HEALTH EFFECTS INSTITUTE

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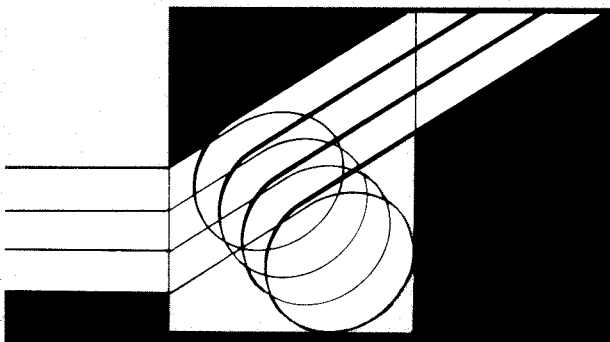
RESEARCH REPORT NO. 14



The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Ashmatic Adolescents

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Includes the Report of the Institute's Health Review Committee

The Health Effects Institute (HEI) is a non-profit corporation founded in 1980 to assure that objective, credible, high-quality scientific studies are conducted on the potential human health effects of motor vehicle emissions.

Funded equally by the U.S. Environmental Protection Agency (EPA) and 27 automotive manufacturers or marketers in the United States, HEI is independently governed. Its research projects are selected, conducted, and evaluated according to a careful public process, including a rigorous peer review process, to assure both credibility and high scientific standards.

HEI makes no recommendations on regulatory and social policy. Its goal, as stated by former EPA Administrator William D. Ruckelshaus, is "simply to gain acceptance by all parties of the data that may be necessary for future regulations."

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ABBREVIATIONS

EIB	Exercise-induced bronchospasm
FEV_1	forced expiratory volume in one second (litres)
FRC	functional residual capacity (litres)
FVC	forced vital capacity (litres)
PFT	pulmonary function tests
R_T	total respiratory resistance (cm H_2O/litre/second)
V50	maximal airflow at 50% of expired vital capacity (litres/sec)
V75	maximal airflow at 75% of expired vital capacity (litres/sec)

PREFACE

THE HEALTH EFFECTS INSTITUTE AND ITS RESEARCH PROCESS

The Health Effects Institute (HEI) is an independent nonprofit corporation which, according to its charter, is "organized and operated...specifically to conduct or support the conduct of, and to evaluate, research and testing relating to, the health effects of emissions from motor vehicles."

It is organized in the following ways to pursue this purpose:

INDEPENDENCE IN GOVERNANCE

HEI is governed by a four-member Board of Directors whose members are William O. Baker, Chairman Emeritus of Bell Laboratories and Chairman of the Board of Rockefeller University; Archibald Cox, Carl M. Loeb University Professor (Emeritus) at Harvard University; Donald Kennedy, President of Stanford University; and Charles Powers, President, Clean Sites, Incorporated. Professor Cox chairs the Board. These individuals, who select their own successors, were chosen initially, after consultation with industry and other individuals, by then Environmental Protection Agency Administrator Douglas M. Costle.

TWO-SECTOR FINANCIAL SUPPORT

The Institute receives half of its funds from the United States government through the Environmental Protection Agency and half from the automotive industry. Twenty-eight leading manufacturers of vehicles or engines that are certified for use on U.S. highways contribute to the Institute's budget, in shares proportionate to the number of vehicles or engines that they sell.

RESEARCH PLANNING AND PROJECT EVALUATION

HEI is structured to define, select, support, and review research that is aimed at investigating the possible health effects of mobile source emissions. Its research program is developed by the Health Research Committee, a multidisciplinary group of scientists knowledgeable about the complex problems involved in determining the health effects of mobile source emissions. The Committee seeks advice from HEI's sponsors and from other sources prior to independently determining the research priorities of the Institute.

After the Health Research Committee has defined an area of inquiry, the Institute announces to the scientific community that research proposals are being solicited on a specific topic. Applications are reviewed first for scientific quality by an appropriate expert panel. Then they are reviewed by the Health Research Committee both for quality and for relevance to the mission-oriented research program. Studies recommended by the Committee undergo final evaluation by the Board of Directors, which also reviews the procedures, independence, and quality of the selection process.

When a study is completed, a draft final report is reviewed by a separate HEI committee, the Health Review Committee. Members are expert scientists representing a broad range of experience in environmental health sciences. The Health Review Committee has no role either in the review of applications or in the selection of projects and investigators for funding. This Committee assesses the scientific quality of each study and evaluates its contribution to unresolved scientific questions.

Each funded proposal is assigned in advance of completion to a member of the Health Review Committee, who acts as "primary reviewer." When the draft report is received, the primary reviewer directs a peer review by technical experts and, when appropriate, a biostatistician. After the investigator has had a chance to comment on the technical evaluations, the primary reviewer drafts a final report review. This document is sent to the investigator for comment. It is subsequently examined by the full Health Review Committee and revised as necessary. The investigator's final report, as well as the Review Committee's report, are then made available to the sponsors and to the public after evaluation by the HEI Board of Directors.

All HEI investigators are urged to publish the results of their work in the peer-reviewed literature. The timing and nature of HEI report releases are tailored to ensure that the Health Review Committee's report does not interfere with the journal publication process. The report of the Health Review Committee will be as thorough as necessary to evaluate any individual report.

INTRODUCTION

In the summer of 1982, HEI issued a Request for Application (RFA 82-3) soliciting proposals on "Susceptible Populations." In January 1983, Dr. Jane Koenig of the University of Washington, Department of Environmental Health, proposed a project entitled, "The Effects of Ozone and Nitrogen Dioxide on Lung Function in Asthmatic Adolescent Subjects." HEI approved the three-year project and authorized expenditure of $265,481.75. The project began in April, 1983, and the final report was accepted by the Health Review Committee in April, 1987. The Health Review Committee report, which follows the investigators' report, is intended to place the investigators' final report in perspective as an aid to the sponsors of HEI and to the public.

