet aerosol nebulizer system for controlled clinical studies in environmental chambers. Aerosol Sci Technol 12:376–385.

Goldman HI, Becklake MR. 1959. Respiratory function tests: Normal values at median altitudes and the prediction of normal results. Am Rev Tuberc 79:457–467.

Grose EC, Gardner DE, Miller FJ. 1980. Response of ciliated epithelium to ozone and H_2SO_4. Environ Res 22:377–385.

Holma B. 1989. Effects of inhaled acids on airway mucus and its consequences for health. Environ Health Perspect 79:109–113.

Horstman DH, Kehrl H, Weinberg P, Roger LJ. 1986. Pulmonary function changes for asthmatics performing exercise while exposed to combined sulfur dioxide and H_2SO_4: An interim report. In: Aerosols: Research, Risk Assessment, and Control Strategies (Lee SD, Schneider T, Grant LD, Verkerk PJ, eds.) pp. 703–709. Lewis Publishers, Chelsea, MI.

Horvath SM, Folinsbee LJ, Bedi JF. 1987. Combined effect of ozone and H_2SO_4 on pulmonary function in man. Am Ind Hyg Assoc J 48:94–98.

Kagawa J. 1986. Experimental studies on human health effects of aerosol and gaseous pollutants. In: Aerosols: Research, Risk Assessment and Control Strategies (Lee SD, Schneider T, Grant LD, Verkerk PJ, eds.) pp. 683–698. Lewis Publishers, Chelsea, MI.

Kinney PL, Özkaynak H. 1991. Associations of daily mortality and air pollution in Los Angeles county. Environ Res 54:99–120.

Koenig JQ, Covert DS, Marshall SG, Van Belle G, Pierson WE. 1987. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 136:1152–1157.

Koenig JQ, Covert DS, Morgan MS, Horike M, Horike N, Marshall SG, Pierson WE. 1985. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 132:648–651.

Koenig JQ, Covert DS, Pierson WE. 1989. Effects of inhalation of acidic compounds on pulmonary function in allergic adolescent subjects. Environ Health 79:173–178.

Koenig JQ, Pierson WE, Norike M. 1983. The effects of inhaled H_2SO_4 on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 128:221–225.

Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, Kozumbo WJ, Becker S, House DE, McDonnell WF. 1989. Ozone-induced inflammation in the lower airways of human subjects. Am Rev Respir Dis 139:407–415.

Kreit JW, Gross KB, Moore TB, Lorenzen TJ, D'Arcy J, Eschenbacher WL. 1989. Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66:217–222.

Kulle TJ, Kerr HD, Farrell BP, Sauder LR, Bermel MS. 1982. Pulmonary function and bronchial reactivity in human subjects with exposure to ozone and respirable H_2SO_4 aerosol. Am Rev Respir Dis 126:996–1000.

Larson TV, Frank R, Covert DS, Holub D, Morgan MS. 1982. Measurements of respiratory ammonia and the chemical neutralization of inhaled H_2SO_4 aerosol in anesthetized dogs. Am Rev Respir Dis 125:502–506.

Last JA, Hyde DM, Chang DPY. 1984. A mechanism of synergistic lung damage by ozone and a respirable aerosol. Exp Lung Res 7:223–235.

Last JA, Hyde DM, Guth DJ, Warren DL. 1986. Synergistic interaction of ozone and respirable aerosols on rat lungs: I. Importance of aerosol acidity. Toxicology 39:247–257.

Lebowitz MD. 1984. The effects of environmental tobacco smoke exposure and gas stoves on daily peak flow rates in asthmatic and non-asthmatic families. Eur J Respir Dis 65:90–97.

Lebowitz MD, Knudson RJ, Burrow B. 1975. Tucson epidemiologic study of obstructive lung disease: I. Methodology and prevalence of disease. Am J Epidemiol 102:137–152.

Lebowitz MD, O'Rourke MK, Dodge R, Holberg CJ, Corrman G, Hoshaw RW, Pinnas JL, Barbee RA, Sneller MR. 1982. The adverse health effects of biological aerosols, other aerosols, and indoor microclimate on asthmatic and non-asthmatics. Environ Int 8:375–380.

Lehmann EL. 1975. Nonparametrics: Statistical Method Based Ranks. Holden-Day, San Francisco, CA.

Leith DE, Mead J. 1973. Principles of body plethysmography. In: Procedures for Standardized Measurements of Lung Mechanics. National Heart and Lung Institutes, Division of Lung Diseases, Bethesda, MD.

Linn WS, Avol EL, Shamoo DA, Whynot JD, Anderson KR, Hackney JD. 1986. Respiratory responses of exercising asthmatic volunteers exposed to H_2SO_4 aerosol. J Air Pollut Control Assoc 36:1323–1328.

Linn WS, Buckley RD, Spier CE, Blessey RL, Jones MP, Fischer DA, Hackney JD. 1978. Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117:835–843.

Lioy PJ, Lippmann, M. 1986. Measurement of exposure to acid sulfur aerosols. In: Aerosols: Research, Risk Assessment, and Control Strategies (Lee SD, Schneider T, Grant LD, Verkerk PJ, eds.) pp. 743–752. Lewis Publishers, Chelsea, MI.

Lippmann M, Schlesinger RB. 1984. Interspecies comparisons of particle deposition and mucociliary clearance in tracheobronchial airways. J Toxicol Environ Health 13:261–289.

Molfino NA, Wright SC, Katz I, Tarlo S, Silverman F, McClean PA, Szalai JP, Raizenne M, Slutsky AS, Zamel N. 1991. Effect of low concentration of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338:199–203.

Morris J, Koski A, Johnson L. 1971. Spirometric standards for healthy nonsmoking adults. Am Rev Respir Dis 103:57–61.

Morrow PE, Utell MJ. 1986. Technology and methodology of clinical exposures to aerosols. In: Aerosols: Research, Risk Assessment, and Control Strategies (Lee SD, Schneider T, Grant LD, Verkerk PJ, eds.) pp. 661–670. Lewis Publishers, Chelsea, MI.

Morrow PE, Utell MJ, Bauer MA, Speers DM, Gibb FR. 1994. Effects of near ambient levels of H_2SO_4 aerosol on lung function in exercising subjects with asthma and COPD. Ann Occup Hyg (in press).

Pope CA III, Kanner RE. 1993. Acute effects of PM_{10} pollution on pulmonary function of smokers with mild to moderate COPD. Am Rev Respir Dis 147:1336–1340.

Salem H. 1987. Good laboratory practices. In: Inhalation Toxicology: Research Methods, Applications, and Evaluations (Salem H, ed.) pp. 385–433. Marcel Dekker, New York, NY.

Schlesinger RB. 1985. Effects of inhaled acids on respiratory tract defense mechanisms. Environ Health Perspect 63:25–38.

Schlesinger RB, Graham JA. 1992. Health effects of atmospheric acid aerosols: A model problem in inhalation toxicology and air pollution risk assessment (Symposium overview). Fundam Appl Toxicol 18:17–24.

Schwartz J, Dockery DW. 1992. Increased mortality in Philadelphia associated with daily air pollution concentrations. Am Rev Respir Dis 145:600–604.

Sheffer AL (chairman). 1991. Guidelines for the diagnosis and management of asthma (part of National Heart, Lung, and Blood Institute, National Asthma Education Program, Expert Panel Report). J Allergy and Clin Immunol Suppl 88(3)part 2:425–534.

Spektor DM, Leikauf GD, Albert RE, Lippmann M. 1985. Effects of submicrometer H_2SO_4 aerosols on mucociliary transport and respiratory mechanics in asymptomatic asthmatics. Environ Res 37:174–191.

Spengler JD, Keeler GJ, Koutrakis P, Ryan PB, Raizenne M, Franklin CA. 1989. Exposures to acidic aerosols. Environ Health Perspect 79:43–51.

Stacy RW, Seal E, House DE, Green J, Roger LJ, Raggio J. 1983. A survey of gaseous and aerosol pollutants on pulmonary function of normal males. Arch Environ Health 38:104–115.

Thurston GD, Kazuhiko I, Kinney PL, Lippmann M. 1992. A multi-year study of air pollution and respiratory hospital admissions in three New York State metropolitan areas: Results for 1988 and 1989 summers. J Expo Anal Environ Epidemiol 2:429–450.

Utell MJ, Mariglio JA, Morrow PE, Gibb FR, Speers DM. 1989. Effects of inhaled acid aerosols on respiratory function: The role of endogenous ammonia. J Aerosol Med 2:141–147.

Utell MJ, Morrow PE, Hyde RW. 1983a. Latent development of airway hyperreactivity in human subjects after H_2SO_4 aerosol exposure. J Aerosol Sci 14:202–205.

Utell MJ, Morrow PE, Hyde RW, Schreck RM. 1984. Exposure chamber for studies of pollutant gases and aerosols in human subjects: Design considerations. J Aerosol Sci 15:219–221.

Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. 1983b. Airway responsiveness to sulfate and H_2SO_4 aerosols in asthmatics. Am Rev Respir Dis 128:444–450.

Utell MJ, Samet JM. 1993. Particulate air pollution and health: New evidence on an old problem (editorial). Am Rev Respir Dis 147:1334–1335.

Utell MJ, Swinburne AJ, Hyde RW, Speers DM, Gibb FR, Morrow PE. 1979. Airway reactivity to nitrates in normal and mild asthmatic subjects. J Appl Physiol: Respirat Environ Exercise Physiol 46:189–196.

Weiss KB, Wagener DK. 1990. Changing patterns of asthma mortality: Identifying target populations at high risk. JAMA 264:1683–1687.

APPENDIX A. Protocols

Day 1

Questionnaire and informed consent

Subject history and physical examination

Baseline pulmonary function tests

- Functional residual capacity
- Specific airway conductance
- Spirometry
- Maximum flow-volume loops

Carbachol in propylene glycol: 0.25% or 0.5% carbachol for healthy subjects; 0.0125%, 0.025%, 0.05%, 0.25%, or 0.5% carbachol for subjects with asthma

Repeat pulmonary function tests after each carbachol exposure until sGaw measures a 40% drop or greater, FEV_1 measures a 15% drop or greater, or FEV_1 measures a 15% drop or greater after bronchodilator inhalation

Exercise with $\dot{V}_E$ and oxygen saturation (4 × resting $\dot{V}_E$)

Day 2

Resting $\dot{V}_E$ and oxygen saturation

Baseline pulmonary function tests

Chamber exposure, 3 hours: NaCl or H_2SO_4 at 100 $\mu g/m^3$

0:00–0:15	Enter and rest
0:15–0:25	Exercise with $\dot{V}_E$ and oxygen saturation
0:25–0:45	Rest
0:45–0:55	Exercise with $\dot{V}_E$ and oxygen saturation
0:55–1:15	Rest
1:15–1:25	Exercise with $\dot{V}_E$ and oxygen saturation
1:25–1:45	Rest
1:45–1:55	Exercise with $\dot{V}_E$ and oxygen saturation
1:55–2:15	Rest
2:15–2:25	Exercise with $\dot{V}_E$ and oxygen saturation
2:25–2:45	Rest
2:45–2:55	Exercise with $\dot{V}_E$ and oxygen saturation
3:00–3:05	Pulmonary function tests questionnaire

Day 3 (24 hours later)

Resting $\dot{V}_E$ and oxygen saturation

Baseline pulmonary function tests

Chamber exposure 3 hours: 0.08, 0.12, or 0.18 ppm ozone

0:00–0:15	Enter and rest
0:15–0:25	Exercise with $\dot{V}_E$ and oxygen saturation
0:25–0:45	Rest
0:45–0:55	Exercise with $\dot{V}_E$ and oxygen saturation
0:55–1:15	Rest
1:15–1:25	Exercise with $\dot{V}_E$ and oxygen saturation
1:25–1:30	Rest
1:45–1:55	Exercise with $\dot{V}_E$ and oxygen saturation
1:55–2:15	Rest
2:15–2:25	Exercise with $\dot{V}_E$ and oxygen saturation
2:25–2:45	Rest
2:45–2:55	Exercise with $\dot{V}_E$ and oxygen saturation
2:55–3:05	Pulmonary function tests questionnaire
5:00–5:10	Spirometry
7:00–7:10	Spirometry

Day 4 (2 to 8 weeks later)

Same as Day 2

Day 5 (24 hours later)

Same as Day 3

Days 6, 7, 8, and 9 (2 to 8 weeks later)

Repeat Day 2 and Day 3 sequence using a different ozone level

APPENDIX B. Symptomatology Questionnaires

Height ______

Weight ______

(for office use only)

For Office Use

Card #030

Person # ______

1. WHAT IS YOUR FULL NAME? ______

1a. Address ______

2. WHAT IS YOUR SOCIAL SECURITY NUMBER? ______

3. WHAT SEX ARE YOU?
   1. Male
   2. Female

4. WHAT IS YOUR ETHNIC GROUP OR ANCESTRY?
   1. White
   2. Mexican-American
   3. Black
   4. Other

5. WHAT IS YOUR MARITAL STATUS?
   1. Married
   2. Separated
   3. Never married
   4. Widowed
   5. Divorced

6. HOW MANY YEARS OF FORMAL EDUCATION OR SCHOOLING HAVE YOU HAD? (For example, completion of high school ... 12) ______

7. WHAT IS YOUR BIRTHDATE? ___ mo ___ day ___ year

8. HOW TALL ARE YOU (without shoes)? ___ ft ___ inches

9. HOW MUCH DO YOU WEIGH? ___ pounds

For Office Use

10. HAVE YOU EVER HAD ANY OF THE FOLLOWING DISEASES? *(If uncertain, circle NO)*

a. Arthritis (57)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

b. Ulcer of the stomach or duodenum (58)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

c. Bowel trouble or colitis (59)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

d. Kidney trouble (60)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

e. Liver trouble (61)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

f. Any kind of heart trouble (62)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

g. High blood pressure (63)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

h. Diabetes (sugar in urine) (64)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

i. Stroke (65)
   1. YES, I still have it.
   2. YES, but I no longer have it.
   3. NO.

j. Hardening of the arteries (66)
   1. YES.
   2. NO.

k. A serious skin rash in infancy (eczema) (67)
   1. YES.
   2. NO.

11a. DO YOU USUALLY COUGH FIRST THING IN THE MORNING IN THE BAD WEATHER? (If you usually cough in the morning regardless of the weather, circle YES) (68)
   1. YES.
   2. NO.

11b. DO YOU USUALLY COUGH AT OTHER TIMES DURING THE DAY OR NIGHT IN THE BAD WEATHER? (If you usually cough regardless of the weather, circle YES) (69)
   1. YES.
   2. NO.

IF YES TO EITHER 11a or 11b, ANSWER c AND d

c. DO YOU COUGH ON MOST DAYS FOR AS MUCH AS 3 MONTHS OF THE YEAR? (70)
   1. YES.
   2. NO.

d. FOR HOW MANY YEARS HAVE YOU HAD THIS COUGH? (71)
   1. Less than 2 years.
   2. 2-5 years.
   3. More than 5 years.

12. HOW MUCH ARE YOU BOTHERED BY COUGH COMPARED TO MOST PEOPLE? (72)

1 2 3 4 5

much less than most — much more than most

(circle appropriate number)

For Office Use

13a. DO YOU USUALLY BRING UP PHLEGM, SPUTUM, OR MUCOUS FROM YOUR CHEST FIRST THING IN THE MORNING IN THE BAD WEATHER? (If you usually bring up phlegm from your chest in the morning regardless of the weather, circle YES) (73)
   1. Yes
   2. No

13b. DO YOU USUALLY BRING UP PHLEGM, SPUTUM, OR MUCOUS FROM YOUR CHEST AT OTHER TIMES DURING THE DAY OR NIGHT IN THE BAD WEATHER? (If you usually bring up phlegm from your chest, regardless of the weather, circle YES) (74)
   1. Yes
   2. No

IF YES TO EITHER 13a or 13b, ANSWER c AND d:

c. DO YOU BRING UP PHLEGM, SPUTUM, OR MUCOUS FROM YOUR CHEST ON MOST DAYS FOR AS MUCH AS 3 MONTHS OF THE YEAR? (75)
   1. Yes
   2. No

d. FOR HOW MANY YEARS HAVE YOU RAISED PHLEGM, SPUTUM, OR MUCOUS FROM YOUR CHEST? (76)
   1. Less than 2 years.
   2. 2-5 years.
   3. More than 5 years.

14. HOW OFTEN DO YOU BRING UP PHLEGM FROM YOUR CHEST? (77)

1 2 3 4 5

never — very often

(circle appropriate number)

Card #040 (1-3)

ID DUP (4-10)

15a. DOES YOUR CHEST EVER SOUND WHEEZY OR WHISTLING? (11)
   1. YES.
   2. NO.

IF YES TO 15a:

b. DO YOU GET THIS WITH COLDS? (12)
   1. YES.
   2. NO.

c. DO YOU GET THIS EVEN WHEN YOU DON'T HAVE A COLD? (13)
   1. YES.
   2. NO.

d. DO YOU GET THIS ON MOST DAYS? (14)
   1. YES.
   2. NO.

16a. HAVE YOU EVER HAD ATTACKS OF SHORTNESS OF BREATH WITH WHEEZING? (15)
   1. YES.
   2. NO.

IF YES TO 16a:

b. HOW OFTEN ARE YOU BOTHERED BY SUCH ATTACKS? (16)

1 2 3 4 5

rarely — very often

(circle appropriate number)

For Office Use

17. ARE YOU MORE SHORT OF BREATH THAN MOST PEOPLE YOUR AGE? (17)
   1. YES.
   2. NO.

18. ARE YOU TROUBLED BY SHORTNESS OF BREATH WHEN HURRYING ON LEVEL GROUND OR WALKING UP A SLIGHT HILL? (18)
   1. YES.
   2. NO.

19. DO YOU GET SHORT OF BREATH WALKING WITH OTHER PEOPLE OF YOUR OWN AGE ON LEVEL GROUND? (19)
   1. YES.
   2. NO.

20. DO YOU HAVE TO STOP FOR BREATH WHILE WALKING AT YOUR OWN PACE ON LEVEL GROUND? (20)
   1. YES.
   2. NO.

21a. HAVE YOU EVER HAD ANY KIND OF CHEST TROUBLE? (21)
   1. YES.
   2. NO.

IF YES TO 21a:

b. WHAT SORT OF TROUBLE? ______ (22-57)

c. HAVE YOU HAD THIS DURING THE PAST YEAR? (58)
   1. YES.
   2. NO.

22. DID YOU HAVE ANY RESPIRATORY TROUBLE BEFORE AGE 16? (59)
   1. YES.
   2. NO.

23. DURING THE PAST THREE YEARS, HOW MUCH TROUBLE HAVE YOU HAD WITH ILLNESSES SUCH AS CHEST COLDS, BRONCHITIS, OR PNEUMONIA? (Does not refer to head colds) (60)

1 2 3 4 5

none — a great deal

(circle appropriate number)

24. DURING THE PAST THREE YEARS, HOW OFTEN WERE YOU UNABLE TO DO YOUR USUAL ACTIVITIES BECAUSE OF ILLNESSES SUCH AS CHEST COLDS, BRONCHITIS, OR PNEUMONIA? (Does not refer to head colds) (61)
   1. Never.
   2. During one such illness.
   3. During 2-5 illnesses.
   4. During 6 illnesses or more.

25. DURING THE PAST YEAR, FOR HOW MANY DAYS HAVE YOU BEEN UNABLE TO DO YOUR USUAL ACTIVITIES BECAUSE OF SUCH ILLNESSES? ______ days. (62-63)

26. *BEFORE* THREE YEARS AGO, HOW MUCH TROUBLE DID YOU HAVE WITH ILLNESSES SUCH AS CHEST COLDS, BRONCHITIS, OR PNEUMONIA? (Includes such illnesses during childhood) (64)

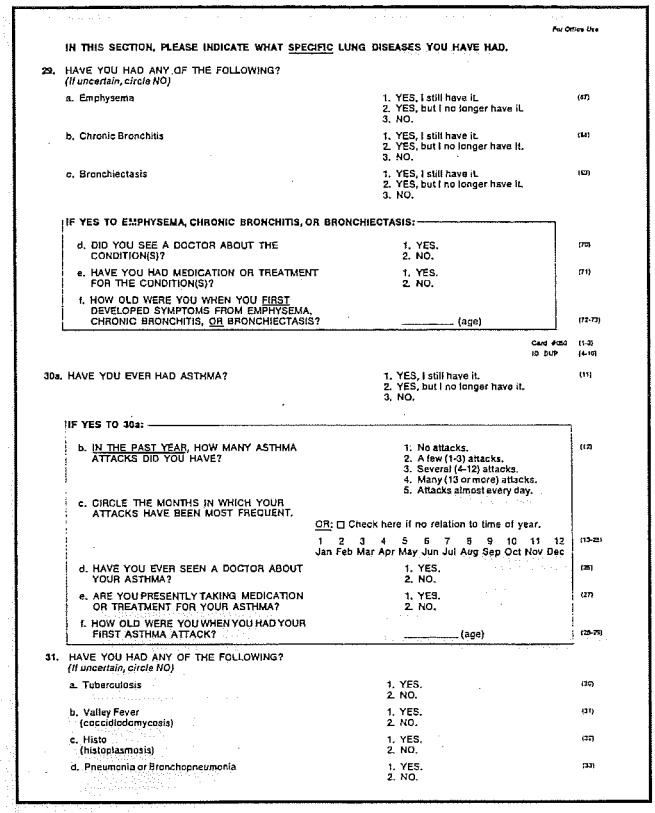
1 2 3 4 5

none — a great deal

(circle appropriate number)

27. DO YOU THINK YOU HAVE EVER HAD ANY OF THESE CHEST DISORDERS—ASTHMA, ANY KIND OF BRONCHIAL TROUBLE, OR EMPHYSEMA? (65)
   1. YES.
   2. NO.