CLEAN AIR ACT

The Environmental Protection Agency (EPA) sets standards for motor vehicle emissions of oxides of nitrogen (and other pollutants) under Section 202 of the Clean Air Act, as amended

in 1977. Section 202 (a)(1) directs the Administrator of EPA 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 judgement cause, or contribute to, air pollution which may reasonably be anticipated to endanger public health or welfare." Section 202(a)(3) and 202(b)(1) impose specific requirements for reductions in motor vehicle emissions of oxides of nitrogen (and other pollutants), and provide 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 to protect the public health. The current standards include those for ozone and nitrogen dioxide. The legislative history of the Act makes it clear that, in setting ambient air quality standards, EPA is required to consider the health of particularly sensitive subgroups of the population. The Senate report on the legislation states: "An ambient air quality standard... should be the maximum permissible air level of an air pollution agent or class of such agents (related to a period of time) which will protect the health of any group of the population" (U.S. Senate, 1970).† The identification of such groups is not clear, but the report does specify that "included among those persons whose health should be protected by the ambient standard are particularly sensitive citizens such as bronchial asthmatics and emphysematics who in the normal course of daily activity are exposed to the ambient environment" (U.S. Senate, 1970).† The report further states that "in establishing an ambient standard necessary to protect the health of these persons, reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group" (U.S. Senate, 1970).† Research on the sensitivity of adolescent asthmatics can contribute knowledge useful in determining whether that subgroup needs special protection under a national ambient air quality standard.

BACKGROUND

Fuel combustion from vehicles is a major source of the air pollutants, nitrogen dioxide (NO_2) and ozone (O_3). The primary target of these pollutants is the lung. Nitrogen oxides react with hydrocarbons and oxygen in sunlight to yield nitrogen dioxide, ozone, and oxidized hydrocarbons in a reversible reaction. Both nitrogen dioxide and ozone gases are chemically reactive but relatively insoluble. They penetrate deep into the lungs and reach the alveoli where the exchange of oxygen and carbon dioxide takes place. Animal studies suggest that on exposure to these pollutants, the ciliated cells (which are important in the normal clearance of airways and in the removal of foreign particles), as well as Type I alveolar epithelial cells (which line the alveolar sacs at the blood-air barrier of the lungs), suffer most damage (U.S. Environmental Protection Agency, 1986a).† One of the most important causes of damage is believed to be the oxidation of unsaturated lipids in the cellular membranes. Disruption of the cellular membrane follows; this probably accounts for many of the biological effects observed at high level exposures to these pollutants (bronchial spasms, pulmonary edema, morphological damage, hyperplasia).

The current National Air Ambient Quality Standard (NAAQS) for nitrogen dioxide is 0.053 ppm averaged over one year. Although the nitrogen dioxide standard is being met generally, peak one-hour concentrations continue to exceed the standard in many parts of the country where levels of between 0.15 ppm and 0.3 ppm are reached 10 to 20 days of the year (U.S. Environmental Protection Agency, 1986b).†

The current one-hour NAAQS for ozone is 0.12 ppm. This level is regularly exceeded in many urban areas, with daily maximum one-hour values of 0.34 ppm being recorded in several metropolitan areas of less than two million inhabitants. (A detailed analysis of ozone levels in metropolitan areas is provided in the 1986 ozone criteria document, U.S. Environmental Protection Agency, 1986b.)†

In this report, Koenig et al. have examined the effects of acute exposure to near ambient levels of nitrogen dioxide and ozone (0.12 ppm and 0.18 ppm, respectively) in resting and exercising individuals who were either healthy or suffered from the respiratory disorder known as allergic asthma. This study was undertaken because of: (1) a paucity of controlled human studies involving healthy and susceptible members of the population at near ambient levels, and (2) uncertainty about the lowest exposure levels of oxidative pollutants that cause health effects.

Variations exist in the susceptibility of different healthy individuals to adverse health effects caused by nitrogen dioxide and ozone; the causes of the observed variation is not well understood. When Dr. Koenig's study was initiated, no group of individuals defined by age or sex was known to have greater susceptibility than any other group to these pollutants.

Asthma is a respiratory disease that affects about twelve million people (about five percent of the population of the United States). It is characterized by episodes of overt airway obstruction caused by involuntary contractions of the smooth muscle surrounding the airways. Symptoms of asthma are heterogeneous but commonly include chest tightness, coughing, wheezing, and shortness of breath; differing severity and frequency of episodes are observed in different individuals. The types of asthma fall into two broad categories:

(a) Allergic (or extrinsic) asthma arises from a hypersensitive response to environmental agents (allergens), such as pollen or dust. Antibodies of the class IgE are made against these allergens by the affected person; IgE activates the release of histamine and an allergic reaction follows. An extrinsic asthma reaction occurs initially after exposure to an allergen either through skin or through the airways. This may be followed by a "late" hyperreactive response, which results in edema and inflammation of the airways that can persist for days or weeks.

† See References on page 27.

(b) Non-allergic (or intrinsic) asthma occurs in response to a variety of factors including viral or bacterial infections, chemicals, and exercise. There is no evidence of specific IgE involvement in this group.

Acute exposure to oxidant pollutants, such as nitrogen dioxide and ozone, can stimulate a "late" non-specific hyperreactive response in asthmatics with or without an "early" stimulus of an allergen (Bromberg, 1987)†. Possible mechanisms for this effect include the direct stimulation of airway epithelial cells to release relevant mediators, which in turn stimulate local nerve endings and result in the contraction of smooth muscle in a bronchial spasm. Alternatively, alteration of epithelial permeability induced by these pollutants could enable inhaled particles to gain access to sensitized cells, which would result in bronchoconstriction.

Human studies are governed by ethical and practical considerations. Those studies carried out on asthmatic individuals have used subjects who are asymptomatic or lightly symptomatic, and who are not on medication. In the current study, the more severely affected asthmatics who were on maintenance doses of anti-asthmatic drugs were chosen. These individuals were allowed to take medication until four hours before an experiment and were not sensitized by a primary stimulus. Exercise-induced bronchospasm occurs in these asthmatics but not in the healthy non-asthmatic controls. Attention also was paid to characterizing the form and extent of the asthma of the individuals, so that some homogeneity of the sample groups was achieved.

The controlled exposures used in this study are important because they can provide accurate measurements of exposure levels and conditions for a single pollutant or simple combination of pollutants. Valuable information can be obtained by selecting individual parameters of exposure encountered in the environment. It should be noted, however, that the environment consists of a variety of acute and chronic exposure conditions (U.S. Environmental Protection Agency, 1986a)†; continually changing exposure levels and combinations of multiple pollutants for varying lengths of time cause a range of adverse health effects that may exceed those observed in a controlled clinical study.

† See References on page 27.

INVESTIGATORS' REPORT

by Jane Q. Koenig, Ph.D., William E. Pierson, M.D., David S. Covert, Ph.D., Susan G. Marshall, M.D., Michael S. Morgan, Sc.D., Gerald van Belle, Ph.D.

The Effects of Ozone and Nitrogen Dioxide on Lung Function in Healthy and Asthmatic Adolescents.

ABSTRACT

The aim of this project was to investigate whether or not well characterized groups of healthy adolescents and adolescents with asthma differed in their sensitivity to ozone and nitrogen dioxide at near ambient concentrations of these pollutants. The project was divided into three phases. In each phase, ten healthy and ten asthmatic adolescents were exposed via a mouthpiece to three different atmospheres (filtered air, ozone, and nitrogen dioxide, at either 0.12 or 0.18 ppm) on separate days at least one week apart.

During Phase I, subjects at rest inhaled the test atmospheres at 0.12 ppm for two 30-minute periods. The following pulmonary functional values were measured before, during, and after exposure: peak flow, total respiratory resistance, thoracic gas volume at functional residual capacity, maximal flow at 50 and 75 percent of expired vital capacity (performed with both room air and a helium-oxygen mixture), and forced expiratory volume in one second. Pulmonary function was not consistently altered in either the asthmatic or the healthy non-asthmatic adolescents as a result of the exposures. As a result, the study was repeated with the addition of ten minutes of exercise to the 30-minute rest exposure period (Phase II).

In Phase II, small but significant increases in total respiratory resistance to all test atmospheres were seen after exposure at 0.12 ppm during exercise in both healthy and asthmatic adolescents. However, the increase in resistance between the groups of subjects was not statistically different. On the basis of these results, Phase III was conducted at higher concentrations of the pollutants (0.18 ppm).

In Phase III, statistically significant changes were seen in average total respiratory resistance values in both healthy and asthmatic adolescents exposed to 0.18 ppm ozone while exercising. Again, the difference between the groups was not significant. Small decreases in average forced expiratory volume were found in healthy subjects exposed to ozone and filtered air. After exposure to nitrogen dioxide there was a 3 percent decrease in the forced expiratory volume in one second in asthmatic subjects. This change was not significant.

It is concluded that there were no differences in pulmonary function responses between asymptomatic, allergic asthmatic adolescents and healthy adolescents exposed to either ozone or nitrogen dioxide under the conditions of these studies. However, an increase in total respiratory resistance was observed in both asthmatic and healthy adolescent subjects after their exercise exposure to 0.18 ppm ozone. In addition, a dose-related increase in respiratory resistance in response to different levels of ozone (0.12 ppm and 0.18 ppm) is suggested. A subsequent study is required, however, to confirm this hypothesis.

INTRODUCTION

The six ambient air pollutants regulated by the U.S. Environmental Protection Agency are sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone (O_3), suspended particulate matter, carbon monoxide, and airborne lead. The EPA is required to set ambient air quality standards for these pollutants on the basis of the public health effects revealed in reviews of the scientific literature. Several laboratories, including ours, have been studying the effects of acute exposures to near ambient concentrations of these pollutants on pulmonary function.

There is compelling evidence that subjects with asthma are much more sensitive to the effects of inhaled sulfur dioxide than are healthy, non-asthmatic subjects. Jaeger and co-workers (1979) exposed normal and asthmatic subjects to 0.5 ppm sulfur dioxide or ambient air for three hours. Subsequent to the sulfur dioxide exposures, midmaximal expiratory flow rates decreased in the asthmatic subjects. Koenig and co-workers (1981;1983b) showed that when asthmatic adolescent subjects inhaled either 0.5 or 1.0 ppm sulfur dioxide during moderate exercise, changes in pulmonary function were approximately 20 times greater than those seen in healthy adolescent subjects under similar conditions (Koenig et al., 1982a). This increased sensitivity to sulfur dioxide also has been shown in adult subjects with asthma (Sheppard et al.,1981; Linn et al., 1983). In addition, Koenig and co-workers (1982b) showed that allergic non-asthmatic adolescent subjects, whose only sign of airway hypersensitivity is exercise-induced bronchospasm, are sensitive to the effects of inhaled sulfur dioxide. Asthmatic adolescent subjects also have a heightened sensitivity to 100 $\mu g/m^3$ of sulfuric acid (H_2SO_4), a common atmospheric transformation product of sulfur dioxide (Koenig et al., 1983a). However, Avol and co-workers (1979) found no difference in the pulmonary response of normal adult or asthmatic adult subjects who inhaled 100 $\mu g/m^3$ of sulfuric acid. Utell and associates (1983) also studied adult asthmatic subjects and found that airway resistance increased after inhalation of 1000 $\mu g/m^3$ sulfuric acid; however, they found no changes after the 100 $\mu g/m^3$ exposure. On the basis of these results, adolescent asthmatic subjects appear to be more sensitive than adult asthmatic subjects to inhaled sulfuric acid aerosol, a form of suspended particulate matter.

This increase in sensitivity to an air pollutant in persons with allergic disease has not been shown for the other four criterion pollutants (carbon monoxide, nitrogen dioxide, ozone, or lead). Neither carbon monoxide nor lead are assumed to affect the lung as a primary target organ.

Several groups have studied pulmonary function in subjects exposed to nitrogen dioxide in environmental chambers, with varying results. Orehek and co-workers (1976) reported that adult asthmatics had an exaggerated response to a carbachol challenge after they inhaled 0.1 ppm nitrogen dioxide. Kleinman and co-workers (1983) found that the bronchial reactivity of adult asthmatics changed in a variable manner after exposure to 0.2 ppm nitrogen dioxide. In the group as a whole, pulmonary function values did not change significantly; however, the response to graded doses of methacholine in 20 of the 31 subjects was enhanced after the nitrogen dioxide exposures.