28. HAS A DOCTOR EVER TOLD YOU THAT YOU HAD ASTHMA, SOME KIND OF BRONCHIAL TROUBLE, OR EMPHYSEMA? (66)
   1. YES.
   2. NO.



For Office Use

IN THIS SECTION, PLEASE INDICATE WHAT SPECIFIC LUNG DISEASES YOU HAVE HAD.

29. HAVE YOU HAD ANY OF THE FOLLOWING? *(If uncertain, circle NO)*

a. Emphysema — 1. YES, I still have it. 2. YES, but I no longer have it. 3. NO. (67)

b. Chronic Bronchitis — 1. YES, I still have it. 2. YES, but I no longer have it. 3. NO. (68)

c. Bronchiectasis — 1. YES, I still have it. 2. YES, but I no longer have it. 3. NO. (69)

IF YES TO EMPHYSEMA, CHRONIC BRONCHITIS, OR BRONCHIECTASIS:

d. DID YOU SEE A DOCTOR ABOUT THE CONDITION(S)? — 1. YES. 2. NO. (70)

e. HAVE YOU HAD MEDICATION OR TREATMENT FOR THE CONDITION(S)? — 1. YES. 2. NO. (71)

f. HOW OLD WERE YOU WHEN YOU FIRST DEVELOPED SYMPTOMS FROM EMPHYSEMA, CHRONIC BRONCHITIS, OR BRONCHIECTASIS? _____ (age) (72-73)

Card #050 (1-3) ID DUP (4-10)

30a. HAVE YOU EVER HAD ASTHMA? — 1. YES, I still have it. 2. YES, but I no longer have it. 3. NO. (11)

IF YES TO 30a:

b. IN THE PAST YEAR, HOW MANY ASTHMA ATTACKS DID YOU HAVE? — 1. No attacks. 2. A few (1-3) attacks. 3. Several (4-12) attacks. 4. Many (13 or more) attacks. 5. Attacks almost every day. (12)

c. CIRCLE THE MONTHS IN WHICH YOUR ATTACKS HAVE BEEN MOST FREQUENT.
OR: ☐ Check here if no relation to time of year.
1 2 3 4 5 6 7 8 9 10 11 12
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec (13-23)

d. HAVE YOU EVER SEEN A DOCTOR ABOUT YOUR ASTHMA? — 1. YES. 2. NO. (25)

e. ARE YOU PRESENTLY TAKING MEDICATION OR TREATMENT FOR YOUR ASTHMA? — 1. YES. 2. NO. (27)

f. HOW OLD WERE YOU WHEN YOU HAD YOUR FIRST ASTHMA ATTACK? _____ (age) (28-29)

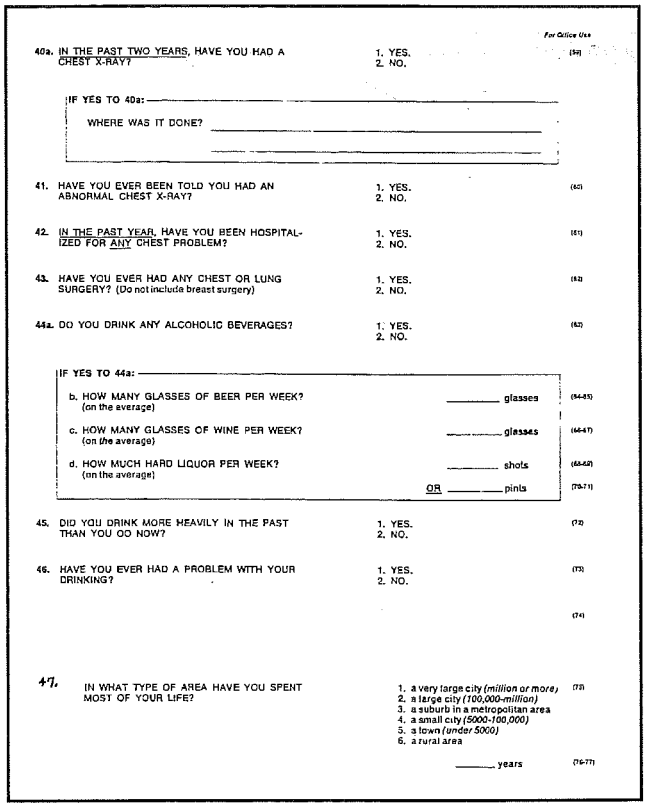
31. HAVE YOU HAD ANY OF THE FOLLOWING? *(If uncertain, circle NO)*

a. Tuberculosis — 1. YES. 2. NO. (30)

b. Valley Fever (coccidiodomycosis) — 1. YES. 2. NO. (31)

c. Histo (histoplasmosis) — 1. YES. 2. NO. (32)

d. Pneumonia or Bronchopneumonia — 1. YES. 2. NO. (33)



For Office Use

40a. IN THE PAST TWO YEARS, HAVE YOU HAD A CHEST X-RAY? — 1. YES. 2. NO. (59)

IF YES TO 40a:

WHERE WAS IT DONE? _____

41. HAVE YOU EVER BEEN TOLD YOU HAD AN ABNORMAL CHEST X-RAY? — 1. YES. 2. NO. (60)

42. IN THE PAST YEAR, HAVE YOU BEEN HOSPITALIZED FOR ANY CHEST PROBLEM? — 1. YES. 2. NO. (61)

43. HAVE YOU EVER HAD ANY CHEST OR LUNG SURGERY? (Do not include breast surgery) — 1. YES. 2. NO. (62)

44a. DO YOU DRINK ANY ALCOHOLIC BEVERAGES? — 1. YES. 2. NO. (63)

IF YES TO 44a:

b. HOW MANY GLASSES OF BEER PER WEEK? (on the average) _____ glasses (64-65)

c. HOW MANY GLASSES OF WINE PER WEEK? (on the average) _____ glasses (66-67)

d. HOW MUCH HARD LIQUOR PER WEEK? (on the average) _____ shots (68-69) OR _____ pints (70-71)

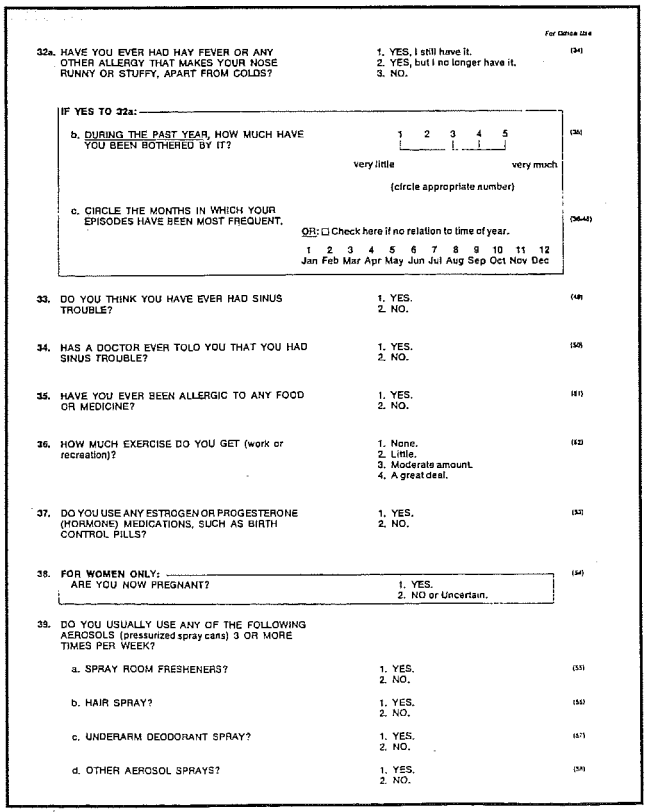
45. DID YOU DRINK MORE HEAVILY IN THE PAST THAN YOU DO NOW? — 1. YES. 2. NO. (72)

46. HAVE YOU EVER HAD A PROBLEM WITH YOUR DRINKING? — 1. YES. 2. NO. (73)

(74)

47. IN WHAT TYPE OF AREA HAVE YOU SPENT MOST OF YOUR LIFE? — 1. a very large city *(million or more)* 2. a large city *(100,000-million)* 3. a suburb in a metropolitan area 4. a small city *(5000-100,000)* 5. a town *(under 5000)* 6. a rural area (75)

_____ years (76-77)



For Office Use

32a. HAVE YOU EVER HAD HAY FEVER OR ANY OTHER ALLERGY THAT MAKES YOUR NOSE RUNNY OR STUFFY, APART FROM COLDS? — 1. YES, I still have it. 2. YES, but I no longer have it. 3. NO. (34)

IF YES TO 32a:

b. DURING THE PAST YEAR, HOW MUCH HAVE YOU BEEN BOTHERED BY IT? 1 2 3 4 5 (35)
very little — very much
(circle appropriate number)

c. CIRCLE THE MONTHS IN WHICH YOUR EPISODES HAVE BEEN MOST FREQUENT. (36-46)
OR: ☐ Check here if no relation to time of year.
1 2 3 4 5 6 7 8 9 10 11 12
Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

33. DO YOU THINK YOU HAVE EVER HAD SINUS TROUBLE? — 1. YES. 2. NO. (48)

34. HAS A DOCTOR EVER TOLD YOU THAT YOU HAD SINUS TROUBLE? — 1. YES. 2. NO. (50)

35. HAVE YOU EVER BEEN ALLERGIC TO ANY FOOD OR MEDICINE? — 1. YES. 2. NO. (51)

36. HOW MUCH EXERCISE DO YOU GET (work or recreation)? — 1. None. 2. Little. 3. Moderate amount. 4. A great deal. (52)

37. DO YOU USE ANY ESTROGEN OR PROGESTERONE (HORMONE) MEDICATIONS, SUCH AS BIRTH CONTROL PILLS? — 1. YES. 2. NO. (53)

38. FOR WOMEN ONLY: ARE YOU NOW PREGNANT? — 1. YES. 2. NO or Uncertain. (54)

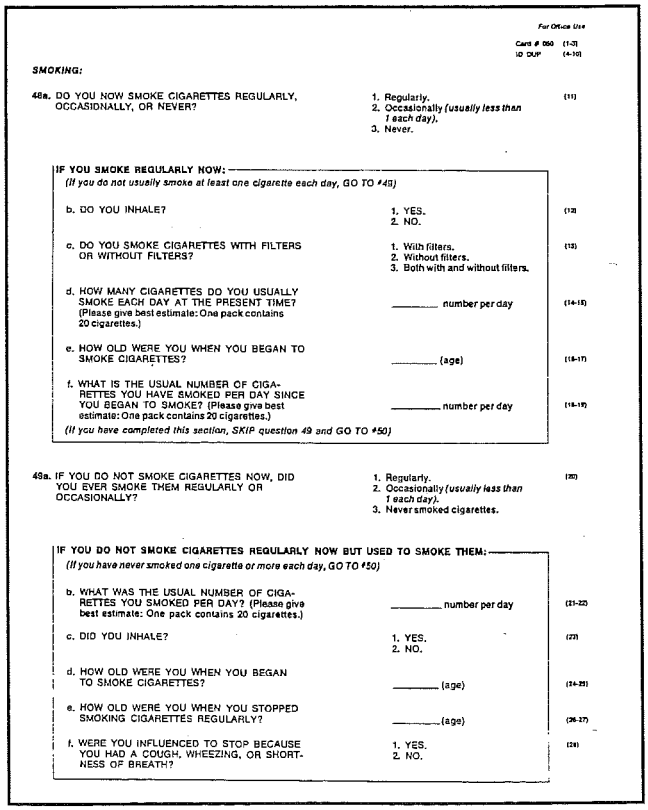
39. DO YOU USUALLY USE ANY OF THE FOLLOWING AEROSOLS (pressurized spray cans) 3 OR MORE TIMES PER WEEK?

a. SPRAY ROOM FRESHENERS? — 1. YES. 2. NO. (55)

b. HAIR SPRAY? — 1. YES. 2. NO. (56)

c. UNDERARM DEODORANT SPRAY? — 1. YES. 2. NO. (57)

d. OTHER AEROSOL SPRAYS? — 1. YES. 2. NO. (58)



For Office Use

Card # 050 (1-3) ID DUP (4-10)

SMOKING:

48a. DO YOU NOW SMOKE CIGARETTES REGULARLY, OCCASIONALLY, OR NEVER? — 1. Regularly. 2. Occasionally *(usually less than 1 each day)*. 3. Never. (11)

IF YOU SMOKE REGULARLY NOW:
(If you do not usually smoke at least one cigarette each day, GO TO #49)

b. DO YOU INHALE? — 1. YES. 2. NO. (12)

c. DO YOU SMOKE CIGARETTES WITH FILTERS OR WITHOUT FILTERS? — 1. With filters. 2. Without filters. 3. Both with and without filters. (13)

d. HOW MANY CIGARETTES DO YOU USUALLY SMOKE EACH DAY AT THE PRESENT TIME? (Please give best estimate: One pack contains 20 cigarettes.) _____ number per day (14-15)

e. HOW OLD WERE YOU WHEN YOU BEGAN TO SMOKE CIGARETTES? _____ (age) (16-17)

f. WHAT IS THE USUAL NUMBER OF CIGARETTES YOU HAVE SMOKED PER DAY SINCE YOU BEGAN TO SMOKE? (Please give best estimate: One pack contains 20 cigarettes.) _____ number per day (18-19)

(If you have completed this section, SKIP question 49 and GO TO #50)

49a. IF YOU DO NOT SMOKE CIGARETTES NOW, DID YOU EVER SMOKE THEM REGULARLY OR OCCASIONALLY? — 1. Regularly. 2. Occasionally *(usually less than 1 each day)*. 3. Never smoked cigarettes. (20)

IF YOU DO NOT SMOKE CIGARETTES REGULARLY NOW BUT USED TO SMOKE THEM:
(If you have never smoked one cigarette or more each day, GO TO #50)

b. WHAT WAS THE USUAL NUMBER OF CIGARETTES YOU SMOKED PER DAY? (Please give best estimate: One pack contains 20 cigarettes.) _____ number per day (21-22)

c. DID YOU INHALE? — 1. YES. 2. NO. (23)

d. HOW OLD WERE YOU WHEN YOU BEGAN TO SMOKE CIGARETTES? _____ (age) (24-25)

e. HOW OLD WERE YOU WHEN YOU STOPPED SMOKING CIGARETTES REGULARLY? _____ (age) (26-27)

f. WERE YOU INFLUENCED TO STOP BECAUSE YOU HAD A COUGH, WHEEZING, OR SHORTNESS OF BREATH? — 1. YES. 2. NO. (28)

For Office Use

50a. DO YOU NOW SMOKE PIPES OR CIGARS REGULARLY, OCCASIONALLY, OR NEVER?
1. Regularly.
2. Occasionally *(usually less than 1 each day).*
3. Never. (25)

IF YOU SMOKE PIPES OR CIGARS REGULARLY NOW:
(If you do not usually smoke at least one cigar or pipeful each day, GO TO #51)

b. HOW MANY PIPEFULS OR CIGARS DO YOU USUALLY SMOKE EACH DAY? _____ number each day (30-31)

c. HOW OLD WERE YOU WHEN YOU FIRST SMOKED? _____ (age) (32-33)

d. DO YOU USUALLY INHALE WHEN YOU SMOKE EITHER PIPES OR CIGARS?
1. YES.
2. NO. (34)

(If you completed this section, SKIP question 51 and GO TO #52)

51a. IF YOU DO NOT SMOKE CIGARS OR PIPES NOW, DID YOU EVER SMOKE THEM REGULARLY OR OCCASIONALLY?
1. Regularly.
2. Occasionally *(usually less than 1 each day).*
3. Never. (35)

IF YOU DO NOT SMOKE PIPES OR CIGARS REGULARLY NOW BUT USED TO SMOKE THEM:
(If you have never smoked at least one cigar or pipeful each day, GO TO #52)

b. HOW MANY PIPEFULS OR CIGARS DID YOU USUALLY SMOKE EACH DAY? _____ number each day (36-37)

c. HOW OLD WERE YOU WHEN YOU FIRST SMOKED PIPES OR CIGARS? _____ (age) (38-39)

d. HOW OLD WERE YOU WHEN YOU STOPPED SMOKING PIPES OR CIGARS? _____ (age) (40-41)

e. DID YOU USUALLY INHALE WHEN YOU SMOKED EITHER PIPES OR CIGARS?
1. YES.
2. NO. (42)

(55) Does anyone in your immediate family (parents, brothers, sisters, or children) have asthma now or a history of asthma in the past.

(1) Yes
(2) No

(56) In the past six weeks have you had any symptoms of a cold, "flu", or any other respiratory infection.

(1) Yes
(2) No

(57) What is your occupation?

(1) Student
(2) Other - Please describe briefly

(58) Are you routinely exposed to any fumes or dusts that make you cough, wheeze, or short of breath?

(1) Yes
(2) No

(59) Are you presently taking any medications?

(1) Yes
(2) No

(60) If answer Yes to number (59), list medications and dosage.

(61) If you have asthma and are not currently taking medication, what medication have you taken in the past 5 years?

(62) If you have asthma, when did your last attack occur?

(63) Do you take any Vitamin C or Vitamin E (including what might be in a multivitamin)?

(1) Yes
(2) No

(64) If the answer to number (63) is "Yes" please write the doses of Vitamin C and Vitamin E you take and how often you take them. If you take a multivitamin, give the brand name and the dose of Vitamin C and Vitamin E in it. (If you do not know them off-hand please find them out and get back to us.)

For Office Use

Card # 078 (1-3)
ID DUP (4-10)

52. MAY WE INFORM YOUR DOCTOR OF THE RESULTS OF THIS STUDY?
1. YES.
2. NO. (11)

53. MAY WE OBTAIN INFORMATION FROM YOUR DOCTOR REGARDING YOUR HEALTH?
1. YES.
2. NO. (12)

54. WHAT IS YOUR DOCTOR'S NAME AND LOCATION? _____ (13-41)

_____ (42-73)

FOR OFFICE USE:

55. [ ] PMO CODE (74-77)

56. R-FORM (78)
1 2 3 4

57. ADMIN. (79)
I R

58. NI ____ (80)

3/15/72

(65) Are there any gas burning appliances in use in your home on a regular basis or that have been in use in the past few weeks? (Stoves, unvented heaters, etc.)

(1) Yes
(2) No

(66) Has there been a kerosene heater in use in your home in the past few weeks?

(1) Yes, frequently used
(2) Yes, occasionally used

(3) If yes, how long ago was it used (Days or weeks)

(4) No

(67) Are you getting desensitization injection?
(1) Yes
(2) No

Have you ever had shots?
(1) Yes
(2) No
(3) If yes, when?

(68) Have you ever had formal skin testing?
(1) Yes
(2) No
(3) If yes, when?

(69) Do you now smoke marijuana regularly, occasionally, or never?
(1) Regularly
(2) Occasionally (usually less than once each week)
(3) Never

(70) If you do not smoke marijuana now, did you ever smoke it regularly or occasionally?
(1) Regularly
(2) Occasionally (usually less than once each week)
(3) Never smoked marijuana

(71) Do you presently reside with anyone who smokes cigarettes regularly?
(1) Yes
(2) No

APPENDIX C. Consent Form

THE UNIVERSITY OF ROCHESTER
MEDICAL CENTER
SCHOOL OF MEDICINE AND DENTISTRY · SCHOOL OF NURSING
STRONG MEMORIAL HOSPITAL

RSRB # 3320

601 ELMWOOD AVENUE
ROCHESTER, NEW YORK 14642
AREA CODE 716

CONSENT FORM

Title: Influence of Sulfate Aerosols on Exposure-Response Characteristics of Ozone on Human Lung Function

Investigators: Mark J. Utell, M.D.
Professor of Medicine

Mark W. Frampton, M.D.
Assistant Professor of Medicine

Paul E. Morrow, Ph.D.
Emeritus Professor of Toxicology

UNIVERSITY OF ROCHESTER APPROVED 10/13/88 10/5/89 9/18/90 RSRB

You are invited to participate in a study to evaluate the effects of various common forms of air pollution on the lungs.

It is important that you read and understand the following general statements that apply to all participants in this study: a) Participation is entirely voluntary, b) Personal therapeutic benefit may not result from participation in this study, although knowledge gained may be beneficial to others, and c) Withdrawal from this study may be accomplished at any time without prejudice or jeopardy to your health care.