Other investigators were not able to demonstrate greater pulmonary function response or increased bronchial sensitivity to nitrogen dioxide in asthmatics (Hazucha et al., 1983). Bylin and co-workers (1985) exposed eight normal and eight asthmatic adults to approximately 0.25 and 0.5 ppm nitrogen dioxide for 20 minutes. Bronchial reactivity to histamine, airway resistance, and thoracic gas volume were measured before, during, and after exposure. Airway resistance tended to increase in asthmatics, but the changes were not significant. The response of normal subjects varied; airway resistance increased significantly after exposure to 0.25 ppm nitrogen dioxide and decreased significantly after exposure to 0.5 ppm nitrogen dioxide. Bronchial reactivity also increased in asthmatics who were exposed to 0.5 ppm nitrogen dioxide. The same exposure had no significant effects on bronchial reactivity in normal subjects. Linn and co-workers (1985) exposed both healthy and asthmatic young subjects (20 to 36 years of age) to a relatively high concentration of nitrogen dioxide (4 ppm), but saw no significant changes in pulmonary function. A recent study of adult asthmatics reported that when 0.3 ppm nitrogen dioxide was inhaled via a mouthpiece, forced expiratory volume in one second (FEV_1) decreased, and airway hyperreactivity was enhanced after cold air provocation (1986).

Although ozone is toxic to the human respiratory system (EPA, 1978), reports show that adult asthmatic subjects are no more sensitive than healthy adult subjects to inhaled ozone. Holtzman and co-workers (1979) exposed nine atopic and seven non-atopic adult subjects (selected on the basis of medical history and allergen skin testing) to 0.6 ppm of ozone for two hours. One hour after the subjects who were wearing noseclips breathed ozone in a chamber, their bronchial response to histamine or methacholine challenge was measured and compared to the values obtained before exposure. In both groups, ozone exposure was associated with an increase in bronchial reactivity, but the increase was similar in both groups. The authors concluded that neither the inducibility nor the time course of bronchial hyperreactivity after exposure to ozone was enhanced by the presence of atopy.

Linn and associates (1980) compared the pulmonary responses of 34 normal and 30 asthmatic adult subjects (18 to 55 years of age) after they were exposed to approximately 0.2 ppm ozone for two hours in a chamber. Only slight effects were seen and there seemed to be no difference between the two groups. However, the health status of the subjects was not well-controlled. Some of the normal subjects had allergic symptoms. The asthmatic subjects were clinically quite heterogeneous and represented a spectrum from those who had no asthma since childhood to those who may have had chronic obstructive pulmonary disease as well as asthma. Therefore, it is difficult to evaluate the response of asthmatic subjects to inhaled ozone on the basis of this study.

The data of Silverman (1979) indicate that asthmatic subjects are sensitive to ozone, although the subjects were not compared to healthy control subjects. Seventeen adult asthmatic subjects were exposed for two hours to 0.25 ppm ozone in an environmental chamber. Although responses varied, the maximal flow at 50 percent of expired vital capacity changed more after ozone exposure than after air exposure in approximately one-third of the asthmatic subjects. However, none of the above studies conclusively showed that response to ozone was enhanced in asthmatic subjects as compared with healthy subjects.

SPECIFIC AIMS

The purpose of this study was to investigate systematically, in a well defined group of subjects, sensitivity to ozone in healthy and asthmatic individuals. The subjects, from the clinic of William E. Pierson, MD and associates, were atopic patients who had documented histories of extrinsic asthma and exercise-induced bronchospasm. The comparison group of healthy adolescents was screened carefully for absence of signs or symptoms of allergic disease or for hypersensitive airways. Our hypothesis was that asthmatic adolescent subjects would be more susceptible (in terms of lung function) to inhaled oxidants (ozone and nitrogen dioxide) than healthy adolescents.

The investigation was expected to help determine whether or not adolescent atopic subjects are more sensitive then non-atopic subjects to the effects of inhaled ozone or nitrogen dioxide. The asthmatic adolescent subjects used to test this hypothesis are comparable to subjects used in another project that demonstrated that they were much more sensitive to the effects of inhaled sulfur dioxide than were healthy adolescent subjects (Koenig et al., 1981). The results of this present study add important new data about the effects of ozone in controlled human studies.

METHODS

This project consisted of three separate studies, referred to as Phase I, Phase II, and Phase III.

EXPOSURES AND TEST ATMOSPHERES

All subjects were exposed to the test atmopheres via a rubber mouthpiece, and had nose clips on to ensure mouth breathing.

In Phase I, ten healthy and ten asthmatic adolescent subjects were exposed at rest to filtered air, 0.12 ppm ozone, or 0.12 ppm nitrogen dioxide on three separate days for two 30-minute periods. This protocol was repeated in Phase II with the substitution of 10 minutes of moderate exercise for the second exposure period. In Phase III, the concentration of ozone and nitrogen dioxide was increased to 0.18 ppm; in other respects, the protocol was identical to that of Phase II. Recording - exposure protocols for Phases I, II and III are shown schematically in Figures 1, 2, and 3. All exposures were conducted at approximately 75 percent relative humidity and 22°C.

The initial ozone concentration was set at 0.12 ppm, since that is the current one-hour national ambient air quality standard for ozone. This concentration is exceeded in the summer months in the Seattle area. Nitrogen dioxide was set at the same concentration for comparison of the two oxidant pollutants. At the beginning of the study, it was decided to carry out Phase III exposures at 0.06 or 0.18 ppm, depending on the results of Phases I and II. Since no consistent adverse changes in pulmonary function were seen at 0.12 ppm, the concentration was increased to 0.18 ppm.

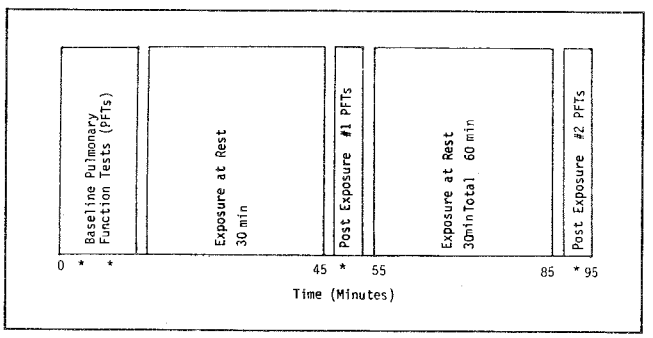

Figure 1. Schematic diagram of recording-exposure protocol for Phase I. *Indicates PFT recordings.

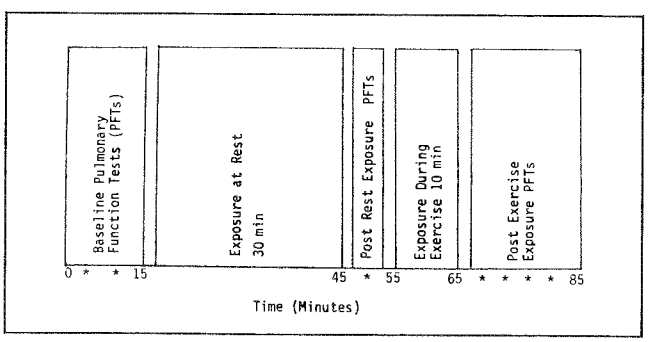

Figure 2. Schematic diagram of recording-exposure protocol for Phase II. *Indicates PFT recordings.

SUBJECT SELECTION

The asthmatic adolescent subjects selected from the clinical practice of Dr. Pierson and his associates had allergic asthma and exercise-induced bronchospasm (EIB). They fulfilled the following four screening criteria: (a) reversible obstructive airway disease documented with spirometry; (b) immunoglobulin E level outside the normal range of 0.5 to 120 International Units(IU)/ml; (c) immediate (Type I) hypersensitivity to skin tests for inhalant and pollen factors; and (d) airway responsiveness to both exercise and methacholine challenge. Exercise-induced bronchospasm was defined as a greater than 15 percent drop in forced expiratory volume per second (FEV_1) after 6 minutes of exercise on a Quinton treadmill at 85 percent or greater maximum oxygen consumption (Eggleston et al., 1979). All asthmatic subjects responded positively to a methacholine challenge. Methacholine was inhaled from a Rosenthal dosimeter. Subjects took five tidal breaths at each dose given in six steps from 0.025 mg/ml to 25 mg/ml. One FEV_1 was performed at 1½ and 3 minutes after methacholine inhalation. A sustained drop in FEV_1 of 20 percent or more following methacholine challenge is defined as a positive test. Only subjects whose FEV_1 decreased more than 20 percent at some point during the test were selected.

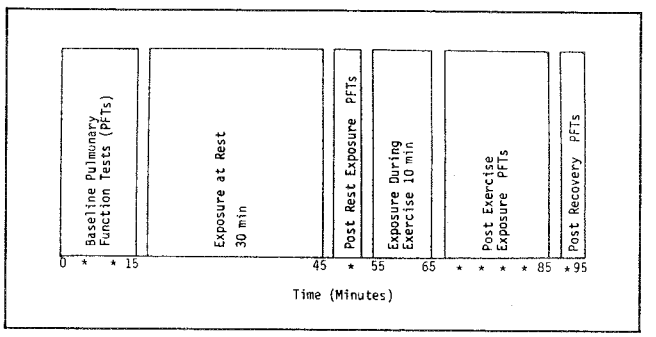

Figure 3. Schematic diagram of recording-exposure protocol for Phase III. *Indicates PFT recordings.

The asthmatic subjects were studied in the afternoon (after 2:00 pm), and were allowed to take their early morning medication if necessary. No medication was allowed within four hours of the study. Our policy is to study asthmatic subjects who are using appropriate, medically prescribed therapy and to determine whether or not, even under ideal conditions, the lung response of this group to ambient levels of common air pollutants is still deleterious. Thus, all asthmatic subjects continued to take their usual medication for asthma. They were asymptomatic at the time they were studied. By complying with these criteria, a refined homogeneous group of adolescent asthmatic subjects was ensured.

Both males and females, ranging in age from 11 to 19 years, participated in the study. A comparison of the responses of eight female and thirteen male adolescent asthmatics exposed to sulfur dioxide in different studies showed no significant difference in response on the basis of gender.

The healthy adolescent subjects, who were recruited from a private school, had no signs or symptoms of allergic disease or atopic predisposition. An extensive evaluation for any evidence of allergic (Type I) hypersensitivity was completed following a history and physical examination. These included:

- Examination of nasal cytology by light microscopy after differential eosinophil stain (Hansel's stain).

- Pulmonary function evaluation: forced vital capacity, FEV_1, and peak expiratory flow rate.
- Quantitative determination of serum immunoglobulins including IgE. The normal range for IgE for ages 11 to 19 is 0.5 to 120 IU/ml.
- Standardized exercise treadmill test (Eggleston et al., 1979). The criterion for normalcy is less than a 15 percent decrease in FEV_1 after five to six minutes of exercise at greater than 85 percent maximum oxygen consumption.
- Standard six-step methacholine challenge test (described above) to quantify cholinergic sensitivity to aerosolized methacholine. The criterion for normalcy is less than a 20 percent decrease in FEV_1 or forced vital capacity at any time during the test.

Only subjects whose evaluation and test values were normal were included in the study. The healthy subjects were matched as closely as possible to the age and gender of the asthmatic subjects. Table 1 summarizes the criteria used to select subjects.

Table 1. Criteria for Selection of Subject Populations

Characteristics	Asthmatic Group	Healthy Group
Reversible obstructive airways disease	+	–
Elevated serum IgE levels	+	–
Hypersensitivity to skin tests for inhalant & pollen factors	+	–
20% decrease in FEV_1 following a methacholine challenge test	+	–
15% decrease in FEV_1 following an exercise challenge test (EIB)	+	–

Before each study informed consent forms approved by the University of Washington Human Subjects Office, that included a description of the possible benefits and risks of participation in our research, were read and signed by each volunteer and one of his or her parents or guardians.

Subjects were exposed at random to the test atmospheres and were not told the identity of the gas exposure. The person who calculated the pulmonary function values did not know the test atmosphere identities.

Twenty subjects participated in Phase I. Each group consisted of four males and six females. The asthmatic subjects ranged from 11 to 18 years of age and the healthy subjects ranged from 13 to 18 years of age.

Nine of the healthy subjects from Phase I continued in Phase II. The range in age was 14 to 19 years. An additional girl was recruited, bringing the total to three males and seven females. Six of the asthmatic subjects from Phase I continued in Phase II and four new subjects were recruited. The age of the asthmatic subjects in Phase II ranged from 11 to 19 years; there were four males and six females.

Phase III involved seven healthy subjects who continued from Phase II. Two subjects dropped out of the study and one female subject was dropped because her baseline values were not reproducible; three additional subjects were recruited. A male subject from Phase I who could not participate in Phase II came back for Phase III. The four male and six female healthy subjects ranged in age from 15 to 19 years. Four of the asthmatic subjects from Phase II participated in Phase III, and six new subjects were recruited. The age range was 12 to 18 years. There were seven males and three females.