The nature of the study, the risks, inconveniences, discomforts, and other important information about the study are discussed below. Please feel free to ask any questions that may arise as you review this material or at any point in the study, should you decide to participate.

This study is designed to evaluate the potential interaction of various common forms of air pollutants on the respiratory status of healthy volunteers and asthmatics. In this particular study, we wish to evaluate the effects on lung function of ozone following exposure to either an aerosol mist of sodium chloride or sulfuric acid. The concentration of air pollutant particle or gas that will be used for the exposure is low-level and controlled and has been used many times in the past in human investigation without difficulty. The investigations are needed to understand the effects of these widely present air pollutants on the lung in order to define their potential health consequences, help establish guidelines for acceptable limits of exposure, and try to determine the mechanism of their actions.

You will receive a physical examination and review of your medical history upon admission to the study (Day 1). Breathing tests will be performed; these tests will require a series of breathing maneuvers and are entirely non-invasive. Response to a carbachol aerosol will be assessed by these breathing maneuvers. Carbachol is a standard drug used to test for asthma. For the asthmatic volunteers, we will determine if you are allergic by a blood test (IgE levels) and routine skin tests to common inhalants.

Title: Influence of Sulfate Aerosols on Exposure-Response Characteristics of Ozone on Human Lung Function

Three tablespoons of blood will be obtained in the usual fashion from the vein. The risks from blood drawing are minimal and include possible bleeding into the skin or fainting. Skin tests to 10 inhalants are performed by first puncturing the skin with a needle with the solution to be tested (puncture test). If this test is negative, a second test is performed by injecting a small amount of the material into the skin (an intradermal test). A positive reaction to either the puncture or intradermal test is indicated by hives at the test site. If the puncture test in positive, the intradermal test will not be performed. No systemic reaction is expected from the skin testing but fainting rarely occurs.

On a second day (Day 2), you will undergo a 3-hour exposure to either an aerosol of sodium chloride or sulfuric acid. The sulfates inhaled are at low concentrations (0.1 mg/m^3) and well below currently recommended levels in industrial plants. The exposure will be done in an environmental chamber at Strong Memorial Hospital. Exposures to the low levels of the pollutants have been performed safely in normal and asthmatic volunteers many times in the past. They are pollutants to which you are often exposed in city air. There is always the possibility that the exposure can be irritating to the airways and cause coughing or shortness of breath. These possibilities are unlikely. The exposures will be done in a room-sized environmental chamber during which time you will be free to be active within the confines of the room. We will have you perform light exercise on a stationary bicycle for 10 minutes on several occasions during the exposure. Your heart rate and breathing will be monitored during these exercise periods. Breathing tests will be performed before and after the exposure. Following the exposure, you may return to your usual activities but should attempt to avoid exposure to irritating fumes or other pollutants.

On the next morning (Day 3), you will undergo a 3-hour exposure to ozone. The ozone concentration ranges from below the ambient standard to slightly above the standard; these low concentrations have not produced any changes in lung function during exposures of up to 3 hours. We are interested in determining whether the aerosol exposure on the previous day increases the pulmonary response to ozone. The protocol in the environmental chamber will be identical to the previous day except that breathing tests will also be performed during exposure and 2 and 4 hours after the exposure.

After at least a 2 week period, you will return to be re-interviewed. If there have been no intervening health problem, you will again undergo a 3-hour exposure to an aerosol of sodium chloride or sulfuric acid (Day 4). (If you were exposed to sodium chloride the first time, you will be exposed to a sulfuric acid aerosol the second time. If you were exposed to the sulfuric acid aerosol the first time, you will be exposed to sodium chloride the second time). The exposures will be done in the same manner as before, including the exercise periods and breathing tests. On the following morning (Day 5), you will be exposed to ozone at the same concentration as on the previous ozone exposure day (Day 3). This will complete the first phase of the study.

After at least a 3 week interval, the study will be repeated using a second level of ozone. The sequence of the ozone exposure levels will be randomized. The entire 4 day exposure sequence (Day 6, 7, 8, and 9) will be repeated with the second ozone exposure concentration.

9/18/90 10/13/88 10/5/89

Title: Influence of Sulfate Aerosols on Exposure-Response Characteristics of Ozone on Human Lung Function

You should understand that although this form enumerates several potential though unlikely risks of this study, it is not comprehensive in that it does not include every possible complication both foreseen and unforeseen. You should also understand that standard methods of medical care will be administered if you have any problem. Strong Memorial Hospital will provide medical care for essential acute, emergency medical treatment for physical injuries incurred that the University determines to be a result of your direct participation in the research. Compensation for injury is not available from Strong Memorial Hospital.

An honorarium will be paid to you after your completion of the study.

All medical records and personal study data are available to each study participant or to any physician they choose. No information identified by name will be released from the study without specific consent from the participant.

By signing this consent form you agree that you have had sufficient opportunity to discuss the research study, to ask questions about risks and benefits of the study, and that you feel competent to decide to participate in it or not. Furthermore, you agree that you understand your consent may be withdrawn at any time, for any reason, and you may gain release from the study without prejudice and without jeopardy to your future medical care.

Volunteer's Signature _____ Date _____

I have fully explained possible complications to the volunteer and the nature of the benefits and risks involved.

Investigator's Signature _____ Date _____

Auditor Witness _____ Date _____

(This witness's signature attests that the above information was provided to the volunteer).

10/13/88 10/5/89 9-18-90

APPENDIX D. Analysis of Variance Tables for Healthy Subjects and Subjects with Asthma

Table D.1. Analysis of Forced Vital Capacity Values After Aerosol Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	19.93	9.96	1.43	0.25
Aerosol	1	8.55	8.55	1.23	0.27
Interaction	2	14.82	7.41	1.06	0.35
Aerosol at 0.08 ppm	1	1.63	1.63	0.23	0.63
Aerosol at 0.12 ppm	1	5.15	5.15	0.74	0.39
Aerosol at 0.18 ppm	1	16.58	16.58	2.38	0.13
Linear × linear	1	14.31	14.31	2.05	0.16
Quad × quad	1	0.51	0.51	0.07	0.79
Subject	29	362.71	12.51	1.79	0.021
Error	85	593.38	6.99		
Corrected total	119	980.93			
Coefficient of determination	39.5%				

Table D.2. Analysis of Forced Vital Capacity Values After Exercise in Ozone for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	19.36	9.68	1.29	0.28
Aerosol	1	3.08	3.08	0.41	0.52
Interaction	2	54.45	27.23	3.62	0.031
Aerosol at 0.08 ppm	1	23.85	23.85	3.17	0.079
Aerosol at 0.12 ppm	1	9.46	9.46	1.26	0.2753
Aerosol at 0.18 ppm	1	24.28	24.28	3.22	0.076
Linear × linear	1	48.07	48.07	6.39	0.013
Quad × quad	1	6.38	6.38	0.85	0.36
Subject	29	464.58	16.02	2.13	0.0039
Error	85	639.23	7.52		
Corrected total	119	1,199.28			
Coefficient of determination	46.7%				

Table D.3. Analysis of Forced Vital Capacity Values After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	17.18	8.59	0.63	0.53
Aerosol	1	0.15	0.15	0.01	0.92
Interaction	2	30.08	15.04	1.11	0.33
Aerosol at 0.08 ppm	1	20.81	20.81	1.53	0.22
Aerosol at 0.12 ppm	1	0.94	0.94	0.07	0.79
Aerosol at 0.18 ppm	1	8.49	8.49	0.63	0.43
Linear × linear	1	27.93	27.93	2.06	0.15
Quad × quad	1	2.15	2.15	0.16	0.69
Subject	29	773.02	26.66	1.97	0.0088
Error	85	1,152.21	13.56		
Corrected total	119	1,969.77			
Coefficient of determination	41.5%				

Table D.4. Analysis of Forced Vital Capacity Values Two Hours After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	27.81	13.90	1.22	0.30
Aerosol	1	7.18	7.18	0.63	0.43
Interaction	2	30.54	15.27	1.34	0.27
Aerosol at 0.08 ppm	1	20.93	20.93	1.84	0.18
Aerosol at 0.12 ppm	1	8.58	8.58	0.76	0.39
Aerosol at 0.18 ppm	1	8.20	8.20	0.72	0.40
Linear × linear	1	27.67	27.67	2.44	0.12
Quad × quad	1	2.87	2.87	0.25	0.62
Subject	29	555.66	19.16	1.69	0.033
Error	85	965.61	11.36		
Corrected total	119	1,606.92			
Coefficient of determination	39.9%				

Table D.5. Analysis of Forced Vital Capacity Values Four Hours After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	22.87	11.44	1.06	0.35
Aerosol	1	8.67	8.67	0.81	0.37
Interaction	2	26.75	13.37	1.24	0.29
Aerosol at 0.08 ppm	1	34.02	34.02	3.16	0.080
Aerosol at 0.12 ppm	1	0.15	0.15	0.01	0.91
Aerosol at 0.18 ppm	1	1.25	1.25	0.12	0.73
Linear × linear	1	24.16	24.16	2.24	0.14
Quad × quad	1	2.59	2.59	0.24	0.63
Subject	29	480.24	16.56	1.54	0.066
Error	85	915.48	10.78		
Corrected total	119	1,453.18			
Coefficient of determination	37.0%				

Table D.6. Analysis of Forced Vital Capacity Linear Trend Values for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	87.27	43.63	0.42	0.66
Aerosol	1	34.35	34.35	0.33	0.57
Interaction	2	122.37	61.19	0.58	0.56
Aerosol at 0.08 ppm	1	8.17	8.17	0.08	0.78
Aerosol at 0.12 ppm	1	17.33	17.33	0.16	0.69
Aerosol at 0.18 ppm	1	131.22	131.22	1.25	0.27
Linear × linear	1	36.94	36.94	0.35	0.55
Quad × quad	1	85.43	85.43	0.81	0.37
Subject	29	7,003.27	241.49	2.30	0.0017
Error	85	8,936.14	105.13		
Corrected total	119	16,147.93			
Coefficient of determination	44.7%				

Table D.7. Analysis of Forced Vital Capacity Quadratic Trend Values for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	9.36	4.68	0.28	0.76
Aerosol	1	2.65	2.65	0.16	0.69
Interaction	2	2.18	1.09	0.06	0.94
Aerosol at 0.08 ppm	1	2.50	2.50	0.15	0.70
Aerosol at 0.12 ppm	1	2.26	2.26	0.13	0.72
Aerosol at 0.18 ppm	1	0.069	0.069	0.00	0.95
Linear × linear	1	1.70	1.70	0.10	0.75
Quad × quad	1	0.48	0.48	0.03	0.87
Subject	29	557.36	19.22	1.14	0.32
Error	85	1435.05	16.88		
Corrected total	119	2,003.49			
Coefficient of determination	28.4%				

Table D.8. Analysis of Forced Vital Capacity Cubic Trend Values for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	415.59	207.80	2.01	0.14
Aerosol	1	32.22	32.22	0.31	0.58
Interaction	2	189.15	94.57	0.92	0.40
Aerosol at 0.08 ppm	1	0.82	0.82	0.01	0.93
Aerosol at 0.12 ppm	1	207.18	207.18	2.01	0.16
Aerosol at 0.18 ppm	1	13.36	13.36	0.13	0.72
Linear × linear	1	3.78	3.78	0.04	0.85
Quad × quad	1	185.37	185.37	1.80	0.18
Subject	29	5,131.12	176.94	1.72	0.030
Error	85	8,767.82	103.15		
Corrected total	119	14,306.53			
Coefficient of determination	38.7%				

Table D.9. Analysis of Values for Forced Expiratory Volume in One Second After Aerosol Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	2.75	1.37	0.31	0.73
Aerosol	1	0.29	0.29	0.07	0.80
Interaction	2	9.19	9.59	1.09	0.36
Aerosol at 0.08 ppm	1	4.07	4.07	0.92	0.34
Aerosol at 0.12 ppm	1	0.58	0.58	0.13	0.72
Aerosol at 0.18 ppm	1	4.83	4.83	1.09	0.30
Linear × linear	1	8.89	8.89	2.01	0.16
Quad × quad	1	0.30	0.30	0.07	0.80
Subject	29	258.00	8.90	2.01	0.007
Error	85	375.71	4.42		
Corrected total	119	646.66			
Coefficient of determination	41.9%				

Table D.10. Analysis of Values for Forced Expiratory Volume in One Second After Exercise in Ozone for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	2.96	1.48	0.20	0.82
Aerosol	1	19.11	19.11	2.63	0.11
Interaction	2	0.46	0.23	0.03	0.97
Aerosol at 0.08 ppm	1	7.86	7.86	1.08	0.30
Aerosol at 0.12 ppm	1	3.88	3.88	0.53	0.47
Aerosol at 0.18 ppm	1	7.84	7.84	1.08	0.30
Linear × linear	1	0.0000084	0.0000084	0.00	1.00
Quad × quad	1	0.46	0.46	0.06	0.80
Subject	29	356.95	12.31	1.70	0.032
Error	85	617.05	7.26		
Corrected total	119	995.79			
Coefficient of determination	38.0%				

Table D.11. Analysis of Values for Forced Expiratory Volume in One Second After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	10.40	5.20	0.64	0.53
Aerosol	1	0.75	0.75	0.09	0.76
Interaction	2	9.09	4.55	0.56	0.57
Aerosol at 0.08 ppm	1	3.26	3.26	0.40	0.53
Aerosol at 0.12 ppm	1	3.85	3.85	0.47	0.49
Aerosol at 0.18 ppm	1	2.73	2.73	0.31	0.56
Linear × linear	1	0.011	0.011	0.00	0.97
Quad × quad	1	9.08	9.08	1.11	0.29
Subject	29	491.15	16.94	2.08	0.0051
Error	85	693.50	8.16		
Corrected total	119	1,200.95			
Coefficient of determination	42.2%				

Table D.12. Analysis of Values for Forced Expiratory Volume in One Second Two Hours After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	23.58	11.79	1.17	0.32
Aerosol	1	0.54	0.54	0.05	0.82
Interaction	2	5.27	2.63	0.26	0.77
Aerosol at 0.08 ppm	1	0.0067	0.0067	0.00	0.98
Aerosol at 0.12 ppm	1	0.78	0.78	0.08	0.78
Aerosol at 0.18 ppm	1	5.02	5.02	0.50	0.48
Linear × linear	1	2.70	2.70	0.27	0.61
Quad × quad	1	2.57	2.57	0.26	0.61
Subject	29	342.48	11.81	1.17	0.28
Error	85	856.04	10.07		
Corrected total	119	1,226.48			
Coefficient of determination	30.0%				

Table D.13. Analysis of Values for Forced Expiratory Volume in One Second Four Hours After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	0.95	0.47	0.05	0.95
Aerosol	1	2.49	2.49	0.27	0.60
Interaction	2	6.01	3.00	0.33	0.72
Aerosol at 0.08 ppm	1	0.37	0.37	0.04	0.84
Aerosol at 0.12 ppm	1	0.30	0.30	0.03	0.86
Aerosol at 0.18 ppm	1	7.82	7.82	0.86	0.36
Linear × linear	1	5.81	5.81	0.64	0.43
Quad × quad	1	0.20	0.20	0.02	0.88
Subject	29	328.11	11.31	1.24	0.22
Error	85	775.81	9.13		
Corrected total	119	1,121.45			
Coefficient of determination	30.8%				

Table D.14. Analysis of Linear Trend Values for Forced Expiratory Volume in One Second for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	1.21	0.61	0.01	0.99
Aerosol	1	68.17	68.17	0.85	0.36
Interaction	2	30.63	15.31	0.19	0.83
Aerosol at 0.08 ppm	1	69.80	69.80	0.87	0.35
Aerosol at 0.12 ppm	1	28.64	28.64	0.36	0.55
Aerosol at 0.18 ppm	1	0.35	0.35	0.00	0.95
Linear × linear	1	30.12	30.12	0.38	0.54
Quad × quad	1	0.51	0.51	0.01	0.94
Subject	29	5,096.09	175.73	2.19	0.0028
Error	85	6,805.38	80.06		
Corrected total	119	12,103.50			
Coefficient of determination	43.8%				

Table D.15. Analysis of Quadratic Trend Values for Forced Expiratory Volume in One Second for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	29.24	14.62	0.89	0.42
Aerosol	1	56.98	56.98	3.46	0.066
Interaction	2	48.55	24.27	1.48	0.23
Aerosol at 0.08 ppm	1	15.34	15.34	0.93	0.34
Aerosol at 0.12 ppm	1	0.11	0.11	0.01	0.93
Aerosol at 0.18 ppm	1	90.08	90.08	5.48	0.022
Linear × linear	1	15.54	15.54	0.94	0.33
Quad × quad	1	33.01	33.01	2.01	0.16
Subject	29	398.88	13.75	0.84	0.70
Error	85	1,398.49	16.45		
Corrected total	119	1,944.26			
Coefficient of determination	28.1%				

Table D.16. Analysis of Cubic Trend Values for Forced Expiratory Volume in One Second for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	66.78	33.39	0.48	0.62
Aerosol	1	10.11	10.11	0.15	0.70
Interaction	2	78.62	39.31	0.56	0.57
Aerosol at 0.08 ppm	1	82.36	82.36	1.18	0.28
Aerosol at 0.12 ppm	1	3.26	3.26	0.05	0.83
Aerosol at 0.18 ppm	1	3.10	3.10	0.04	0.83
Linear × linear	1	58.71	58.71	0.84	0.36
Quad × quad	1	19.90	19.90	0.29	0.59
Subject	29	1,970.43	67.95	0.97	0.51
Error	85	5,923.86	69.69		
Corrected total	119	8,079.55			
Coefficient of determination	26.6%				

Table D.17. Analysis of Specific Airway Conductance Values After Aerosol Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	5.08	2.54	0.05	0.95
Aerosol	1	23.12	23.12	0.44	0.51
Interaction	2	170.52	85.26	1.62	0.20
Aerosol at 0 08 ppm	1	176.15	176.15	3.34	0.071
Aerosol at 0 12 ppm	1	16.77	16.77	0.32	0.57
Aerosol at 0.18 ppm	1	0.72	0.72	0.01	0.91
Linear × linear	1	99.70	99.70	1.89	0.17
Quad × quad	1	70.82	70.82	1.34	0.25
Subject	29	1,181.45	40.74	0.77	0.78
Error	85	4,476.31	52.66		
Corrected total	119	5,859.16			
Coefficient of determination	23.6%				

Table D.18. Analysis of Specific Airway Conductance Values After Ozone Exposure for Healthy Subjects

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	68.99	34.50	0.67	0.51
Aerosol	1	16.51	16.51	0.32	0.57
Interaction	2	404.98	202.49	3.93	0.023
Aerosol at 0.08 ppm	1	4.27	4.27	0.08	0.77
Aerosol at 0.12 ppm	1	83.81	83.81	1.63	0.21
Aerosol at 0.18 ppm	1	333.41	333.41	6.47	0.012
Linear × linear	1	206.58	206.58	4.01	0.048
Quad × quad	1	198.40	198.40	3.85	0.053
Subject	29	1,488.14	51.32	1.00	0.48
Error	85	4,376.79	51.49		
Corrected total	119	6,388.81			
Coefficient of determination	31.5%				

Table D.19. Analysis of Forced Vital Capacity Values After Aerosol Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	67.57	33.78	0.97	0.38
Aerosol	1	98.31	98.31	2.82	0.097
Interaction	2	29.46	14.73	0.42	0.66
Aerosol at 0.08 ppm	1	3.26	3.26	0.09	0.76
Aerosol at 0.12 ppm	1	89.78	89.78	2.57	0.11
Aerosol at 0.18 ppm	1	34.72	34.72	1.00	0.32
Linear × linear	1	8.35	8.35	0.24	0.63
Quad × quad	1	21.10	21.10	0.61	0.44
Subject	29	3,954.82	136.37	3.91	0.0001
Error	85	2,963.90	34.87		
Corrected total	119	7,101.33			
Coefficient of determination	58.3%				

Table D.20. Analysis of Forced Vital Capacity Values After Exercise in Ozone for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	143.87	71.93	1.56	0.22
Aerosol	1	9.63	9.63	0.21	0.65
Interaction	2	143.80	71.90	1.56	0.22
Aerosol at 0.08 ppm	1	0.026	0.026	0.00	0.98
Aerosol at 0.12 ppm	1	30.71	30.71	0.67	0.42
Aerosol at 0.18 ppm	1	122.70222885	122.70	2.66	0.11
Linear × linear	1	63.14	63.14	1.37	0.25
Quad × quad	1	80.66	80.66	1.75	0.19
Subject	29	4,087.12	140.94	3.06	0.0001
Error	85	3,920.50	46.12		
Corrected total	119	8,187.65			
Coefficient of determination	52.1%				