AIR CHEMISTRY

The system that generated the exposure atmospheres is shown schematically in Figure 4. 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.

The main air flow then was turbulently mixed with a small flow of ozone or nitrogen dioxide and routed to the subject. Ozone was produced by ultraviolet irradiation of clean air (OREC Model 03V1-0, Ozone Research and Equipment Co., Phoenix, AZ) and monitored with an ultraviolet photometric analyzer (Model CSI 3100, Columbia Scientific Industries, Austin, TX).

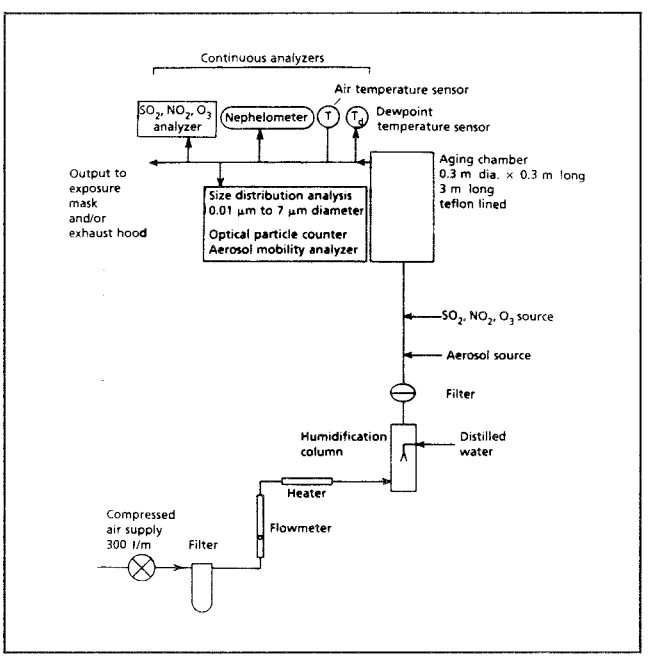

Figure 4. A schematic representation of the gas-aerosol generating system.

Nitrogen dioxide was supplied from a gas cylinder (0.397 mole percent v/v; balance nitrogen, Scott-Marrin, Riverside, CA), and output concentration was monitored with a chemiluminescent NO-NOx analyzer (Model 8840, Monitor Labs, Inc., San Diego, CA).

Ozone concentrations were monitored at the subject's mouthpiece; nitrogen dioxide concentrations and relative humidity were monitored at the output of the test atmosphere generator.

The zero and span calibrations of the monitoring instruments were checked each week. Audits of the zero, span, and linearity of the instruments, transfer standards, and cylinders were made yearly (Washington State Department of Ecology, Redmond, WA).

PULMONARY FUNCTION MEASUREMENTS

The recording-exposure protocol is outlined chronologically in Table 2. All physiological measurements except peak flow were recorded with a thermal recorder or X-Y plotter, with the subject seated in a pressure-compensated volume-displacement body plethysmograph. The following were measured:

- Peak flow with a Vitalograph peak flow meter.
- Total respiratory resistance, using the forced pressure oscillatory technique at 3 Hz (Goldman et al., 1970). Values were based on an average of 10 breaths.
- Thoracic gas volume at functional residual capacity using the gas compression technique (Dubios et al., 1956).
- Maximum flow calculated at 50 percent and 75 percent of expired vital capacity, from a maximal flow-volume curve, performed both with room air and with a helium-oxygen mixture.
- Forced expiratory volume in one second calculated from the same maximal flow-volume maneuvers. Forced vital capacity also was calculated from this maneuver.

Deep inspirations are known to affect the bronchial smooth-muscle tone of both asthmatic and non-asthmatic subjects (Gayrard et al., 1975; Fish et al., 1981). The flow-volume measures were done at the end of each recording session so that they would not influence other functional parameters.

The FEV_1, expired vital capacity, and functional residual capacity tests were measured in triplicate. The mean value was used to assess functional changes. Measurement of FEV_1 maneuvers was repeated if the triplicates were not within 10 percent of each other. Pressure and flow signals were processed by a microcomputer that calculates total respiratory resistance.

Table 2. Ozone and NO_2 Experimental Procedure *(See Abbreviations for Key)*

Baseline 1

Peak Flow with Vitalograph (3 Trials)
Inspiratory Capacity (IC) (1)
R_T (10 Breaths)
FRC (3) Combined Baseline
Flow-Volume ($\dot{V}$-V), FEV_1 (2)

Baseline 2

IC (1)
R_T (10 Breaths)
FRC (3)
$\dot{V}V$, FEV_1 (2)
Helium $\dot{V}V$ (2)

Baseline SaO_2 for 10 Minutes (Phase I and Phase II)

Exposure 1
On mouthpiece for 30 minutes at rest
record:
- SaO_2, O_2 saturation of arterial blood (ear oximetry)
- CO_2
- 10 minute sample of resting ventilation ($\dot{V}_E$)

Post-Rest Exposure (Phase I) PR1

IC (1)
R_T (10 Breaths)
FRC (3)
$\dot{V}V$, FEV_1 (2)

Exposure 2

On mouthpiece for 30 minutes at rest (Phase I)
On mouthpiece for 10 minutes of moderate exercise (Phase II, Phase III)
record:
- SaO_2
- CO_2
- 10 minute sample of exercise ventilation

Post-Rest PR2 (Phase I)

IC (1)
R_T (10 breaths)
FRC (3)
$\dot{V}V$, FEV_1 (2)

Post-Exercise Exposure (Phase II and III)

FEV_1 (3) PE1
R_T (10 breaths)
FRC (3)

$\dot{V}V$, FEV_1 (3) PE2
R_T (10 breaths)
FRC (3)

$\dot{V}V$,FEV_1 PE3
Peak Flow

During the baseline period, peak flow was measured before the subject entered the plethysmograph. Next, two sets of pulmonary functional measurements were recorded, which required a total of approximately 15 minutes. After the baseline measurements were recorded, exposure was begun (via a rubber mouthpiece with nose-clips on the subject to ensure mouth breathing). During the first stage of exposure, the subject sat at rest for 30 minutes outside the plethysmograph. The following parameters were monitored throughout exposure: minute ventilation tidal volume times the number of breaths per minute (Hewlett-Packard respiratory integrator) and end tidal carbon dioxide tension (Beckman gas analyzer). The mean minute ventilation for a 10-minute period during the rest exposure was chosen as that subject's resting value. This was followed by a brief interruption of exposure (5 to 7 minutes) for pulmonary function measurements to be recorded while the subject sat in the plethysmograph.