Table D.21. Analysis of Forced Vital Capacity Values After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	9.28	4.64	0.15	0.86
Aerosol	1	45.42	45.42	1.47	0.23
Interaction	2	344.65		172.33	5.57
Aerosol at 0.08 ppm	1	257.69	257.69	8.33	0.0050
Aerosol at 0.12 ppm	1	31.87	31.87	1.03	0.31
Aerosol at 0.18 ppm	1	100.51		100.51	3.25
Linear × linear	1	340.036	340.036	10.99	0.0014
Quad × quad	1	4.62	4.62	0.15	0.70
Subject	29	5,367.33		185.08	5.98
Error	85	2,630.67	30.95		
Corrected total	119	8,391.99			
Coefficient of determination	68.7%				

Table D.22. Analysis of Forced Vital Capacity Values Two Hours After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	11.94	5.97	0.20	0.82
Aerosol	1	6.08	6.08	0.21	0.65
Interaction	2	77.68	38.84	1.32	0.27
Aerosol at 0.08 ppm	1	74.31	74.31	2.53	0.12
Aerosol at 0 12 ppm	1	4.49	4.49	0.15	0.70
Aerosol at 0 18 ppm	1	4.96	4.96	0.17	0.68
Linear × linear	1	58.84	58.84	2.00	0.16
Quad × quad	1	18.84	18.84	0.64	0.43
Subject	29	2,425.66	83.64	2.85	0.0001
Error	85	2,496.77	29.37		
Corrected total	119	5,032.20			
Coefficient of determination	50.4%				

Table D.23, Analysis of Forced Vital Capacity Values Four Hours After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	95.71	47.85	1.08	0.35
Aerosol	1	3.82	3.82	0.09	0.77
Interaction	2	324.26	162.13	3.65	0.030
Aerosol at 0.08 ppm	1	89.95	89.95	2.02	0.16
Aerosol at 0.12 ppm	1	5.65	5.65	0.13	0.72
Aerosol at 0.18 ppm	1	232.47	232.47	5.23	0.025
Linear × linear	1	305.82	305.82	6.88	0.010
Quad × quad	1	18.44	18.44	0.41	0.52
					0.0008
Subject	29	3,142.66	108.37	2.44	
Error	85	3,778.49	44.45		
Corrected total	119	7,261.86			
Coefficient of determination	48.0%				

Table D.24. Analysis of Forced Vital Capacity Linear Trend Values for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	65.15	32.58	0.06	0.94
Aerosol	1	0.68	0.68	0.00	0.97
Interaction	2	741.22	370.61	0.73	0.48
Aerosol at 0.08 ppm	1	421.86	421.86	0.84	0.36
Aerosol at 0.12 ppm	1	297.83	297.83	0.59	0.44
Aerosol at 0.18 ppm	1	22.22	22.22	0.04	0.83
Linear × linear	1	318.86	318.86	0.63	0.43
Quad × quad	1	422.36	422.36	0.84	0.36
Subject	29	32,098.83	1,106.86	2.19	0.0029
Error	85	42,938.72	505.16		
Corrected total	119	75,812.62			
Coefficient of determination	43.3%				

Table D.25. Analysis of Forced Vital Capacity Quadratic Trend Values for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	272.11	136.06	1.72	0.18
Aerosol	1	203.46	203.46	2.58	0.11
Interaction	2	239.66	119.83	1.52	0.22
Aerosol at 0.08 ppm	2	225.85	225.85	2.86	0.094
Aerosol at 0.12 ppm	1	19.30	19.30	0.24	0.62
Aerosol at 0.18 ppm	1	197.97	197.97	2.51	0.12
Linear × linear	1	0.46	0.46	0.01	0.94
Quad × quad	1	239.20	239.20	3.03	0.085
Subject	29	2,652.72	91.47	1.16	0.30
Error	85	6,708.45	78.92		
Corrected total	119	9,835.02			
Coefficient Of determination	31.8%				

Table D.26. Analysis of Forced Vital Capacity Cubic Trend Values for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	39.60	19.80	0.07	0.94
Aerosol	1	195.09	195.09	0.66	0.42
Interaction	2	1,969.88	984.94	3.35	0.040
Aerosol at 0.08 ppm	1	999.97	999.97	3.40	0.069
Aerosol at 0.12 ppm	1	405.31	405.31	1.38	0.24
Aerosol at 0.18 ppm	1	759.68	759.68	2.58	0.11
Linear × linear	1	1,751.41	1,751.41	5.95	0.017
Quad × quad	1	218.46	218.46	0.74	0.39
Subject	29	18,162.66	626.30	2.13	0.0040
Error	85	25,017.00	294.32		
Corrected total	119	45,466.89			

Table D.27. Analysis of Values for Forced Expiratory Volume in One Second After Aerosol Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	16.18	8.09	0.16	0.85
Aerosol	1	126.31	126.31	2.55	0.11
Interaction	2	15.79	7.90	0.16	0.85
Aerosol at 0.08 ppm	1	10.57	10.57	0.21	0.65
Aerosol at 0.12 ppm	1	62.71	62.71	1.27	0.26
Aerosol at 0.18 ppm	1	68.83	68.83	1.39	0.24
Linear × linear	1	12.72	12.72	0.26	0.61
Quad × quad	1	3.07	3.07	0.06	0.80
Subject	29	8,448.83	291.34	5.89	0.0001
Error	85	4,203.76	49.46		
Corrected total	119	12,820.65			
Coefficient Of determination	67%				

Table D.28. Analysis of Values for Forced Expiratory Volume in One Second After Exercise in Ozone for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	81.49	40.74	0.42	0.66
Aerosol	1	3.93	3.93	0.04	0.84
Interaction	2	67.87	33.94	0.35	0.70
Aerosol at 0.08 ppm	1	57.44	57.44	0.60	0.44
Aerosol at 0.12 ppm	1	14.22	14.22	0.15	0.70
Aerosol at 0.18 ppm	1	0.14	0.14	0.00	0.97
Linear × linear	1	31.61	31.61	0.33	0.57
Quad × quad	1	36.26	36.26	0.38	0.54
Subject	29	7,240.28	249.66	2.60	0.0004
Error	85	8,159.79	96.00		
Corrected total	119	15,488.44			
Coefficient of determination	47.3%				

Table D.29. Analysis of Values for Forced Expiratory Volume in One Second After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	82.53	41.26	0.67	0.52
Aerosol	1	42.91	42.91	0.69	0.41
Interaction	2	121.79	60.89	0.98	0.38
Aerosol at 0.08 ppm	1	135.95	135.95	2.20	0.14
Aerosol at 0.12 ppm	1	13.18	13.18	0.21	0.65
Aerosol at 0.18 ppm	1	15.56	15.56	0.25	0.62
Linear × linear	1	121.76	121.76	1.97	0.16
Quad × quad	1	0.034	0.034	0.00	0.98
Subject	29	13,932.31	480.92	7.77	0.0001
Error	85	5,257.30	61.85		
Corrected total	119	19,360.75			
Coefficient of determination	72.8%				

Table D.30. Analysis of Values for Forced Expiratory Volume in One Second Two Hours After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	150.94	75.47	1.46	0.24
Aerosol	1	70.59	70.59	1.36	0.25
Interaction	2	102.65	51.32	0.99	0.38
Aerosol at 0.08 ppm	1	10.67	10.67	0.21	0.65
Aerosol at 0.12 ppm	1	56.65	56.65	1.09	0.30
Aerosol at 0.18 ppm	1	105.92	105.92	2.04	0.16
Linear × linear	1	91.91	91.91	1.77	0.19
Quad × quad	1	10.74	10.74	0.21	0.65
Subject	29	8,156.19	281.25	5.42	0.0001
Error	85	4,406.69	51.84		
Corrected total	119	12,808.65			
Coefficient of determination	65.6%				

Table D.31. Analysis of Values for Forced Expiratory Volume in One Second Four Hours After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	488.80	244.40	3.33	0.040
Aerosol	1	229.72	229.72	3.13	0.081
Interaction	2	325.47	162.74	2.22	0.12
Aerosol at 0.08 ppm	1	12.41	12.41	0.17	0.68
Aerosol at 0.12 ppm	1	61.37	61.37	0.84	0.36
Aerosol at 0.18 ppm	1	481.40	481.40	6.55	0.012
Linear × linear	1	324.21	324.21	4.41	0.037
Quad × quad	1	1.26	1.26	0.02	0.90
Subject	29	9,473.54	326.67	4.45	0.0001
Error	85	6,243.26	73.45		
Corrected total	119	16,397.01			
Coefficient of determination	61.9%				

Table D.32. Analysis of Linear Trend Values for Forced Expiratory Volume in One Second for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	2,611.12	1,305.56	1.31	0.28
Aerosol	1	2,967.16	2,967.16	2.97	0.089
Interaction	2	5,138.10	2,569.05	2.57	0.083
Aerosol at 0.08 ppm	1	620.68	620.68	0.62	0.43
Aerosol at 0.12 ppm	1	2,113.67	2,113.67	2.11	0.15
Aerosol at 0.18 ppm	1	5,370.92	5,370.92	5.37	0.023
Linear × linear	1	4,821.62	4,821.62	4.82	0.031
Quad × quad	1	316.48	316.48	0.32	0.58
Subject	29	64,510.73	2,224.51	2.22	0.0024
Error	85	84,984.50	999.82		
Corrected total	119	160,103.59			
Coefficient of determination	46.9%				

Table D.33. Analysis of Quadratic Trend Values for Forced Expiratory Volume in One Second for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	323.60	161.80	1.22	0.30
Aerosol	1	233.72	233.72	1.76	0.19
Interaction	2	180.34	90.17	0.68	0.51
Aerosol at 0.08 ppm	1	360.29	360.29	2.71	0.10
Aerosol at 0.12 ppm	1	0.029	0.029	0.00	0.99
Aerosol at 0.18 ppm	1	53.75	53.75	0.40	0.53
Linear × linear	1	67.86	67.86	0.51	0.48
Quad × quad	1	112.48	112.48	0.85	0.36
Subject	29	5,656.81	195.06	1.47	0.09
Error	85	11,302.42	132.97		
Corrected total	119	17,395.58			
Coefficient of determination	35.1%				

Table D.34. Analysis of Cubic Trend Values for Forced Expiratory Volume in One Second for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	233.016	116.51	0.27	0.76
Aerosol	1	1,003.88	1,003.88	2.33	0.13
Interaction	2	801.53	900.77	0.93	0.40
Aerosol at 0.08 ppm	1	1,316.49	1,316.49	3.06	0.08
Aerosol at 0.12 ppm	1	478.21	478.21	1.11	0.29
Aerosol at 0.18 ppm	1	10.71	10.71	0.02	0.87
Linear × linear	1	782.36	782.36	1.82	0.18
Quad × quad	1	19.17	19.17	0.04	0.83
Subject	29	38,106.75	1,314.03	3.05	0.0001
Error	85	36,575.53	430.30		
Corrected total	119	76,996.08			
Coefficient of determination	52.5%				

Table D.35. Analysis of Specific Airway Conductance Values After Aerosol Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	234.17	117.09	0.34	0.71
Aerosol	1	22.19	22.19	0.06	0.80
Interaction	2	290.95	145.48	0.42	0.66
Aerosol at 0.08 ppm	1	130.41	30.41	0.38	0.54
Aerosol at 0.12 ppm	1	122.07	122.07	0.35	0.55
Aerosol at 0.18 ppm	1	60.66	60.66	0.18	0.68
Linear × linear	1	6.59	6.59	0.02	0.89
Quad × quad	1	284.36	284.36	0.83	0.37
Subject	29	54,100.90	1,865.55	5.42	0.0001
Error	85	29,243.43	344.04		
Corrected total	119	84,748.84			
Coefficient of determination	65.5%				

Table D.36. Analysis of Specific Airway Conductance Values After Ozone Exposure for Subjects with Asthma

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	371.04	185.52	0.26	0.77
Aerosol	1	2.44	2.44	0.00	0.90
Interaction	2	1,018.54	509.27	0.70	0.50
Aerosol at 0.08 ppm	1	45.13	45.13	0.06	0.80
Aerosol at 0.12 ppm	1	399.72	399.72	0.55	0.46
Aerosol at 0.18 ppm	1	576.14	576.14	0.79	0.38
Linear × linear	1	471.88	471.88	0.65	0.42
Quad × quad	1	546.66	546.66	0.75	0.39
Subject	29	99,927.31	3,445.77	4.75	0.0001
Error	85	61,660.96	725.42		
Corrected total	119	163,062.27			
Coefficient of determination	62.2%				

Table D.37. Analysis of Forced Vital Capacity Values After Ozone Exposure for All Subjects

Source	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	6.03	3.01	0.14	0.87
Aerosol	1	25.42	25.42	1.14	0.29
Group[a]	1	922.15	922.15	8.59	0.0048
Subject (Group)	58	6,140.34	105.87	4.76	0.0001
Ozone × Aerosol	2	281.68	140.84	6.33	0.0022
Ozone × Group	2	20.43	10.21	0.46	0.63
Aerosol × Group	1	20.16	20.16	0.91	0.34
Ozone × Aerosol × Group	2	93.06	46.53	2.09	0.13
Contrast	1	86.53	86.53	3.89	0.05
Model	69	7,501.03	108.71	4.89	0.0001
Error	170	3,782.87	22.25		
Corrected total	239	11,283.91			
Coefficient of determination	66.5%				

[a] Group indicates either subjects with asthma or healthy subjects.

Table D.38. Analysis of Forced Vital Capacity Values Four Hours After Ozone Exposure for All Subjects

Source	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	26.27	13.14	0.48	0.62
Aerosol	1	0.49	0.49	0.02	0.89
Group[a]	1	87.98	87.98	1.39	0.24
Subject (Group)	58	3,622.90	62.46	2.26	0.0001
Ozone × Aerosol	2	254.56	127.28	4.61	0.011
Ozone × Group	2	92.30	46.15	1.67	0.19
Aerosol × Group	1	12.00	12.00	0.43	0.51
Ozone × Aerosol × Group	2	96.45	48.22	1.75	0.18
Contrast	1	79.03	79.03	2.86	0.093
Model	69	4,109.04	59.55	2.16	0.0001
Error	170	4,693.97	27.61		
Corrected total	239	8,803.01			
Coefficient of determination	46.7%				

[a] Group indicates either subjects with asthma or healthy subjects.

Table D.39. Analysis of Values for Forced Expiratory Volume in One Second Four Hours After Ozone Exposure for All Subjects

Source	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	264.92	132.46	3.21	0.093
Aerosol	1	92.20	92.20	2.23	0.14
Group[a]	1	50.27	50.27	0.29	0.57
Subject (Group)	58	9,801.65	168.99	4.09	0.0001
Ozone × Aerosol	2	121.85	60.92	1.48	0.23
Ozone × Group	2	224.83	112.42	2.72	0.069
Aerosol × Group	1	134.00	140.00	3.39	0.067
Ozone × Aerosol × Group	2	209.63	104.82	2.54	0.082
Contrast	1	208.40	208.40	5.05	0.026
Model	69	10,549.67	152.90	3.70	0.0001
Error	170	7,019.06	41.29		
Corrected total	239	17,568.73			
Coefficient of determination	60.0%				

[a] Group indicates either subjects with asthma or healthy subjects.

Table D.40. Analysis of Linear Trend Values for Forced Expiratory Volume in One Second for All Subjects

Source	Degrees of Freedom	Sum of Squares	Mean Square	*F* Value	$p > F$
Ozone	2	1,294.94	647.47	1.20	0.30
Aerosol	1	1,967.42	1,967.42	3.64	0.058
Group[a]	1	4,699.52	4,699.52	3.88	0.054
Subject (Group)	58	69,606.82	1,200.12	2.22	0.0001
Ozone × Aerosol	2	2,216.05	1,108.02	2.05	0.13
Ozone × Group	2	1,317.39	658.69	1.22	0.30
Aerosol × Group	1	1,067.91	1,067.91	1.98	0.16
Ozone × Aerosol × Group	2	2,952.68	1,476.34	2.73	0.068
Contrast	1	2,806.93	2,806.93	5.20	0.023
Model	69	85,116.72	1,233.58	2.28	0.0001
Error	170	91,789.89	539.94		
Corrected total	239	176,906.61			
Coefficient of determination	48.1%				

[a] Group indicates either subjects with asthma or healthy subjects.

APPENDIX E. Research Team

PERSONNEL

M.J. Utell, Professor of Medicine and Environmental Medicine, coprincipal investigator

P.E. Morrow, Emeritus Professor of Environmental Medicine, coprincipal investigator

M.W. Frampton, Associate Professor of Medicine and Environmental Medicine, coinvestigator

P.C. Levy, Assistant Professor of Medicine, coinvestigator

C. Cox, Associate Professor of Biostatistics and Environmental Medicine, consultant in statistics

F.R. Gibb, Associate in Environmental Medicine, investigator

D.M. Speers, Senior Technical Associate in Medicine (Pulmonary Research)

H.E. Beiter, Laboratory Research Technician in Biophysics

W. Kremer, Technical Associate (Electronics) in Biophysics

DESIGNATED RESPONSIBILITIES

Exposure, analytical, and physical plant team: Morrow and Gibb

Clinical evaluations and physiologic assessments: Utell, Frampton, Levy, and Speers

Physical plant engineering: Gibb

Statistical analyses: Cox and Speers

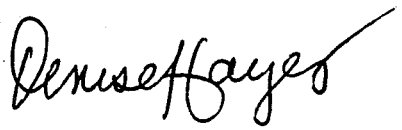
Quality assurance: Beiter and Morrow

Electronics and instrumentation maintenance: Gibb and Kremer

APPENDIX F. External Quality Assurance Report

The conduct of this study has been subjected to periodic audits by the Quality Assurance Officer at the University of Rochester, Ms. Ellen Miles. The audits included in-process observations of study activities and audits of the data. The results of the audits were reported to the Director of Research of the Health Effects Institute and to the Principal Investigator.

The activities of the university's Quality Assurance Officer were overseen by HEI's Quality Assurance Officer, Ms. Denise Hayes of Arthur D. Little, Inc., Cambridge, MA. These audits included reviews of the quality assurance and study procedures, and audits of the study data. Observations made during these visits indicate that the study is well documented, and that the report describes the methods used and reflects the raw data.



Denise Hayes
Quality Assurance Officer
Arthur D. Little

Table F.1. Audits by Quality Assurance Officers

Date of Audit	Focus of Audit	Conducted By
March 22, 1989	Audit of exposure chamber, atmosphere monitoring, and pulmonary function test data	Ellen Miles
April 17, 1989	Review of study protocol and procedures, and quality assurance procedures	Denise Hayes
April 25, 1989	Observation of standards analyses by ion chromatography	Ellen Miles
May and June 1989	Observation of exposure of subjects and pulmonary function tests	Ellen Miles
October 5, 1989	Observation of pulmonary function tests	Ellen Miles
November 2 and 7, 1989	Observation of sample analyses by ion chromatography	Ellen Miles
January through March, 1990	Interim data audits	Ellen Miles
April 10, 1990	Review of study and quality assurance procedures	Denise Hayes
June and September 1990	Audit of aerosol analyses data	Ellen Miles
November 1990	Audit of subject files	Ellen Miles
December 1990 and April 1991	Audit of exposure chamber data	Ellen Miles
March 1992	Audit of aerosol analyses and data base files	Ellen Miles
April 8, 1992	Audit of pulmonary function test data	Denise Hayes and Ellen Miles
May 1994	Review of final report	Denise Hayes

APPENDIX G. Raw Data for Pulmonary Function Measurements

The raw data for all pulmonary function measurements for each subject are presented in this appendix. It is available on request from the Health Effects Institute, 141 Portland Street, Suite 7300, Cambridge, MA 02139.

ABOUT THE AUTHORS

Mark J. Utell is Professor of Medicine and Environmental Medicine and Director of the Pulmonary/Critical Care and Occupational/Environmental Medicine Divisions at the University of Rochester Medical Center. He is the Associate Chairman of the recently established Department of Environmental Medicine. He received his M.D. degree from Tufts University School of Medicine in 1972. His research interests have centered on the effects of environmental pollutants on the human respiratory tract.

Paul E. Morrow received his B.S. and M.S. degrees in chemistry from the University of Georgia and his Ph.D. in pharmacology from the University of Rochester in 1951. He received postdoctoral training at the University of Göttingen (1959) and the University of Zurich (1960) and spent sabbatical leaves with the M.R.C. Toxicology Unit, Carshalton, England, and the Comitato Nacionale Energie Nucleare (Casaccia), Rome, Italy, in 1968 and 1969. He served on the University of Rochester faculty from 1952 to 1985, at which time he retired as Emeritus Professor of Toxicology in Biophysics. Dr. Morrow's primary research interest is the pulmonary toxicology of inhaled substances.

Mark W. Frampton is Associate Professor of Medicine and Environmental Medicine at the University of Rochester Medical Center. He received his M.D. degree from the New York University School of Medicine in 1973. After several years in the practice of internal medicine, he obtained training in pulmonary medicine at the University of Rochester from 1985 to 1988. His primary research interest is the effects of inhaled pollutants on respiratory defense against infection.

Christopher Cox is Associate Professor of Biostatistics and Environmental Medicine. He also holds an appointment in the University of Rochester Center for Biomedical Ultrasound. He received his Ph.D. degree in mathematics from the University of Illinois in 1972. He collaborates actively with a number of research groups at the University of Rochester Medical Center. His research interests are in the area of exponential family regression models.

Paul C. Levy is Assistant Professor of Medicine and Director of Clinical Services of the Pulmonary and Critical Care Unit

at the University of Rochester Medical Center. He received his M.D. degree from Ohio State University in 1982. His primary research interest is characterization of membrane receptors for immunoglobulin.

PUBLICATIONS RESULTING FROM THIS RESEARCH

Samet JM, Utell MJ. 1991. The environment and the lung: Changing perspectives. JAMA 266:670–675.

Utell MJ, Frampton MW. 1991. Sulfur dioxide and acidic aerosols. In: Environmental and Occupational Medicine (WN Rom, ed.) pp. 519–527. Little, Brown & Co., Boston, MA.

Utell MJ, Frampton MW, Morrow PE. 1991. Air pollution and asthma: Clinical studies with H_2SO_4 aerosols. Allergy Proc 12:385–388.

Frampton MW, Morrow PE, Cox C, Levy PC, Speers DM, Gibb FR, Condemi JJ, Utell MJ. 1992. Does pre-exposure to acidic aerosols alter airway responses to ozone in humans? (abstract). Am Rev Respir Dis 145(4):A428.

Utell MJ. 1993. Susceptible populations: Lessons from controlled exposure studies of inhaled pollutants. In: Advances in Controlled Clinical Inhalation Studies (U Mohr, DV Bates, H Fabel, MJ Utell, eds.) pp. 143–150. Springer-Verlag, New York, NY.

Utell MJ, Frampton MW, Morrow PE. 1993. Quantitative clinical studies with defined exposure atmospheres. In: Toxicology of the Lung (DE Gardner, JD Crapo, RO McClellan, eds.) pp. 283–309. Raven Press, New York, NY.

Utell MJ, Samet JM. 1993. Particulate air pollution and health: New evidence on an old problem (editorial). Am Rev Respir Dis 147:1334–1335.

Utell MJ, Warren J, Sawyer RF. 1994. Risks from automotive emissions. Annu Rev Public Health (15:157-178).

ABBREVIATIONS

ANOVA	analysis of variance
BTPS	body temperature and pressure, saturated with water vapor
CRC	Clinical Research Center
EPA	U.S. Environmental Protection Agency
FEV_1	forced expiratory volume in one second
FVC	forced vital capacity
H^+	hydrogen ion
H_2SO_4	sulfuric acid
IgE	immunoglobulin E
MMAD	mass median aerodynamic diameter
NAAQS	National Ambient Air Quality Standard
NaCl	sodium chloride
NH_3	ammonia
NH_4HSO_4	ammonium bisulfate
NO_2	nitrogen dioxide
NO_x	nitrogen oxides
O_3	ozone
PM_{10}	particulate matter equal to or less than 10 μm in aerodynamic diameter
ppb	parts per billion
ppm	parts per million
QA	quality assurance
R^2	squared multiple correlation coefficient
SD	standard deviation
SEM	standard error of the mean
sGaw	specific airway conductance
σ_g	geometric standard deviation
SO_2	sulfur dioxide
$\dot{V}_E$	expired volume of ventilation /min (minute ventilation)

INTRODUCTION

Epidemiologic studies suggest a possible link between asthma attacks and episodes of air pollution (reviewed by Balmes 1993). However, it has been difficult to establish a causal relation between asthma and individual air pollutants, such as ozone, nitrogen dioxide (NO_2)*, or aerosols; controlled exposure studies in clinical settings have yielded equivocal results. Some investigators have postulated that the discrepancies between the epidemiologic and clinical findings is due to the additive or synergistic effects of the multiple pollutants found in urban pollution. Thus, although exposures to many pollutants are common during summer smog, the majority of controlled human studies have evaluated the effects of individual pollutants; only a few studies have attempted to mimic the combined pollutant exposures of urban smog. The two studies discussed in this report evaluate the potential interactions of pollutants found in urban smog and their effect on healthy human subjects and subjects with asthma.

In 1987, the Health Effects Institute (HEI) issued a Request for Applications (RFA 87-1) that solicited proposals to study the "Acute and Chronic Effects of Atmospheric Oxidants." In response to this RFA, Dr. Jane Q. Koenig of the University of Washington submitted a proposal entitled "Pulmonary Effects of Oxidants Combined with Sulfuric and Nitric Acid in Subjects with Asthma." Her three-year project began in February 1989 and total expenditures were $516,602. Dr. Koenig's Investigators' Report was received for review at Health Effects Institute in July 1992, and a revised report was accepted by the Health Review Committee in February 1993.

A second proposal, entitled "Influence of Sulfate Aerosols on Exposure-Response Characteristics of Ozone on Human Lung Function," was submitted by Dr. Mark J. Utell of the University of Rochester. Dr. Utell's project began in November 1988 and was funded for three years at a cost of $722,274. His final report was submitted to Health Effects Institute in July 1993 and was approved for publication in April 1994. During the review process, the Review Committee and the investigators had the opportunity to exchange comments and to clarify issues in the Investigators' Reports and in the Review Committee's Commentary.

Because both studies deal with the health effects of combinations of oxidants and acid aerosols in human subjects with asthma, HEI chose to publish both Investigators' Reports in one Research Report. The following Commentary compares the protocols, results, and conclusions of these two studies. It is intended to aid the sponsors of HEI and the public by highlighting both the strengths and limitations of the two studies and by placing the Investigators' Reports into scientific and regulatory perspective.

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

REGULATORY BACKGROUND

The U.S. Environmental Protection Agency (EPA) sets standards for oxidants (and other pollutants) under Section 202 of the Clean Air Act, as amended in 1990. Section 202 (a)(1) directs the Administrator to "prescribe (and from time to time revise) . . . standards applicable to the emission of any air pollutant from any class or classes of new motor vehicles or new motor vehicle engines, which in his judgment cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Sections 202(a), (b)(1), (g), and (h), and Sections 207(c)(4), (5), and (6) impose specific requirements for reducing motor vehicle emissions of certain oxidants (and other pollutants) and, in some cases, provide the EPA with limited discretion to modify those requirements.

In addition, Section 109 of the Clean Air Act provides for the establishment of National Ambient Air Quality Standards (NAAQS) to protect the public health. The current primary and secondary NAAQS for ozone is 0.12 parts per million (ppm). This standard is met when no more than 1 day per year has a maximum hourly average concentrations of ozone above 0.12 ppm. Section 181 of the Act classifies nonattainment areas according to the degree that they exceed the NAAQS, and assigns a primary standard attainment date for each classification. The current primary NAAQS for nitrogen dioxide is 0.053 ppm as an annual arithmetic mean concentration. This means that the 12-month average value of NO_2 at any specific site should not exceed 0.053 ppm.

There are no NAAQS for sulfuric acid (H_2SO_4) or nitric acid (HNO_3). These are secondary pollutants that are formed through the oxidation of the primary inorganic pollutants, nitrogen oxides), and sulfur oxides. Thus, ambient levels of H_2SO_4 and HNO_3 are indirectly affected by the NAAQS for nitrogen dioxide and sulfur dioxide. Sulfur dioxide has a 24-hour NAAQS of 0.14 ppm, which is not to be exceeded more than once per year, and an annual arithmetic mean standard of 0.03 ppm. The only standard that directly applies to H_2SO_4 acid is set by the Occupational Safety and Health Administration; the permissible expo-

sure limit of 1 mg/m^3 is based on an eight-hour averaging time and a 40-hour work week (Air Contaminants Rule 1993).

Because determining appropriate standards for emissions of air pollutants and their precursors depends, in part, on an assessment of the health risks that they present, research into the health effects of oxidants and secondary pollutants in studies like those reported here is essential to the informed regulatory decision-making required by the Clean Air Act.

SCIENTIFIC BACKGROUND

OXIDANTS AND ACID AEROSOLS

Constituents of photochemical smog include oxidants such as ozone and NO_2, and acid aerosols such as HNO_3 and H_2SO_4. Ozone is produced in the atmosphere in the presence of ultraviolet light by a complex series of reactions among nitrogen oxides and volatile organic compounds that are released from natural sources (e.g., trees) and from mobile and stationary combustion sources. While ambient ozone levels have been steadily decreasing over the last ten years, it is estimated that approximately 45 million Americans live in counties that do not meet the ozone standard (U.S. Environmental Protection Agency 1992).

Nitrogen dioxide is a component of both indoor and outdoor air pollution and is produced by the oxidation of atmospheric nitrogen during combustion processes at high temperatures. Outdoors, it is formed from combustion associated with motor vehicles, power plants, and industrial sources; indoors it is generated by heating and cooking sources fueled by natural gas, kerosene, coal, or wood. In urban areas, outdoor NO_2 concentrations are characterized by morning and afternoon peaks that are associated with motor vehicle traffic.

The acid aerosols H_2SO_4 and HNO_3 are secondary pollutants that are formed through the atmospheric oxidation of sulfur oxides and nitrogen oxides, respectively. Sulfur oxides are mainly produced by the combustion of coal and residual fuel oil in industrial processes and in generating electrical power. Nitrogen oxides are generated though the combustion of fossil fuels. Daytime acid aerosol concentrations are typically higher in the summer months than in the winter months (Spengler et al. 1990). Measurements of peak H_2SO_4 can reach 100 μg/m^3 of air, and 24-hour averages of 20 μg/m^3 have been reported (Spengler et al. 1989). Sulfuric acid pollution events occur most frequently in states just west of the Appalachian mountains, such as West Virginia, Tennessee, Kentucky, and Ohio, and span as far north as southern Ontario. Nitric acid is more prevalent in the western sections of the United States; peak hourly averages of HNO_3 as high as 67μg/m^3 have been reported in the Los Angeles basin.

The following background is intended to provide a brief overview of the scientific literature related to the health effects of oxidants and acid aerosols, and focuses on projects in which mixtures of these pollutants were studied.

EPIDEMIOLOGIC STUDIES

Epidemiologic evidence suggests that air pollution is associated with asthma attacks, hospital admissions due to respiratory distress, and mortality. However, it has been difficult to establish causal links to specific pollutants because many pollutants (oxidant gases, acid aerosols, and particles) are usually involved. Early studies in California (Schoettlin et al. 1961; Whittemore et al. 1980) indicated that more asthma attacks occurred on days when the maximum one-hour concentration of oxidants exceeded 0.2 ppm; and several studies reported an association between hospital admissions and ambient concentrations of oxidant gases (Sterling et al. 1966, 1967; Bates et al. 1983, 1987, 1989; Goldsmith et al. 1983). However, other investigations reported no association between ambient ozone levels and hospital admissions (Sunyer et al. 1991; Tseng et al. 1992); however, these studies did not control for factors such as the day of the week, the season, or long-wave periodicities (seasonal variations in morbidity and mortality). In more recent studies where these issues have been addressed, exacerbation of asthma and increases in hospital admissions for respiratory illness were related to summer haze pollutants (including ozone) and reaction products of acid aerosols (hydrogen ion [H^+] and sulfate) (Thurston et al. 1992). Furthermore, Kinney and colleagues (1991, 1992) found a statistically significant association between ambient ozone concentrations and mortality rates the following day.

Several groups have evaluated the relations among respiratory symptoms, pulmonary function, and ambient ozone levels in people with asthma. Ambient ozone levels were reported to be related to increased symptoms such as wheeze and cough, and decreased peak flow rates in subjects with asthma, but not in healthy control subjects (Lebowitz et al. 1987). Decreases in peak flow rates (Krzyzanowski et al. 1992) and increases in cough, wheezing, and the total number of daily inhaled bronchodilator treatments (Thurston et al. 1992, 1993) also were found to be associated with elevated ambient ozone concentrations.

A series of studies in summer camps have provided evidence of an association between ambient ozone levels

and decrements in lung function in healthy children (Spektor et al. 1988a). In fact, greater lung function decrements have been reported in children playing at camp while exposed to ambient ozone concentrations than in subjects exercising in exposure chambers with the same ozone concentrations (Spektor et al. 1988a). This suggests that ozone exposure may have cumulative effects, or that other pollutants may act in concert with ozone.

During episodes of acute air pollution, such as occurred in Belgium in 1930 and in Donora, PA in 1948, acid aerosols from steel mills and H_2SO_4 plants were implicated in the excessive levels of respiratory illness and mortality. Until recently, acid aerosols have not been specifically monitored as components of smog, although surrogates of H_2SO_4 (total sulfates, sulfur dioxide, sulfate, and H^+ concentrations) have been measured (Lippmann 1992). Coal smoke containing H_2SO_4 has long been thought to be responsible for the deaths that occurred during the London fog episodes of the 1950s and 1960s. This assumption has been confirmed by a recent assessment of the London 1963-1972 mortality data, which revealed that mortality was closely related to the acid concentration of ambient air on the previous day (Thurston et al. 1989).

Elevated ambient levels of sulfate have been associated with an increase in annual mortality rates, respiratory morbidity (Ostro 1990), chronic bronchitis, and lower respiratory tract illness (Ware et al. 1986; Dockery 1989; Speizer 1989). Most recently, in a study of subjects with asthma in Colorado (Ostro et al. 1989, 1991), the concentration of H^+ was found to have a strong association with the occurrence of reported asthma. An analysis of the data from over 600 subjects with asthma in the vicinity of the titanium oxide manufacturing facility in Japan indicated that H_2SO_4 was the causal pollutant responsible for respiratory morbidity (Kitagawa 1984). The plant emitted H_2SO_4 mist, and geographic analyses revealed that the pattern of disease coincided with the pattern of acid deposition.

Research on the potential health effects of NO_2 exposure has focused on the relation of this pollutant to infectious respiratory diseases. The early epidemiologic studies of the health effects of indoor or outdoor NO_2 exposure yielded inconsistent findings and are difficult to interpret because of methodological flaws, including confounding by other pollutants, and poor assessment of exposure and disease outcomes. In some studies, the presence of a gas stove in the home (a potential source of NO_2) was significantly associated with an increase in respiratory symptoms and disease (Melia et al. 1977, 1979; Speizer et al. 1980) or with hospitalization for respiratory illness (Ekwo et al. 1983). However, others reported no statistically significant association between the presence of a gas stove in the home and changes in respiratory symptoms, respiratory illness, hospitalization for respiratory illness, or the duration of upper or lower respiratory illness (Melia et al. 1983; Ware et al. 1984; Ogston et al. 1985; Samet et al. 1993). A few studies have reported associations between the prevalence of respiratory illness in children and levels of NO_2 in their bedrooms (Florey et al. 1979; Goldstein et al. 1979), combined lower respiratory symptoms and indoor NO_2 levels (Neas et al. 1991), and the incidence of upper respiratory illness and outdoor NO_2 levels (Rutishauser et al. 1990; Braun-Fahrlander et al. 1992). However, in other studies in which measurements of indoor NO_2 were made directly, no significant association between indoor NO_2 levels and respiratory illness, duration of upper or lower respiratory illness (Samet et al. 1993), or respiratory tract symptoms (Dijkstra et al. 1990; Rutishauser et al. 1990; Braun-Fahrlander et al. 1992) were found. A metanalysis of the studies published before 1992 revealed that the odds of children experiencing respiratory illness after extended exposures to NO_2 increased by 18% for each additional increment 0.015 ppm beyond normal ambient levels (Hasselblad et al. 1992).

CONTROLLED HUMAN STUDIES

An extensive data base exists that documents the effects of controlled ozone exposure on pulmonary function in humans. In resting subjects, 0.5 ppm was the lowest concentration of ozone that caused a statistically significant decrease in measurements of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) (Folinsbee et al. 1978; Horvath et al. 1979). However, the level of ozone at which pulmonary function effects occur is reduced if ventilation is increased during periods of exercise. Numerous studies (Folinsbee et al. 1975; Hackney et al. 1975; Silverman et al. 1976; DeLucia et al. 1977) indicate that these pulmonary effects are dependent on the effective dose of ozone (the product of concentration, time, and ventilation). Thus, as the exercise level increases, the concentration or time necessary to reach the "effective dose" decreases (Adams et al. 1983; McDonnell et al. 1983; Avol et al. 1984; Folinsbee et al. 1984; Kulle et al. 1985; Gong et al. 1986; Linn et al. 1986). During heavy intermittent exercise, concentrations of ozone as low as 0.08 ppm have been associated with statistically significant reductions in FEV_1 (reviewed by Lippmann 1989, 1993, and U.S. EPA 1993).

Surprisingly, in most controlled human studies, subjects with asthma have responded to low concentrations of ozone (0.12 to 0.25 ppm) with decrements in pulmonary function similar to those exhibited by healthy subjects (Linn et al. 1978; Silverman 1979; Koenig et al. 1985, 1987, 1988). A greater response to ozone was reported in subjects with

asthma when higher ozone concentrations and increased levels of exercise were examined. For example, Kreit and coworkers (1989) found that the decrement in some measures of pulmonary function was greater in subjects with asthma exposed to 0.4 ppm ozone during heavy exercise than in healthy subjects with the same exposure protocol.

The results of controlled exposures of humans to acid aerosols are inconsistent. In general, most investigators did not find decrements in spirometric measurements or increases in airway resistance or airway responsiveness after exposure to H_2SO_4 in healthy adults (Avol et al. 1979; Leikauf et al. 1981, 1984; Horvath et al. 1982, 1987) using a range of concentrations (10 to 1,000 $\mu g/m^3$) and particle sizes (0.1 to 10 μm) of H_2SO_4. Some exceptions are the study by Horvath and associates (1982), which showed that exposure to high doses of H_2SO_4 (1,000 $\mu g/m^3$) while subjects were exercising was associated with a significant decrease in FEV_1, and the study by Ericsson and Cramner (1983), which demonstrated an increase in respiratory rate and tidal volume after exposure to H_2SO_4 at 350 to 500 $\mu g/m^3$. These effects of high doses have not been replicated by other investigators even when the same exposure concentrations were used (Leikauf et al. 1981; Horvath et al. 1987; Avol et al. 1988a,b).

Some evidence indicates that subjects with asthma may be more sensitive to acid aerosol exposures than healthy subjects; however, these results are also inconsistent. Koenig and coworkers reported statistically significant decreases in FEV_1 in adolescent subjects with allergies when they were exposed to H_2SO_4 at either 68 $\mu g/m^3$ (Koenig et al. 1989) or at 100 $\mu g/m^3$ (Koenig et al. 1983). Utell and coworkers (1983a) reported decreases in specific airway conductance and FEV_1 in subjects with asthma who were exposed at rest for 16 minutes to high concentrations of H_2SO_4 (1,000 $\mu g/m^3$); airway conductance also was reduced with exposure to H_2SO_4 at 450 $\mu g/m^3$. However, no statistically significant effects were observed with exposure to H_2SO_4 at 100 $\mu g/m^3$. Linn and coworkers (1989) described decreases in FEV_1, increases in airway resistance, and increases in symptoms after subjects with asthma (but not healthy subjects) were exposed for one hour to H_2SO_4 at 2 $\mu g/m^3$. Linn and coworkers (1989) also noted that the symptom responses of healthy subjects increased as the aerosol droplet size increased; however, the responses of subjects with asthma were independent of droplet size.

These effects of H_2SO_4 in subjects with asthma have not been confirmed by other investigators when lower acid aerosol concentrations have been tested. When Avol and colleagues (1979) exposed healthy subjects and subjects with asthma to H_2SO_4 at 75 $\mu g/m^3$ for two hours with intermittent exercise, they found no changes in lung function, airway resistance, or time to onset of symptoms. Similar negative results were reported by Linn and associates (1986) and by Avol (1988b). Furthermore, in a study of children with asthma, Avol and coworkers (1990) found that H_2SO_4 exposure (at 50 $\mu g/m^3$ or at 150 $\mu g/m^3$) had no effect on lung function, specific airway resistance, or FEV_1. In an attempt to replicate Dr. Koenig's procedures of 1983, Avol (1990) exposed subjects with asthma to H_2SO_4 at 150 $\mu g/m^3$ through a rubber mouth piece; he found no statistically significant changes in lung function or reported symptoms, possibly due to the acid in the chamber being neutralized by body ammonia.

Human studies of controlled exposure to NO_2 have generally yielded negative results, except when exposure concentrations were very high. One- to three-hour exposures of healthy adults to NO_2 (at concentrations less than 1 ppm) are not typically associated with changes in specific airway resistance or lung function. Some studies have reported an increase in specific airway resistance and airway responsiveness after brief exposures to NO_2 at concentrations in excess of 1 ppm (Linn et al. 1985; Kulle and Clements 1988; Mohsenin 1988). Studies of human subjects with asthma also have produced equivocal results. One study reported enhanced airway responsiveness to a bronchoconstrictor challenge after short-term exposures to NO_2 (0.1 to 1 ppm) (Orehek et al. 1976; Bylin et al. 1985; Bauer et al. 1986; Mohsenin 1987); however, most investigators have not found statistically significant changes in airway reactivity or lung function after exposure to 0.1 to 0.5 ppm NO_2 (Bylin et al. 1985; Koenig et al. 1985, 1987; Utell 1989).

Only a few controlled human studies have been conducted on the effects of combined exposure to ozone and acid aerosols. Those studies have varied considerably in their protocols and pollutant combinations.