During the second stage of the exposure, the subject either sat again for 30 minutes at rest or walked on a treadmill for 10 minutes. The speed and elevation were adjusted for each subject to produce a five- to six-fold increase in the mean resting minute ventilation (approximately 2.5 mph and 16 percent grade). The treadmill settings for each subject were repeated each day of exposure; thus, the work load for each day was nearly identical. Immediately after the second exposure, the subject returned to the plethysmograph for 20 minutes of nearly continuous pulmonary functional measurements. During Phases I and II, oxygen saturation of arterial blood was monitored during exposures with an ear oximeter (Hewlett-Packard). However, the quality of these measurements was poor during exercise, and they were discontinued.

Any session in which the baseline values varied by more than 10 percent was rescheduled. This occurred twice in Phase I, once in Phase II, and twice in Phase III. Baseline mean values from atmosphere to atmosphere were compared using the paired t test, to be certain that differences among baselines within a given group were not significant.

A symptom rating scale for coughing, substernal pain, sore throat, wheezing, shortness of breath, unusual taste or smell, fatigue, headache, and nasal discharge, was scored by the subject at the end of the experiment (see Appendix A). The form was taken home and scored later that day and on the following day. The peak flow meter also was taken home and measurements were made one, two, three, and four hours after exposure to detect delayed effects of the exposure.

STATISTICAL ANALYSIS

The design of each phase was a repeated measures design. Data from each phase were examined by means of analysis of covariance for a repeated measures design, using baseline observations as covariates (see Figures 1 through 3). Repeated baseline measurements were averaged to produce a single measurement. Similarly, post-exposure measurements within two minutes of each other were averaged to produce single readings. If a mean square in the analysis of covariance was significant, the effect was examined for direction.

An alternative endpoint was change from baseline. Results of analyses using this endpoint were very similar to those of the analysis of covariance. The latter approach was chosen since the change from baseline is a special case of analysis of covariance, with zero intercept and a slope of one. In both approaches, the main effects for conditions (recording periods; post-rest, post-exercise 1, and so forth), the interaction effect for conditions by atmospheres (air, ozone, nitrogen dioxide), and the interaction effect for conditions by atmospheres by groups (asthmatics or normal) are the same, since they do not involve the covariate.

All analyses of covariance were carried out using the BMDP statistical software for a micro-computer. When possible, orthogonal polynomial decomposition of effects was carried out, but are not reported here.

RESULTS

PHASE I

The mean oxidant concentrations during Phase I exposures were 119.8 $\pm$ 1.62 ppb ozone and 120.2 $\pm$ 1.75 ppb nitrogen dioxide for asthmatic subjects, and 120.9 $\pm$ 1.91 ppb ozone and 120.2 $\pm$ 0.78 ppb nitrogen dioxide for healthy subjects. The physical characteristics of the ten healthy and ten asthmatic subjects are summarized in Table 3. There were no consistent significant changes in pulmonary functional parameters after exposure at rest to 0.12 ppm ozone or to 0.12 ppm nitrogen dioxide in either the healthy or asthmatic adolescent subjects. The means and standard deviations for the pulmonary functional values in the asthmatic and healthy adolescent subjects are shown in Tables 4 and 5. From an analysis of the adjusted cell means, it was concluded that, after adjusting for baseline values, the only differential effect was that on total respiratory resistance: the healthy subjects showed a slight increase in resistance after exposure and the asthmatic subjects showed a slight decrease. This effect was independent of atmospheric exposure.

There were no changes in the oxygen saturation of arterial blood attributable to the exposures (data not shown). Also, there were no significant differences in symptom scores. However, asthmatic subjects reported higher ratings on the symptom scale than healthy subjects, a total of 34, 56, and 67, after air, ozone and nitrogen dioxide exposures, respectively, compared with totals of 11, 14, and 7, reported by healthy subjects. (Symptom score analyses are not given here but are available by request from the authors.)

Ambient concentrations of ozone and nitrogen dioxide in the Seattle area during Phase I (October, 1983 through May, 1984) were low. During this period, the maximal hourly averages were 0.03 to 0.04 ppm for ozone and 0.03 to 0.07 ppm for nitrogen dioxide. The subjects, therefore, were not exposed

Table 3. Physical Characteristics of Asthmatic and Healthy Adolescent Subjects: Phase I

Subject #	Sex	Age	Ht.(cm)	Baseline FEV_1	Medication use	Response to methacholine*
Asthmatic						
1121	M	18	175	4.00	Albuterol; Theodur	10.0 mg/ml
1129	M	14	164	3.20	Theodur	2.5 mg/ml
1227	F	11	155	2.10	Albuterol	5.0 mg/ml
0216	M	14	165	3.10	Albuterol	2.5 mg/ml
0322	F	14	157	3.20	Albuterol	0.25 mg/ml
0402	F	16	167	2.60	Theodur; Albuterol	not tested
0316	M	14	180	4.10	Albuterol	not tested
0406	F	12	142	2.10	Slophyllin; Alupent	not tested
0418	F	17	158	1.95	Theodur; Albuterol	0.25 mg/ml
0427	F	14	164	2.75	Theodur	0.25 mg/ml
Healthy						
1012	M	17	180	4.70		
1116	M	18	182	4.50		
1019	F	13	154	2.80		
0416	M	13	163	3.30		
1027	F	13	156	2.80		
1103	F	13	157	3.20		
0214	M	14	178	4.90		
1028	F	13	150	2.50		
0223	F	13	159	3.20		
0302	F	13	156	3.30		

* Concentration of methacholine at which FEV_1 decreased by 20% or greater.