Kulle (1982) investigated the effect of preexposure to 0.3 ppm ozone on the response of healthy adults to H_2SO_4 at 100 $\mu g/m^3$. Although bronchial reactivity to a methacholine challenge decreased after the H_2SO_4 exposure, the change was not statistically significant. Horvath and coworkers (1987) exposed healthy male subjects for two hours, including 60 minutes of moderate exercise, to a combination of 0.25 ppm ozone and H_2SO_4 (1,200 to 1,600 $\mu g/m^3$). They found no changes in FVC, FEV_1, or airway resistance.

To mimic the effects of southern California coastal fog (where acid fog typically precedes peak ozone), Aris and coworkers (1991) exposed ozone-sensitive, healthy, athletic subjects for two hours (while exercising) to a fog containing HNO_3 at 0.5 mg/m^3, and then to 0.2 ppm ozone

during three hours of exercise. Unexpectedly, the reduction in FEV_1 and FVC typically associated with ozone exposure diminished when ozone exposure followed exposure to either the HNO_3 fog or the H_2O fog (control).

Linn and associates (1991) found a statistically significant interaction between ozone concentration and H_2SO_4 concentration for mean specific airway resistance in both healthy subjects and subjects with asthma; however, ozone was responsible for most of the measured respiratory dysfunction and increased bronchial reactivity. When FEV_1 data from both groups were combined, the analysis showed a statistically significant decrease in FEV_1 in response to exposure to ozone and H_2SO_4 combined. The authors suggested that ozone injury to the airways allows H_2SO_4 to act as a secondary irritant, thereby reducing lung function. It is interesting to note that the ozone-induced decrements in pulmonary function were less on the second day of exposure, and that the effects from the combined exposure also did not occur.

The health effects of exposure to combined oxidant air pollutants also have been examined. Adams and coworkers (1987) exposed healthy subjects to a combination of 0.30 ppm ozone and 0.60 ppm NO_2 for one hour during heavy exercise. In all but one of the pulmonary function parameters measured, no differences were found between exposure to ozone and exposure to combined NO_2 and ozone; only specific airway resistance was lower after the combined exposure than after exposure to ozone. Koenig (1988) also found no effects of short (1 hour and 15 minutes) combined exposures to 0.12 ppm ozone and 0.30 ppm NO_2 on pulmonary function in healthy subjects and subjects with asthma.

RATIONALE FOR FUNDING THE KOENIG AND UTELL STUDIES

In issuing RFA 87-1, the Health Effects Institute was interested in supporting research to improve our understanding of the health effects of exposure to oxidant gases (ozone and NO_2), including the interactive effects of these gases with other pollutants. The HEI Research Committee was most interested in studies that would examine the consequences of inhaling oxidants at concentrations comparable to ambient levels. The main objectives of the RFA were to support studies that would investigate (1) the relations among oxidant exposure, the acute effects of oxidant injury, and later manifestations of pulmonary disease; (2) the possible interactive effects of atmospheric oxidants and other pollutants; and (3) the variability in individual sensitivity to oxidant exposure.

Drs. Jane Q. Koenig and Mark J. Utell each submitted a proposal in response to this RFA. Both investigators were interested in exploring the human health effects of combined pollutant exposure and the responses of a susceptible population, people with asthma. They both hypothesized that exposure to two pollutants would cause greater pulmonary function changes than exposure to a single pollutant.

Dr. Koenig and her colleagues proposed to investigate the pulmonary responses of adolescents with asthma to a mixture of ozone and NO_2, and to this mixture combined with H_2SO_4 or with HNO_3. As discussed earlier, Dr. Koenig and her colleagues had previously reported that, compared with healthy subjects, adolescents with asthma exhibited increased airway responsiveness after exposure to certain acidic air pollutants such as sulfur dioxide and H_2SO_4. The proposed exposures would test atmospheres that mimicked the acidic summer haze conditions that persist in urban areas during periods of air stagnation.

Dr. Utell and his colleagues proposed to investigate whether prior exposure to H_2SO_4 aerosol sensitized the airways of human subjects to subsequent ozone exposure. They noted that ozone and H_2SO_4 frequently coexist in the environment and that inhalation of each one has been reported to produce effects on breathing. The rationale for their study was supported by previously published data from their laboratory that indicated that subjects experience enhanced airway responsiveness 24 hours after exposure to H_2SO_4 aerosols (Utell et al. 1983a).

As discussed earlier, few investigators have examined the effects of exposures to combinations of pollutants on human subjects; in those studies that have been done, the exposures were usually simultaneous, not sequential. The key features of both studies are presented in Table 1 of this Commentary.

TECHNICAL EVALUATION OF DR. KOENIG'S STUDY

SPECIFIC AIMS AND ATTAINMENT OF STUDY OBJECTIVES

Dr. Koenig's study had two main objectives:

- to determine whether two consecutive days of exposure to a mixture of ozone and NO_2 produced greater effects on the pulmonary function of subjects with asthma than a single day of exposure; and
- to compare the effects of adding either H_2SO_4 or HNO_3 to the oxidant gas mixture in order to determine whether the addition of an acid aerosol produced greater toxicity than the oxidant gas mixture alone.

Table 1. Key Features of Two Clinical Studies of Exposure to Oxidant Gases Plus Acid Aerosols

Study Parameter	Utell Study	Koenig Study
Research questions	• Does prior exposure to an acidic aerosol increase airway responsiveness to ozone in healthy adults and in subjects with asthma? • Do ozone dose-response curves differ between normal subjects and subjects with asthma?	• Do two days of exposure to air pollutants produce greater effects than one day of exposure? • Do mixtures of an acid aerosol with oxidants produce greater toxicity than oxidants alone?
Subjects	• 30 Healthy adults (16 females, 14 males) • 30 Adults with asthma (10 females, 20 males) (1 subject left the study)	• 28 Adolescents with allergic asthma (15 females, 7 males) (6 subjects left the study)
Criteria for subject selection	• History of intermittent bronchospasm • Abnormally low specific airway resistance • >15% Drop in FEV_1 with administration of isoproterenol, or >40% drop in specific airway resistance after carbachol challenge • Positive skin tests	• Reversible obstructive airway disease • History of allergic asthma • >15% Drop in FEV_1 (exercise-induced bronchoconstriction) • Airway reactivity to methacholine
Pollutant exposure regimen	• Either H_2SO_4 or NaCl aerosol (100 $\mu g/m^3$) followed 24 hours later by either 0.08, 0.12, or 0.18 ppm O_3 • Two to four weeks later, the aerosol not previously administered followed 24 hours later by the same concentration of O_3 • One three-hour exposure with a total of 1 hour of intermittent exercise (minute ventilation increased four-fold)	Subjects exposed, in random order, to each of the following: • Filtered air • 0.12 ppm ozone + 0.3 ppm NO_2 • 0.12 ppm ozone + 0.3 ppm NO_2 + H_2SO_4 (68 $\mu g/m^3$) • 0.12 ppm ozone + 0.3 ppm NO_2 + 0.05 ppm HNO_3 • One 90-minute exposure with a total of 45 minutes of intermittent exercise (minute ventilation increased three-fold)
Exposure protocol	• 9 Days (4 of 6 possible test atmospheres × 2 days/atmosphere plus 1 training day) • Lemonade mouthwash	• 12 Days (4 test atmospheres × 3 days/atmosphere)
Pulmonary function tests	• FEV_1, FVC, specific airway resistance	• FEV_1, FVC, total respiratory resistance, maximum flow at 50% and 75% of expired vital capacity
Seasonal restrictions	• Exposures from mid-September through mid-June to avoid Northeast seasonal peaks in exposure pollutants	• Exposures from April through August to limit the study to one pollen season
Medication restrictions	• 6 Hours before protocol	• 4 Hours of oral inhalers before protocol and no use of other medications on test days.
Results	Healthy Adults • No effect of exposure to H_2SO_4, NaCl, or O_3 on pulmonary function tests, and no interactions between acid aerosols and O_3 Adults with Asthma • H_2SO_4 Exposure had no direct effect on pulmonary function responses • Preexposure to H_2SO_4 enhanced the small mean decrements in FVC that occurred in response to exposure to 0.18 ppm ozone	Adolescents with Asthma • No effects on pulmonary function after exposure to any of the test atmospheres on either the first or second day of the test protocol.

The investigators accomplished their specific aims for 22 of the 28 subjects enrolled in the study; six subjects did not complete the experimental protocol. The extent to which the subjects leaving the study affected the results and subsequent interpretation of the study data cannot be determined.

STUDY DESIGN AND METHODS

Twenty-eight male and female subjects with asthma between the ages of 12 and 19 comprised the original study pool. The criteria for subject selection are clearly described in the Investigators' Report. The investigators evaluated four test atmospheres:

- Filtered air;
- 0.12 ppm ozone + 0.3 ppm NO_2;
- 0.12 ppm ozone + 0.3 ppm NO_2 + H_2SO_4 at 68 $\mu g/m^3$; and
- 0.12 ppm ozone + 0.3 ppm NO_2 + 0.05 ppm HNO_3.

Subjects were exposed to each of the test atmospheres in random order, and each three-day exposure protocol was separated from the next one by at least one week. The effects of each test atmosphere were evaluated on three consecutive days. On each of the first two days, subjects inhaled the test atmosphere via a mouthpiece for 90 minutes. The protocol included alternating 15-minute periods of rest and moderate exercise on a treadmill. The exercise level was adjusted to increase the measurement of resting minute ventilation by approximately three-fold. Pulmonary function tests, peak flow, measurements of oral ammonia, and symptom evaluation forms were completed by the subjects before and after each exposure. In the evening, subjects measured their peak flow at home with the same instrument used during the exposures. On the third day, the subjects were not exposed to pollutants, but baseline pulmonary function tests and peak flow measurements were performed. The subjects then underwent methacholine challenge tests to determine the effects of the previous two days of exposure on the responsiveness of their airways. A total of 12 protocol days (4 test atmospheres × 3 days/test atmosphere) were required to complete the testing of each subject, in addition to the week between each successive exposure.

This study was performed by a team of investigators with considerable experience in clinical respiratory environmental research. Their protocol and subject selection were well-defined. The sample size necessary for sufficient power for reliable detection of changes in lung function due to exposures was calculated prior to subject selection. Sample size was based on projected changes in FEV_1. Estimates of within-subject variability were obtained from three previous studies using analysis of variance of repeated baseline observations. Using sample size calculations, Dr. Koenig and the study statistician estimated that a sample of 12 subjects would be sufficient to detect a 0.14-mL change in FEV_1. Therefore, a change of 5% in FEV_1 should have been detectable with this sample size.

Experiments were conducted between April and August each year in order to restrict the study to one season. The pollutant levels selected by the investigators were well-justified. The ozone exposure concentration (0.12 ppm) is the current one-hour NAAQS for ozone. The NO_2 exposure concentration (0.3 ppm) is higher than typical ambient concentrations of this pollutant (0.02-0.15 ppm), but peak NO_2 concentrations of 0.3 ppm or higher can occur in indoor and outdoor settings. The H_2SO_4 concentration (68 $\mu g/m^3$) was in the same range as maximum four- to eight-hour values observed in the atmosphere. The HNO_3 concentration (0.5 ppm) was, however, higher than typical ambient levels.

It is noteworthy that the subjects inhaled the pollutant mixtures through a mouthpiece. This system has been used by Dr. Koenig's group for many years. A mouthpiece, however, also has been shown to alter breathing patterns (McCool and Paek 1993). Increased tidal volume, decreased breathing frequency, and increased minute ventilation have been associated with the use of a mouthpiece at rest; and both increased and decreased tidal volume have been reported when a mouthpiece is used during exercise (Sackner et al. 1980; Stark et al. 1988).

Standard pulmonary function tests were performed and symptoms were recorded. In the first year of testing, the technician who recorded data knew the composition of the test atmosphere. In the second and third years, the studies were performed in a completely double-blinded manner; neither the investigators, the technicians, nor the subjects knew the test atmosphere composition.

The clinical criteria used for subject selection are described in the Investigators' Report. Initially, the asthma status of most of the subjects was characterized by their sensitivity to inhaled methacholine. Using generally accepted criteria, six subjects were characterized with severe asthma, thirteen with moderate asthma, and eight with mild asthma. Twenty-two subjects completed the study. The six subjects who left the study before completing the test exposures offered a variety of reasons, ranging from respiratory infections to loss of stamina during daily sports activities; it is noteworthy that these subjects had either moderate or severe asthma, and left the study after a pollutant exposure, not a clean-air control exposure.

There are several questions regarding the characterization of the subjects for this study. Although the investigators describe their subjects as a homogeneous study population of

subjects with asthma, the variable drug regimen used by the study subjects to treat their asthma symptoms (see Table 3 of the Investigators' Report) contradict this description. Thus, the effects observed in this study population may not accurately predict responses to similar exposures by individuals with asthma in the general population.

Another issue to be addressed is the procedure the investigators used for restricting medications for the study participants on a test day. The protocol called for restricting oral inhalers for four hours before testing and allowed no other asthma medication to be used on test days. In practice, subjects who were exposed in the morning had their medication restricted for more than eight hours before exposure, whereas those exposed in the afternoon were restricted for only four hours. In most clinical studies of subjects with asthma, the subject guidelines restrict personal medication use for at least eight hours before testing. The extent to which the subjects' use of or restriction from medications altered their responses to the test atmospheres in this study remains undetermined.

Concerns also arise regarding the doses of methacholine selected for the airway challenge tests performed on the third day of each exposure regimen. These doses were low compared with accepted clinical testing procedures. In support of her selection of the methacholine challenge doses, Dr. Koenig stated that she wanted to avoid adverse airway reactions in this susceptible study population. She also noted that the doses were increased in the third year of the study to concentrations that were closer to the dose for each individual that would provoke a 20% decrease in FEV_1. As a result, several subjects experienced some discomfort. However, the higher methacholine challenge dose did not improve the investigators' ability to differentiate individual responses to the test atmospheres.

STATISTICAL METHODS

The investigators selected a repeated measures design to analyze their data. However, rather than performing a comprehensive repeated measures analysis of variance, they expressed each post-exposure measurement as a change relative to baseline measurements. They then took the difference between the change produced by pollutant exposure on day one or day two and the change during exposure to air. These "differences of differences" then were analyzed by a one-sample t test to test the null hypothesis that a change produced by the pollutant exposure was equal to the change produced by the filtered-air exposure. The authors recognized that these tests would be applied to data from three test atmospheres, two days of exposure, and two sets of post-exposure measurements, yielding a total of 12 tests. Therefore, they used a Bonferroni correction factor for multiple comparisons and required a p value of 0.0042 (0.05/12) before declaring any single comparison as statistically significant. These analyses were accompanied by nonparametric signed-rank tests and by graphical analyses using histograms and boxplots.

The investigators appropriately selected a series of paired t tests to analyze their data; a clear explanation of these methods was presented in the text. Alternatively, they could have used a mixed-model repeated-measures analysis of variance that assumes constant variance and interobservation correlation over the entire set of observations. However, in that sense, the mixed-model analysis is a more restrictive and less suitable statistical analysis than the series of paired t tests that were used by these investigators. Multivariate repeated-measures analysis of variance also would have been problematic in this case because the number of observations exceeded the number of subjects.

The authors corroborated that their study had sufficient power to detect changes at the 5% level by comparing the observed standard errors with those anticipated for the results. As a helpful addition to facilitate the readers' detection of exceedences above or below the 5% level, the authors plotted dashed lines on each of their boxplots.

RESULTS AND INTERPRETATION

Dr. Koenig and coworkers originally hypothesized that deficits in pulmonary function would be observed after two days of consecutive exposure to oxidants, or oxidants plus an acid aerosol. However, they found no significant effects after exposures to any of the test atmospheres. The data from the pulmonary function tests summarized in Figures 2 through 6 of the Investigators' Report indicate that the pollutant exposures produced changes of only a few hundred milliliters in lung volume, and these changes could be attributed to normal test variability.

The negative results of this study are intriguing because these investigators previously had observed statistically significant changes in pulmonary function after a 45-minute exposure of subjects with asthma to a range of H_2SO_4 concentrations (51 to 176 $\mu g/m^3$) (Koenig et al. 1983; Hanley et al. 1992). In their previous studies, exposure to H_2SO_4 was associated with a decrease in pulmonary function measurements when subjects were tested immediately after the 45-minute exposure. No effect was observed, however, when exposure to H_2SO_4 was extended to 90 minutes (Koenig et al. 1992). Thus, the investigators speculated that a pollutant-induced effect may have been present but was "lost" at the 90-minute time point. In support of this hypothesis are other findings of Koenig and associates indicating that the effects induced by H_2SO_4 were statistically

significant only when measured immediately after the end of a 45-minute exposure, and that a substantial lessening of effects was observed within 20 minutes of exposure termination (Hanley et al. 1992; Koenig et al. 1992). However, these results also are difficult to interpret because of the inverse dose dependence that was reported. A statistically significant decrease in FEV_1 was observed after exposure to H_2SO_4 at 35 $\mu g/m^3$, but not after exposure to H_2SO_4 at 70 $\mu g/m^3$. Moreover, neither low-dose nor high-dose exposures to H_2SO_4 produced significant changes in pulmonary function if the duration of the exposures was increased from 45 to 90 minutes. One possible explanation for the loss of H_2SO_4 effect over time is a buildup of oral ammonia levels, which would act to neutralize the acid aerosol. In the present study, oral ammonia levels were consistently 40% to 60% higher than baseline levels after air and pollutant exposures.

As noted earlier, the fact that six subjects left this study before completing all of the exposures atmospheres seriously complicates the interpretation of the results. On the basis of the subjects' airway responsiveness, as assessed by methacholine challenge tests (see Table 3), five of these six subjects were categorized as having moderate or severe asthma, and the sixth subject was not categorized. A review of the reasons given by the subjects who left the study suggests that aggravation of asthma symptoms was not an apparent cause for withdrawal from the exposure regimen. However, the investigators noted that all six of the subjects who left did so after a pollutant exposure rather than after exposure to clean air. In any case, the net result is that the data from a group of subjects, representing approximately 20% of the original subjects, were not included in the final data analyses. Accordingly, the conclusions of the study may have been based on a group of subjects more tolerant to oxidants, acid aerosols, or both, than those constituting the original study group. The issue of the subjects who left the study raises concerns about extrapolating the results of this study and using them to anticipate the responses of the general population of individuals with asthma to similar pollutant exposures.

In summary, the lack of any effects after subjects with asthma were exposed to the combinations of oxidants and acid aerosols was unexpected in this population of adolescent subjects that has, in Dr. Koenig's laboratory, previously exhibited increased airway responsiveness to at least one component of these pollutant mixtures. Given the sample size, the results are meaningful for subjects with asthma as a group, but are not definitive for all people with asthma. These limitations must, however, be balanced with the fact that studies that involve sensitive populations are difficult to do. Because the exposures were designed to simulate ambient conditions of acidic summer haze, these findings provide some reassurance for public health concerns regarding exposures to these pollutants. Nonetheless, the impact of the loss of data from the subjects who left on the study results remains indeterminable and seriously limits extending these findings to subjects with moderate or severe asthma.

TECHNICAL EVALUATION OF DR. UTELL'S STUDY

SPECIFIC AIMS AND ATTAINMENT OF STUDY OBJECTIVES

Dr. Utell's major objective was to investigate whether prior exposure to an aerosol of H_2SO_4 alters the airway responsiveness of healthy subjects or subjects with asthma to subsequent ozone exposure. The investigators had three specific aims:

- to evaluate the interactive effects of ozone with a commonly coexistent pollutant, H_2SO_4 aerosol;
- to determine whether the sensitizing effect of prior acid aerosol exposure changes the threshold level of ozone that causes physiological effects or alters the dose-response profile; and
- to provide additional information on the effects of ozone exposure on asthmatic subjects.

The investigators achieved their primary goal, which was to determine if preexposure to H_2SO_4 modifies the effects of ozone exposure on pulmonary function in healthy subjects or subjects with asthma. It is noteworthy that they were successful in completing nearly 100% of their testing. The one subject who left the study did so because of a change in residence. The investigators implemented careful laboratory and quality assurance procedures, and consistently achieved a high level of scientific rigor in the design and conduct of the experiment.

STUDY DESIGN AND METHODS

Thirty healthy subjects and thirty subjects with asthma (males and females, between 18 and 45 years of age) were exposed to each test atmosphere for three hours in an exposure chamber. Subject selection is clearly described in the Methods section of the Investigators' Report. The exposure protocol was designed to determine exposure-response relations by using three different concentrations of ozone. Using random assignment, the first exposure protocol consisted of an aerosol of either H_2SO_4 at 100 $\mu g/m^3$, or sodium chloride (NaCl) at 100 $\mu g/m^3$, followed 24 hours

later by exposure to either 0.08, 0.12, or 0.18 ppm ozone. Two to four weeks later, the exposure protocol included the aerosol not previously administered and the same concentration of ozone that was administered the first time. All exposures were conducted in an environmental chamber, and the subjects exercised for 10 minutes out of every 30 minutes at a work load sufficient to quadruple the rate of their resting minute ventilation. Studies were discontinued from mid-June through mid-September to avoid the periods of highest ambient ozone and acid aerosol concentrations in the eastern part of the United States.

Dr. Utell's study incorporated an incomplete block design such that each subject was exposed to four of the six possible acid aerosol–ozone combinations. A total of nine days (four test atmospheres × two days per test atmosphere plus one training day) were required of each subject to complete the protocol. Pulmonary function tests, which included FVC, FEV_1, and specific airway conductance, were performed before, during, immediately after, and two and four hours after exposure. Dr. Utell chose a sequential rather than combined exposure protocol because (1) previous studies using combined oxidant–acid aerosol exposures had not revealed synergistic effects, and (2) simultaneous exposures to two or more pollutants increases the probability of airborne reactions between the pollutants, thereby changing the speciation of the test atmosphere.

The protocol for this study was well-described and the exposures were carefully executed. The investigators used conventional equipment for measuring pulmonary function and airway resistance. The ozone exposure concentrations are comparable to reported ambient levels; however, the concentration of H_2SO_4 selected for the study (100 $\mu g/m^3$) is on the high end of the range of measurements for polluted ambient air. The pulmonary function testing was expertly performed by this experienced team of investigators, and the quality assurance program was followed rigorously.

STATISTICAL METHODS

The statistical methods employed in this study were appropriate and thoughtful. The authors used an incomplete block design, which randomly assigned a subset of four of the six study exposures to each participant. This made it possible to obtain comparative data on six exposures and yet limit the burden of participation for individual subjects. This imaginative solution to the potential problem of excessive participant burden may partially explain the investigators' success in retaining participants. Every participant remained in the study until its conclusion and every primary measurement was obtained as planned.

Three-way mixed-models analysis of variance was used to analyze separately the healthy subjects and subjects with asthma. In addition, both groups were analyzed simultaneously by a four-way analysis of variance. These methods are appropriate. Longitudinal analysis of polynomial coefficients would also have been appropriate for repeated-measures data, although the results can be difficult to interpret.

Had the data been incomplete, the investigators would have needed to use specialized statistical software to carry out the proper analysis. Because there were no missing observations, standard statistical software produced a valid analysis that took proper account of the correlation among repeated measures on individual subjects.

RESULTS AND INTERPRETATION

The main findings of Dr. Utell's study were as follows: In healthy subjects, no statistically significant effects of acid aerosol exposure were observed for any of the lung function measurements, nor did preexposure to H_2SO_4 aerosol affect the subject's response to subsequent ozone exposure. Similar results were obtained for measurements of FEV_1, and specific airway resistance in subjects with asthma. However, Dr. Utell found statistically significant mean decrements in FVC measurements for subjects with asthma exposed to the highest concentration (0.18 ppm) of ozone. This effect was dependent on dose and was enhanced by preexposure to H_2SO_4.

An interesting observation from this study is that the changes reported for the subjects with asthma were changes in lung volume, specifically FVC, rather than the changes in flow rate, such as FEV_1, that have been reported by numerous other investigators following exposure of subjects to ozone. In addition, Dr. Utell's team did not observe episodes of bronchospasm after H_2SO_4 and ozone exposures. Because even the changes in lung function that were reported for subjects with asthma were relatively modest, the possibility exists that the three-hour exposure to even the highest ozone concentration (0.18 ppm) may have been too short to reflect adequately the extent of changes that could occur after longer exposures to ozone under ambient conditions. Moreover, although the investigators carefully avoided subject exposures during the periods of high ambient pollution, they did not similarly control for allergen blooms that could have affected the responsiveness of the subjects with asthma. Other explanations for these unexpected results include a residual effect from bronchodilator therapy, exercise-induced bronchodilation, or desensitization from ambient air pollution.

A notable finding of this study was the heterogeneity in the results among the subjects with asthma exposed to the

H_2SO_4 aerosol and 0.18 ppm ozone (illustrated in Figure 21 of the Investigators' Report). These data indicate that the linear trends reported for the responses of the subjects with asthma to the various acid aerosol–ozone exposures represent mean values overlying a background of heterogenous responses to the exposures. For example, Figure 21 indicates that four subjects with asthma exhibited greater than 10% increases in FEV_1 after the exposure, although the other subjects exhibited a range of decreases in this same parameter. It is not clear whether these results distinguish a susceptible population of subjects with asthma because it is uncertain if these individual responses are repeatable. It is also not clear whether this heterogeneity is similarly observed in other pulmonary function measurements, after preexposure with NaCl rather than H_2SO_4, or if it occurs in the healthy subject population. Unfortunately, no predictive characteristics can be gleaned from these results because they were not correlated with age, gender, serum IgE levels, or baseline lung function. Interestingly, a strong correlation between reactivity to carbachol and responsiveness to H_2SO_4 was observed in a previous study by this same group (Utell et al. 1983b).

The authors chose to use NaCl aerosol as a control instead of clean air because its particle size is similar to that of H_2SO_4. However, preexposure to NaCl aerosol had paradoxical effects. In both normal subjects and subjects with asthma, it appears to have mitigated the decrements in pulmonary function expected to occur at high ozone exposure concentrations. This trend is noted in measurements of both FVC and FEV_1. The authors attribute the apparently protective effects of NaCl aerosol exposure to exercise-induced bronchodilation in some subjects. Furthermore, they suggest that after preexposure to H_2SO_4, bronchodilation occurred in some subjects at only the lowest ozone concentration because the higher ozone concentrations attenuated the exercise-induced bronchodilation. However, it is difficult to understand how preexposure to NaCl could reverse the well-described, dose-dependent effects of ozone on pulmonary function. Regardless of the explanation, the fact that NaCl reduced a well-described physiologic response to ozone exposure is surprising. The apparently beneficial consequences of preexposure to NaCl aerosol and the variability in the observed changes in FVC make the deleterious effects of H_2SO_4 exposure somewhat less convincing.

It is noteworthy that fogs of both HNO_3 and H_2O have been shown to attenuate the decreases in pulmonary function induced by ozone (Aris et al. 1991). Aris postulated that such fogs may act by either physically coating and thereby protecting the airway epithelium, or by stimulating the secretion of protective mucus. An alternative explanation is that fogs stimulated the production of antioxidants that could modify the effects of ozone. In retrospect, an additional control exposure to clean air in the Utell study might have provided insight into these complex relationships, and it is unfortunate that it was not practical to add it to the protocol. If such data were available, they would have provided a means of evaluating any exercise-induced functional changes that might have occurred, especially in the subjects with asthma.

In summary, this was a thorough and informative study; it highlights the complexities associated with controlled human studies, and raises many questions about the design and direction of future studies.

DISCUSSION OF THE RESULTS OF THE STUDIES BY DRS. KOENIG AND UTELL

Drs. Koenig and Utell and their collaborators investigated the pulmonary function effects of exposure to mixtures of air pollutants that commonly occur in photochemical smog. Both investigators focused on individuals with asthma, a potentially susceptible population. They designed their experiments to mimic ambient ozone and acid aerosol concentrations and included exercise in their protocols in order to imitate normal outdoor activity. As indicated in Table 1 of this Commentary, both investigator groups used standard methods for exposing human subjects to controlled atmospheres and measuring the outcome variables.

Two approaches were used to assure the quality of data in these two studies. First, standard operating procedures were adopted for all aspects of both studies to ensure that the collected data were accurate and precise; this was primarily a quality control effort. Second, an external contractor, Arthur D. Little, Inc., was given the specific responsibility for overall quality assurance for both studies. Representatives of Arthur D. Little reviewed the standard operating procedures, and periodically audited the test centers to assure that the investigators were adhering to the protocols and procedures for each study. These two approaches are closely related, and both affect the quality and reliability of the data. During the periodic visits to the test centers, the Arthur D. Little audit team checked whether or not the data were being recorded accurately and appropriately on standardized forms. Reports from these periodic visits were sent to the HEI Director of Research and any necessary corrective action was requested by the HEI Staff Scientist responsible for monitoring each research project. A summary of the external quality assurance report can be found in Appendices A and F of the Koenig and Utell Investigators' Reports, respectively.

Although most of the findings of these studies were negative, some differences in the reported results are apparent. This is not surprising given the differences in experimental designs. Dr. Koenig found no statistically significant changes in pulmonary function in adolescent subjects with asthma after any of the simultaneous exposures to mixtures of oxidants and acid aerosols that she tested. In contrast, Dr. Utell found some statistically significant changes in pulmonary function in subjects with asthma after sequential exposures to H_2SO_4 and 0.18 ppm ozone. The most obvious difference in design was the order of administration of pollutants. Dr. Koenig administered two oxidants (ozone, NO_2) and one of two acid aerosols (H_2SO_4, HNO_3) simultaneously; Dr. Utell first administered either H_2SO_4 or NaCl (as a control atmosphere), and then ozone one day later. Other differences included the subjects' age range, the dose of pollutants and duration of exposure, the control atmosphere, the testing season, and, perhaps, the severity of subjects' asthma (see Table 1). Another difference between the two studies was the mode of pollutant administration. Dr. Koenig administered pollutants by a mouthpiece, and Dr. Utell exposed his subjects in an environmental chamber. Furthermore, the exercise levels were different, as evidenced by a three-fold increase in ventilation in Dr. Koenig's study, and a four-fold increase in Dr. Utell's study.

The results of both studies suggest that a subpopulation of subjects with asthma may be more susceptible than others to the effects of smog components such as acid aerosols and ozone. This population is more evident in Dr. Utell's study, in which the responses of subjects with asthma to H_2SO_4 and ozone exposure showed a great heterogeneity in the range of responses. Figure 21 of Dr. Utell's report indicates that some subjects with asthma experienced large decrements in FVC induced by pollutant exposure, whereas others had increased FVC. It is not known whether this is a reproducible response. Because the pulmonary function data were not presented for individual subjects in Dr. Koenig's report, the range of responses cannot be determined. However, as noted previously, the subjects who left Dr. Koenig's study were characterized as having moderate or severe asthma, and they left the study after exposure to a pollutant rather than to clean air. It is possible that these subjects may have represented a subpopulation of sensitive subjects with asthma. If this is true, subjects with asthma who are more sensitive to pollutant exposure may be identifiable by their response to methacholine challenge and the associated rating of asthma severity. However, in the remaining subjects, methacholine challenge did not correlate with any changes in FEV_1 induced by pollutant exposure. It is apparent from these studies that identifying a subpopulation of subjects with asthma who are more sensitive to ozone, if one exists, is not a simple matter. This is true not only because of the difficulties of designing and interpreting controlled human studies, but also because no criteria have been established by which to identify such a subpopulation.

REMAINING UNCERTAINTIES AND IMPLICATIONS FOR FUTURE RESEARCH

These studies provide insights into possible directions for future research. First, the criteria for selecting a study population of subjects with asthma must be considered carefully. Although both studies used state-of-the-art criteria for subject selection, it is apparent that these criteria did not allow for identifying a subpopulation of subjects with asthma who are particularly sensitive to ozone, if such a subpopulation exists. A study design that plans for potentially sensitive subpopulations or a study in which sensitive and nonsensitive subjects are carefully tested and differentiated might be considered. Consistent responses would support the existence of truly sensitive subjects with asthma, and could identify how other such individuals can be identified.

Another aim of future studies involving subjects with asthma might be to reduce potential sources of variability in the data. One approach would be to standardize medication, for example, by not including individuals on different medication regimens. This would reduce the variability of effects caused by different drugs and simplify the interpretation of the observed effects. Investigators also could consider establishing a standard restriction (e.g., 24 hours) on subject's medication usage before pollutant exposure. The 24-hour restriction is used most often in clinical studies, and its use could reduce confounding factors that may be attributable to specific effects of medication. Clinical considerations, however, may prevent this length of restriction except in subjects with minimal symptoms. A multicenter study of a large number of subjects with asthma also might provide greater understanding of the responses of individual subjects than would clinical investigations of 20 to 30 subjects. This option, of course, depends upon a significant level of funding.

Other goals of future studies could be to define more clearly the dose duration and sequence variables for combined pollutant exposures. This could be accomplished by increasing the duration of the exposures or by employing a range of exposure concentrations. Focusing on subjects with asthma and prolonging the exposure to each ozone

concentration might produce more striking effects than were observed in Dr. Utell's study. However, increasing these parameters also may be a questionable plan for a potentially susceptible population. Other biological endpoints beyond those investigated in the present studies also could be evaluated.

Other questions that need to be considered are: Is FVC a better indicator of functional alterations than FEV_1? and What is the best way to control for confounding factors, such as seasonal allergen blooms and ambient levels of oxidant pollutants and particles?

The results of these studies emphasize the importance of improving our understanding of the mechanisms that underly the range of human responsiveness to inhaled oxidant gases and acid aerosols. This is true not only for healthy individuals, but also for those groups of individuals, such as subjects with asthma, who are potentially more susceptible to the adverse health effects of air pollutants.

SUMMARY AND CONCLUSIONS

The results of these two studies of exposures to oxidant gases and acid aerosols have yielded interesting but somewhat equivocal results. Dr. Koenig found no changes in pulmonary function associated with exposure to oxidants (ozone, NO_2) in combination with acid aerosol (H_2SO_4 or HNO_3) in adolescent subjects with asthma. Interpretation of these results is limited, however, by the fact that approximately 20% of the subject population did not complete the study. Dr. Utell reported no statistically significant changes in symptoms or pulmonary function after sequential exposures to H_2SO_4 and ozone in healthy subjects. In subjects with asthma, however, a statistically significant mean decrease in one pulmonary function measurement, FVC, was reported after exposure to sequentially administered H_2SO_4 and ozone. However, in clinical terms, the absolute amount of this decrease was small.

An interesting observation that emerges from these data is the potential existence of a subpopulation of subjects with asthma that is particularly sensitive to exposure to oxidants and acid aerosols. The dose-response curves for individual subjects in Dr. Utell's study revealed a great diversity in the pulmonary function responses of the subjects with asthma. Although it is uncertain if these responses can be replicated, this diversity may have masked the response of a sensitive subpopulation. In Dr. Koenig's study, the six subjects with asthma who left the study did so after a pollutant exposure, and all were classified as having moderate or severe asthma. These subjects may represent a sensitive subpopulation, and as such, have important implications for the design of future studies.

Controlled human exposure studies provide information of direct relevance to the process of setting standards for criteria pollutants such as ozone. However, most of the existing data are from exposures to individual pollutant gases; little is known about the interactive effects of multiple pollutants. The results of these two studies suggest that the effects on pulmonary function of exposures (combined or sequential) to mixtures of oxidant gases and acid aerosols did not differ substantially from the effects of individual pollutant exposures. However, these results must be interpreted in the context of the investigators' protocols, which were directed toward simple two- or three-component mixtures, and do not reflect the complex ambient atmosphere. The results suggest, moreover, that a subpopulation of people with asthma may be more seriously affected by exposure to multiple pollutants; future studies should be directed toward identifying and studying this population.

ACKNOWLEDGMENTS

The Review Committee wishes to thank Dr. Brenda Barry and the ad hoc reviewers for their help in evaluating the scientific merit of these Investigators' Reports, and Drs. Nancy Silva and Kathleen Nauss for assisting the Committee in preparing its Commentary. The Committee also acknowledges Virgi Hepner and Valerie Carr for overseeing the publication of this report, and Diane Foster and Mary L. Stilwell for their editorial and administrative support.

REFERENCES

Adams WC, Brookes KA, Schelegle ES. 1987. Effects of NO_2 alone and in combination with ozone on young men and women. J Appl Physiol 62:1698–1704.

Adams WC, Schelegle ES. 1983. Ozone and high ventilation effects on pulmonary function and endurance performance. J Appl Physiol 55:805–812.

Air Contaminants Rule. 1993. 40 Code of Federal Regulations Part 1910. Federal Register 6-30-93.

Aris R, Christian D, Sheppard D, Balmes JR. 1991. The effects of sequential exposure to acidic fog and ozone on pulmonary function in exercising subjects. Am Rev Respir Dis 143:85–91.

Avol EL, Jones MP, Bailey RM, Chang NMN, Kleinman MT, Linn WS, Bell KA, Hackney JD. 1979. Controlled exposures of human volunteers to sulfate aerosols. Am Rev Respir Dis 120:319–327.

Avol EL, Linn WS, Shamoo DA, Anderson KR, Peng RC, Hackney JD. 1990. Respiratory responses of young asthmatics volunteers in controlled exposures to sulfuric acid aerosols. Am Rev Respir Dis 142:343–348.

Avol EL, Linn WS, Venet TG, Shamoo DA, Hackney JD. 1984. Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise. J Air Pollut Control Assoc 34:804–809.

Avol EL, Linn WS, Wightman LH, Whynot JD, Anderson KR, Hackney JD. 1988a. Short-term respiratory effects of sulfuric acid in fog: A laboratory study of healthy and asthmatic volunteers. J Air Pollut Control Assoc 38:258–263.

Avol EL, Linn WS, Whynot JD, Anderson KR, Shamoo DA, Valencia LM, Little DE, Hackney JD. 1988b. Respiratory dose-response study of normal and asthmatic volunteers exposed to sulfuric acid aerosol in the sub-micrometer size range. Toxicol Ind Health 4:173–184.

Balmes JR. 1993. The role of ozone exposure in the epidemiology of asthma. Environ Heath Perspect (Suppl) 101:219–224.

Bates DV, Bell GM, Burnham CD, Hazucha M, Mantha J, Pengelly LD, Silverman F. 1972. Short-term effects of ozone 0on the lung. J Appl Physiol 32:176–181.

Bates DV, Sizto R. 1983. Relationship between air pollutant levels and hospital admissions in Southern Ontario. Can J Public Health 74:117–122.

Bates DV, Sizto R. 1987. Air pollution and hospital admissions in Southern Ontario: The acid summer haze effect. Environ Res 43:317–331.

Bates DV, Sizto R. 1989. The Ontario air pollution study: Identification of the causative agent. Environ Health Perspect 79:69–72.

Bauer MA, Utell MJ, Morrow PE, Speers DM, Gibb FR. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. Am Rev Respir Dis 134:1203–1208.

Bedi JF, Drechsler-Parks DM, Horvath SM. 1985. Duration of increased pulmonary function sensitivity to an initial ozone exposure. Am Ind Hyg Assoc J 46:731–734.

Braun-Fahrlander C, Ackermann-Liebrich U, Wanner H-U, Rutishauser M, Gnehm HE, Minder CE. 1992. Air pollution and respiratory symptoms in preschool children. Am Rev Respir Dis 145:42–47.

Bylin G, Lindvall T, Rehn T, Sundlin B. 1985. Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. Eur J Respir Dis 66:205–217.

DeLucia AJ, Adams WC. 1977. Effects of ozone inhalation during exercise on pulmonary function and blood biochemistry. J Appl Physiol 43:75–81.

Dijkstra L, Houthuijs D, Brunekreef B, Akkerman I, Boleij JSM. 1990. Respiratory health effects of the indoor environment in a population of Dutch children. Am Rev Respir Dis 142:1172–1178.

Dockery DW, Speizer FE, Stram DO, Ware JH, Spengler JD, Ferris BG. 1989. Effects of inhalable particles on respiratory health of children. Am Rev Respir Dis 139:587–594.

Ekwo EE, Weinberger MM, Lachenbruch PA, Huntley WH. 1983. Relationship of parental smoking and gas cooking to respiratory disease in children. Chest 84:662–668.

Ericsson G, Cramner P. 1983. Health effects of sulfur oxides and particulate matter in ambient air. Scand J Work Environ Health 9(S3):1–52.

Florey C du V, Melia RJ, Chinn S, Goldstein BD, Brooks AGF, John HH, Craighead IB, Webster X. 1979. The relation between respiratory illness in primary schoolchildren and the use of gas for cooking: III. Nitrogen dioxide, respiratory illness, and lung infection. Int J Epidemiol 8:347–353.

Folinsbee JJ, Bedi JF, Horvath SM. 1984. Pulmonary function changes after one hour continuous heavy exercise in 0.21 ppm ozone. J Appl Physiol 57:984–988.

Folinsbee LJ, Drinkwater BL, Bedi JF, Horvath SM. 1978. The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In: Environmental Stress: Individual Human Adaptations (Folinsbee LJ, Wagner JA, Borgia JF, Drinkwater BL, Gliner JA, Bedi JF, eds.) pp. 125–145. Academic Press, Orlando, FL.

Folinsbee LJ, Silverman F, Shephard RJ. 1975. Exercise responses following ozone exposure. J Appl Physiol 38:996–1001.

Goldsmith JR, Griffith HL, Detels R, Beeser S, Neumann L. 1983. Emergency room admissions, meteorologic variables, and air pollutants: A path analysis. Am J Epidemiol 118:759–778.

Goldstein BD, Melia RJ, Chinn S, Florey C du V, Clark D, John HH. 1979. The relation between respiratory illness in primary school children and the use of gas cooking: II. Factors affecting nitrogen dioxide levels in the home. Int J Epidemiol 8:339–345.

Gong H Jr., Bradley PW, Simmons MS, Tashkin DP. 1986. Impaired exercise performance and pulmonary function in elite cyclists during low-level ozone exposure in a hot environment. Am Rev Respir Dis 134:726–733.

Hackney JD, Linn WS, Law DC, Karuza SK, Greenberg H, Buckley RD, Pedersen EE. 1975. Experimental studies on human health effects of air pollutants: III. Two-hour exposure to ozone alone and in combination with other pollutant gasses. Arch Environ Health 30:385–390.

Hanley QS, Koenig JQ, Larson TV, Anderson TL, Van Belle G, Rebolledo V, Covert DS, Pierson WE. 1992. Response of young asthmatic patients to inhaled sulfuric acid. Am Rev Respir Dis 145:326–331.

Hasseblad V, Eddy DM, Kotchmar DJ. 1992. Synthesis of environmental evidence: Nitrogen dioxide epidemiology studies. J Air Waste Manage Assoc 42:662–671

Hazucha M, Silverman F, Parent C, Field S, Bates DV. 1973. Pulmonary function in man after short-term exposure to ozone. Arch Environ Health 27:183–188.

Hoek G, Brunekreef B. 1992. Time trends in repeated spirometry in children. Eur J Respir Dis 5:553–559.

Horstman D, Folinsbee LJ, Ives PJ, Abdul-Salaam S, McDonnell WF. 1990. Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, 0.12 ppm. Am Rev Respir Dis 142:1158–1163.

Horvath SM, Folinsbee LJ, Bedi JF. 1982. Effects of large (0.9 μm) sulfuric acid aerosols on human pulmonary function. Environ Res 28:123–130.

Horvath SM, Folinsbee LJ, Bedi JF. 1987. Combined effect of ozone and sulfuric acid on pulmonary function in man. Am Ind Hyg Assoc J 48:94–98.

Horvath SM, Gliner JA, Folinsbee LJ. 1981. Adaptation to ozone: Duration of effect. Am Rev Respir Dis 123:496–499.

Horvath SM, Gliner JA, Matsen-Twisdale JA. 1979. Pulmonary function and maximum exercise responses following acute ozone exposure. Aviat Space Environ Med 50:901–905.

Kinney PL, Özkaynak H. 1991. Associations of daily mortality and air pollution in Los Angeles County. Environ Res 54:99–120.

Kinney PL, Özkaynak H. 1992. Association between ozone and daily mortality in Los Angeles and New York City. Am Rev Respir Dis 145:A95.

Kitagawa T. 1984. Cause analysis of the Yokkaichi asthma episode in Japan. J Air Pollut Control Assoc 34:743–746.

Koenig JQ, Covert DS, Larson TV, Pierson WE. 1992. The effect of duration of exposure on sulfuric acid-induced pulmonary function changes in asthmatic adolescent subjects: A dose-response study. Toxicol Ind Health 8:285–296.

Koenig JQ, Covert DS, Marshall SG, Van Belle G, Pierson WE. 1987. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. Am Rev Respir Dis 136:1152–1157.

Koenig JQ, Covert DS, Morgan MS, Horike M, Horike N, Marshall SG, Pierson WE. 1985. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am Rev Respir Dis 132:648–651.

Koenig JQ, Covert DS, Pierson WE. 1989. Effects of inhalation of acidic compounds on pulmonary function in allergic adolescent subjects. Environ Health Perspect 79:173–178.

Koenig JQ, Covert DS, Smith MS, Van Belle G, Pierson WE. 1988. The pulmonary effects of ozone and nitrogen dioxide alone and combined in healthy and asthmatic adolescent subjects. Toxicol Ind Health 4:521–532.

Koenig JQ, Pierson WE, Horike M. 1983. The effects of inhaled sulfuric acid on pulmonary function in adolescent asthmatics. Am Rev Respir Dis 128:221–225.

Kreit JW, Gross KB, Moore TB, Lorenzen TJ, D'Arcy J, Eschenbacher WL. 1989. Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J Appl Physiol 66:217–222.

Krzyzanowski M, Quackenboss JJ, Lebowitz MD. 1992. Relation of peak expiratory flow rates and symptoms to ambient ozone. Arch Environ Health 47:107–115.

Kulle TJ, Clements ML. 1988. Susceptibility to Virus Infection with Exposure to Nitrogen Dioxide. Research Report Number 15. Health Effects Institute, Cambridge, MA.

Kulle TJ, Kerr HD, Farrell BP, Sauder LR, Bermel MS. 1982. Pulmonary function and bronchial reactivity in human subjects with exposure to ozone and respirable sulfuric acid aerosol. Am Rev Respir Dis 126:996–1000.

Kulle TJ, Sauder LR, Hebel JR, Chatman MD. 1985. Ozone response in healthy nonsmokers. Am Rev Respir Dis 132:36–41.

Lebowitz MD, Collins L, Holberg CJ. 1987. Time series analyses of respiratory responses to indoor and outdoor environmental phenomena. Environ Res 43:332–341.

Leikauf GD, Spektor DM, Albert RE, Lippmann M. 1984. Dose-dependent effects of sub-micrometer sulfuric acid aerosol on particle clearance from ciliated human lung airways. Am Ind Hyg Assoc J 45:285–292.

Leikauf GD, Yeates DB, Wales KA, Spektor DM, Albert RE, Lippmnan M. 1981. Effects of sulfuric acid aerosol on respiratory mechanics and mucociliary particle clearance in healthy nonsmoking adults. Am Ind Hyg Assoc J 42:273–282.

Linn WS, Avol EL, Anderson KR, Shamoo DA, Peng RC, Hackney JD. 1989. Effect of droplet size on respiratory responses to inhaled sulfuric acid in normal and asthmatic volunteers. Am Rev Respir Dis 140:161–166.

Linn WS, Avol EL, Anderson KR, Shamoo DA, Peng RC, Hackney JD. 1991. Respiratory responses of healthy volunteers in prolonged repeated exposures to ozone and sulfuric acid. Am Rev Respir Dis 143:A97.

Linn WS, Avol EL, Shamoo DA, Spier CE, Valencia LM, Venet TG, Fischer DA, Hackney JD. 1986. A dose-response study of healthy, heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. Toxicol Ind Health 2:99–112.

Linn WS, Avol EL, Shamoo DA, Whynot JD, Anderson KR, Hackney JD. 1986. Respiratory responses of exercising asthmatic volunteers exposed to sulfuric acid aerosol. J Air Pollut Control Assoc 36:1323–1328.

Linn WS, Buckley RD, Spier CE, Blessey RL, Jones MP, Fischer DA, Hackney JD. 1978. Health effects of ozone exposure in asthmatics. Am Rev Respir Dis 117:835–843.

Linn WS, Medway DA, Anzar UT, Valencia LM, Spier CE, Rsao FSD, Fischer DA, Hackney JD. 1982. Persistence of adaptation to ozone in volunteers exposed repeatedly for six weeks. Am Rev Respir Dis 125:491–495.

Linn WS, Shamoo DA, Spier CE, Valencia LM, Anzar UT, Venet TG, Avol EL, Hackney JD. 1985. Controlled exposure of volunteers with chronic obstructive pulmonary disease to nitrogen dioxide. Arch Environ Health 40:313–317.

Lippmann M. 1989. Ozone health effects and emerging issues in relation to standards setting. In: Atmospheric Ozone Research and Its Policy Implications (Schneider T, Lee SD, Wolters GJR, Grant LD, eds.) pp 21–33. Elsevier Science Publishing Co., New York, NY.

Lippmann M. 1992. Ozone. In: Environmental Toxicants: Human Exposures and Their Health Effects (Lippmann M. ed.) pp 465–519. Van Nostrand Reinhold, New York, NY.

McCool FD, Paek D. 1993. Noninvasive Methods for Measuring Ventilation in Mobile Subjects: Measurements of Ventilation in Freely Ranging Subjects. Research Report Number 59. Health Effects Institute, Cambridge, MA.

McDonnell WF, Horstman DH, Hazucha MJ, Seal E Jr, Haak ED, Salaam SA, House DE. 1983. Pulmonary effects of ozone exposure during exercise: Dose-response characteristics. J Appl Physiol 54:1345–1352.

McDonnell WF, Kehrl HR, Abdul-Salaam S, Ives PJ, Folinsbee LJ, Devlin RB, O'Neil JJ, Horstman DH. 1991. Respiratory repsonse of humans exposed to low levels of ozone for 6.6 hours. Arch Environ Health 46:145–150.

Melia RJ, Florey C du V, Altman DG, Swan AV. 1977. Association between gas cooking and respiratory disease in children. Br Med J (Clin Res Ed) 2:149–152.

Melia RJ, Florey C du V, Chinn S. 1979. The relation between respiratory illness in primary school children and the use of gas cooking: Results from a national survey. Int J Epidemiol 8:333–338.

Melia RJ, Florey C du V, Sittampalam Y, Watkins C. 1983. The relation between respiratory illness in infants and gas cooking in the U.K.: A preliminary report. In: Proceedings of the 6th World Congress in Air Quality, August 16-20, 1983, pp. 263–269. International Union of Air Pollution Prevention Associations, Paris, France.

Mohsenin V. 1987. Airway responses to nitrogen dioxide in subjects with asthma. J Toxicol Environ Health 22:371–380.

Mohsenin V. 1988. Airway responses to 2.0 ppm nitrogen dioxide in normal subjects. Arch Environ Health 43:242–246.

Neas LM, Dockery DW, Ware JH, Spengler JD, Speizer FE, Ferris BG Jr. 1991. Association of indoor nitrogen dioxide with respiratory symptoms and pulmonary function in children. Am J Epidemiol 134:204–219.

Ogston SA, Florey C du V, Walker CH. 1985. The Tayside infant morbidity and mortality study: Effects on health of using gas for cooking. Br Med J (Clin Res Ed) 290:957–960.

Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. 1976. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. J Clin Invest 57:301–307.

Ostro BD. 1990. Associations between morbidity and alternative measures of particulate matter. Risk Anal 10:421–427.

Ostro BD, Lipsett MJ, Weiner MB, Selner JC. 1991. Asthmatic responses to airborne acid aerosols. Am J Public Health 81:694–702.

Ostro BD, Rothschild S. 1989. Air pollution and acute respiratory morbidity: An observational study of multiple pollutants. Environ Res 50:238–247.

Raizenne ME, Burnett RT, Stern B, Franklin CA, Spengler JD. 1989. Acute lung function responses to ambient acid aerosol exposures in children. Environ Health Perspect 79:179–185.

Rutishauser M, Ackermann U, Braun C, Gnehm HP, Wanner HU. 1990. Significant association between outdoor NO_2 and respiratory symptoms in preschool children. Lung 168(Suppl):347–352.

Sackner JD, Nixon AN, Davis B, Atkins N, Sackner MA. 1980. Effects of breathing through external dead space on ventilation at rest and during exercise. Am Rev Respir Dis 122:933–940.

Samet JM, Lambert WE, Skipper BJ, Cushing AH, Hunt WC, Young SA, McLaren LC, Schwab M, Spengler JD. 1993. Nitrogen Dioxide and Respiratory Illness in Children, Part I, Health Outcomes. Research Report Number 58. Health Effects Institute, Cambridge, MA.

Schoettlin CE, Landau E. 1961. Air pollution and asthmatic attacks in the Los Angeles area. Public Health Rep 76:545–548.

Silverman F. 1979. Asthma and respiratory irritants (ozone). Environ Health Perspect 29:131–136.

Silverman F, Folinsbee LJ, Barnard J, Shephard RJ. 1976. Pulmonary function changes in ozone-interaction of concentration and ventilation. J Appl Physiol 41:859–864.

Speizer FE. 1989. Studies of acid aerosols in six cities and in a new multi-city investigation: Design issues. Environ Health Perspect 79:61–67.

Speizer FE, Ferris BJ, Bishop YM, Spengler J. 1980. Respiratory disease rates and pulmonary function in children associated with NO_2 exposure. Am Rev Respir Dis 121:3–10.

Spektor DM, Lippmann M, Lioy PJ, Thurston GD, Citak K, James DJ, Bock N, Speizer FE, Hayes C. 1988a. Effects of ambient ozone on respiratory function in active, normal children. Am Rev Respir Dis 137:313–320.

Spektor DM, Lippmann M, Thurston GD, Lioy PJ, Stecko J, O'Connor G, Garshick E, Speizer FE, Hayes C. 1988b. Effects of ambient ozone on respiratory function in healthy adults exercising outdoors. Am Rev Respir Dis 138:821–828.

Spengler JD, Brauer M, Koutrakis P. 1990. Acid air and health. Environ Sci Technol 24:946–956.

Spengler JD, Keeler GJ, Koutrakis P, Ryan PB, Raizenne M, Franklin CA. 1989. Exposures to acid aerosols. Environ Health Perspect 79:43–51.

Stark GP, Hodous TK, Hankinson JL. 1988. The use of inductive plethysmography in the study of the ventilatory effects of respiratory wear. Am Ind Hyg Assoc J 49:401–408.

Sterling TD, Phair JJ, Pollack SV, Schumsky DA, DeGroot I. 1966. Urban morbidity and air pollution: A first report. Arch Environ Health 13:158–170.

Sterling TD, Pollack SV, Phair JJ. 1967. Urban hospital morbidity and air pollution: A second report. Arch Environ Health 15:362–374.

Sunyer J, Anto JM, Murillo C, Saez M. 1991. Effects of urban air pollution on emergency room admissions for chronic obstructive pulmonary disease. Am J Epidemiol 134:277:286.

Thurston GD, Ito K, Kinney PL, Lippmann M. 1992. A multi-year study of air pollution and respiratory hospital admissions in three New York State metropolitan areas: Results for 1988 and 1989 summers. J Exp Anal Environ Epidemiol 2:429–450.

Thurston GD, Ito K, Lippmann M, Hayes C. 1989. Reexamination of London, England mortality in relation to exposure to acidic aerosols during 1963-1972 winters. Environ Health Perspect 79:73–82.

Thurston GD, Lippmann M, Bartoszek M, Fine I. 1993. Air pollution associations with asthma exacerbations, peak flow changes and respiratory symptoms in children at a summer asthma camp. Am Rev Respir Dis 147:A633.

Tseng R-Y, Li C-K, Spinks JA. 1992. Particulate air pollution and hospitalization for asthma. Ann Allergy 68:425–432.

U.S. Environmental Protection Agency. 1992. National Air Quality and Emissions Trends Report 1992. EPA-454/R-93-031. Office of Air Quality Planning and Standards, Research Triangle Park, NC.

Utell MJ. 1989. Asthma and nitrogen dioxide: A review of the evidence. In: Susceptibility to Inhaled Pollutants (Utell MJ, Frank R, eds.) pp. 218–223. American Society for Testing and Materials, Philadelphia, PA.

Utell MJ, Morrow PE, Hyde RW. 1983a. Latent development of airway hyperreactivity in human subjects after sulfuric acid aerosol exposure. J Aerosol Sci 14:202–205.

Utell MJ, Morrow PE, Speers DM, Darling J, Hyde RW. 1983b. Airway responses to sulfate and sulfuric acid aerosols in asthmatics. Am Rev Respir Dis 128:444–450.

Ware JH, Dockery DW, Spiro A, Speizer FE, Ferris BG Jr. 1984. Passive smoking, gas cooking, and respiratory health of children in six cities. Am Rev Respir Dis 129:366–374.

Ware JH, Ferris BG, Dockery DW, Spengler JD, Stram DO, Speizer FE. 1986. Effects of ambient sulfur oxides and suspended particles on respiratory health of preadolescent children. Am Rev Respir Dis 133:834–842.

Whittemore AS, Korn EL. 1980. Asthma and air pollution in the Los Angeles area. Am J Public Health 70:687–696.

RELATED HEI PUBLICATIONS

Report No.	Title	Principal Investigator	Publication Date
Ozone, Nitrogen Dioxide, and Pulmonary Function			
HEI Research Reports			
6	Effect of Nitrogen Dioxide, Ozone, and Peroxyacetyl Nitrate on Metabolic and Pulmonary Function	D.M. Drechsler-Parks	1987
14	The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Asthmatic Adolescents	J.Q. Koenig	1988
23	Responses of Susceptible Subpopulations to Nitrogen Dioxide	P.E. Morrow	1989
43	Mechanisms of Nitrogen Dioxide Toxicity in Humans	M.J. Utell	1991
HEI Communications Number 3: Environmental Epidemiology Planning Project, Section on Tropospheric Ozone			
•	Examining Acute Health Outcomes Due to Ozone Exposure and Their Subsequent Relationship to Chronic Disease Outcomes	B.D. Ostro	1994
•	The Role of Ozone Exposure in the Epidemiology of Asthma	J.R. Balmes	1994
•	Identification of Subpopulations That Are Senstitive to Ozone Exposure: Use of End Points Currently Available and Potential Use of Laboratory-Based End Points Under Development	R.B. Devlin	1994
•	Design and Analysis of Studies of the Health Effects of Ozone	A. Munoz	1994
Acid Aerosols			
HEI Research Reports			
38	Synergistic Effects of Air Pollutants: Ozone Plus a Respirable Aerosol	J.A. Last	1991

Copies of these reports can be obtained by writing or calling the Health Effects Institute, 141 Portland Street, Suite 7300, Cambridge, MA 02139. Phone (617) 621-0266. FAX (617) 621-0267. Request a Publications and Documents booklet for a complete listing of publications resulting from HEI-sponsored research.

HEI

The Board of Directors

Archibald Cox *Chairman*
Carl M. Loeb University Professor (Emeritus), Harvard Law School

William O. Baker
Chairman (Emeritus), Bell Laboratories

Douglas Costle
Chairman of the Board and Distinguished Senior Fellow, Institute for Sustainable Communities

Donald Kennedy
President (Emeritus) and Bing Professor of Biological Sciences, Stanford University

Walter A. Rosenblith
Institute Professor (Emeritus), Massachusetts Institute of Technology

Health Research Committee

Bernard Goldstein *Chairman*
Director, Environmental and Occupational Health Sciences Institute

Joseph D. Brain
Chairman, Department of Environmental Health, and Cecil K. and Philip Drinker Professor of Environmental Physiology, Harvard University School of Public Health

Glen R. Cass
Professor of Environmental Engineering and Mechanical Engineering, California Institute of Technology

Seymour J. Garte
Professor and Deputy Director, Department of Environmental Medicine, New York University Medical Center

Leon Gordis
Professor and Chairman, Department of Epidemiology, Johns Hopkins University, School of Hygiene and Public Health

Stephen S. Hecht
Director of Research, American Health Foundation

Meryl H. Karol
Professor of Environmental and Occupational Health, University of Pittsburgh, Graduate School of Public Health

Robert F. Sawyer
Class of 1935 Professor of Energy (Emeritus), University of California at Berkeley

Gerald van Belle
Chairman, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington

Health Review Committee

Arthur Upton *Chairman*
Clinical Professor of Pathology, University of New Mexico School of Medicine

A. Sonia Buist
Professor of Medicine and Physiology, Oregon Health Sciences University

Gareth M. Green
Associate Dean for Education, Harvard School of Public Health

Donald J. Reed
Professor and Director, Environmental Health Sciences Center, Oregon State University

David J. Riley
Professor of Medicine, University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School

Herbert Rosenkranz
Chairman, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh

Robert M. Senior
Dorothy R. and Hubert C. Moog Professor of Pulmonary Diseases in Medicine, Washington University School of Medicine

James H. Ware
Dean of Academic Affairs and Professor of Biostatistics, Harvard School of Public Health

Frederick A. Beland *Special Consultant to the Committee*
Director for the Division of Biochemical Toxicology, National Center for Toxicological Research

Henry A. Feldman *Special Consultant to the Committee*
Senior Research Scientist, New England Research Institute

Edo D. Pellizzari *Special Consultant to the Committee*
Vice President for Analytical and Chemical Sciences, Research Triangle Institute

Officers and Staff

Daniel S. Greenbaum *President*
Richard M. Cooper *Corporate Secretary*
Kathleen M. Nauss *Director for Scientific Review and Evaluation*
Elizabeth J. Story *Director of Finance and Administration*
Jane Warren *Director of Research*
Maria G. Costantini *Senior Staff Scientist*
Chester A. Bisbee *Staff Scientist*
Aaron J. Cohen *Staff Scientist*
Bernard Jacobson *Staff Scientist*
Debra A. Kaden *Staff Scientist*
Martha E. Richmond *Staff Scientist*
Lata Shirnamé-Moré *Staff Scientist*

Gail V. Allosso *Senior Administrative Assistant*
Valerie Anne Carr *Publications Production Coordinator*
Diane C. Foster *Editorial Assistant*
L. Virgi Hepner *Managing Editor*
Debra N. Johnson *Controller*
Teresina McGuire *Accounting Assistant*
Jean C. Murphy *Research Associate*
Malti Sharma *Administrative Assistant*
Mary L. Stilwell *Administrative Assistant*
Stacy Synan *Administrative Assistant*
Susan J. Walsh *Receptionist*

HEI HEALTH EFFECTS INSTITUTE

141 Portland Street, Cambridge, MA 02139 (617) 621-0266

Research Report Number 70

November 1994