Table 4. Pulmonary Functional Measurements in Asthmatic Adolescent Subjects Before, During, and After Exposure: Phase I (N = 10). Mean ± Standard Deviation

Functional Measurement	Exposure Atmosphere +	Baseline	Post-30 min Exposure	Post-60 min Exposure
R_T	Air	4.57 ± 1.39	4.59 ± 1.73	4.33 ± 1.93
$cmH_2O/l/sec$	O_3 +	4.87 ± 1.20	4.54 ± 1.40	4.38 ± 1.16
	NO_2 +	4.27 ± 1.14	4.19 ± 1.50	4.08 ± 1.10
FRC	Air	2.67 ± 0.82	2.41 ± 0.80	2.41 ± 0.64
l	O_3 +	2.62 ± 0.66	2.57 ± 0.62	2.55 ± 0.50
	NO_2 +	2.78 ± 0.76	2.63 ± 0.52	2.76 ± 0.56
$\dot{V}_{max50}$	Air	2.21 ± 1.08	2.19 ± 1.10	2.37 ± 1.03
l/sec	O_3 +	2.24 ± 1.17	2.18 ± 1.19	2.20 ± 1.08
	NO_2 +	2.37 ± 1.18	2.27 ± 1.05	2.45 ± 1.10
$\dot{V}_{max75}$	Air	0.86 ± 0.54	0.82 ± 0.59	0.91 ± 0.57
l/sec	O_3 +	0.87 ± 0.53	0.83 ± 0.53	0.85 ± 0.51
	NO_2 +	0.89 ± 0.55	0.86 ± 0.49	0.91 ± 0.49
FEV_1	Air	2.91 ± 0.76	2.85 ± 0.72	2.92 ± 0.76
l	O_3 +	2.94 ± 0.86	2.91 ± 0.87	2.85 ± 0.74
	NO_2 +	3.02 ± 0.92	2.97 ± 0.85	2.95 ± 0.80
FVC	Air	3.42 ± 0.77	3.44 ± 0.83	3.44 ± 0.81
l	O_3 +	3.43 ± 0.80	3.40 ± 0.80	3.45 ± 0.76
	NO_2 +	3.51 ± 0.82	3.53 ± 0.78	3.48 ± 0.76

\+ O_3 and NO_2 concentration, 0.12 ppm.

Table 5. Pulmonary Functional Measurements in Healthy Adolescent Subjects Before, During, and After Exposure: Phase I (N = 10). Mean ± Standard Deviation

Functional Measurement	Exposure Atmosphere +	Baseline	Post 30 min Exposure	Post 60 min Exposure
R_T	Air	3.30 ± 0.70	3.29 ± 0.59	3.56 ± 0.58
$cmH_2O/l/sec$	O_3 +	3.68 ± 0.75	3.63 ± 0.96	3.85 ± 0.91
	NO_2 +	3.50 ± 0.98	3.51 ± 1.26	3.68 ± 1.38
FRC	Air	3.31 ± 0.64	3.17 ± 0.57	3.13 ± 0.63
l	O_3 +	2.99 ± 0.76	2.76 ± 0.49	2.82 ± 0.60
	NO_2 +	2.86 ± 0.79	2.82 ± 0.84	2.61 ± 0.67
$\dot{V}_{max50}$	Air	3.62 ± 0.86	3.74 ± 0.90	3.60 ± 0.76
l/sec	O_3 +	3.80 ± 0.90	3.61 ± 1.01	4.01 ± 0.98
	NO_2 +	3.83 ± 1.03	3.78 ± 0.99	3.89 ± 0.88
$\dot{V}_{max75}$	Air	1.47 ± 0.45	1.57 ± 0.44	1.44 ± 0.35
l/sec	O_3 +	1.56 ± 0.47	1.50 ± 0.56	1.70 ± 0.50
	NO_2 +	1.62 ± 0.46	1.57 ± 0.48	1.67 ± 0.42
FEV_1	Air	3.52 ± 0.86	3.52 ± 0.82	3.48 ± 0.79
l	O_3 +	3.59 ± 0.90	3.55 ± 0.90	3.59 ± 0.90
	NO_2 +	3.64 ± 0.93	3.52 ± 0.89	3.49 ± 0.89
FVC	Air	3.92 ± 1.09	3.92 ± 1.05	3.91 ± 1.04
l	O_3 +	3.88 ± 1.14	3.86 ± 1.09	3.96 ± 1.12
	NO_2 +	3.90 ± 0.96	3.85 ± 1.13	3.89 ± 1.01

+ O_3 and NO_2 concentration, 0.12 ppm.

to significant concentrations of the oxidants outside of our laboratory. The results of the exposures during Phase I of this project have been published (Koenig et al., 1985).

PHASE II

The mean oxidant concentrations during Phase II exposures were 120.2 ± 1.14 ppb ozone and 119.7 ± 0.5 ppb nitrogen dioxide for asthmatic subjects, and 120.2 ± 0.63 ppb ozone and 120.5 ± 1.08 ppb nitrogen dioxide for healthy subjects. The physical characteristics of the subjects who participated in Phase II of this project are summarized in Table 6. FEV_1 and total respiratory resistance changes during exposures are shown in Figures 5 and 6. The means and standard deviations for the pulmonary functional values in the asthmatic and healthy subjects are shown in Tables 7 and 8. The average exercise ventilation rate was 32.6 ± 6.4 l/minute for healthy subjects and 32.8 ± 7.8 l/minute for asthmatic subjects.

Based on the analysis of covariance of the FEV_1 and the total respiratory resistance values, the F ratios (treatment mean squares divided by error mean squares) for conditions (post-rest, post-exercise-exposure 1 and post-exercise-exposure 2) were highly significant ($p < 0.001$) for both healthy and asthmatic subjects. The F ratio for test atmospheres was not significant. Compared with baseline values, asthmatic subjects showed a decrease in average FEV_1 values after exposure to the air, ozone, and nitrogen dioxide test atmospheres. The decreases were 6 percent in all cases. The decrease in mean FEV_1 seen after ozone exposure was more prolonged than the decrease after air exposure. In one asthmatic subject, FEV_1 decreased markedly (36 percent) after nitrogen dioxide exposure, which is consistent with bronchoconstriction. In contrast, healthy subjects only showed a decrease in average FEV_1 values after exposure to air.

For the asthmatic subjects, the mean total respiratory resistance values increased over baseline means 3 percent, 15 percent, and 12 percent, 7 to 8 minutes post-exercise (PE2) after exposure to air, ozone, and nitrogen dioxide, respectively. In healthy subjects, mean total respiratory resistance values increased over baseline means by 12 percent, 16 percent, and 17 percent, 7 to 8 minutes post-exercise after air, ozone, and nitrogen dioxide exposures, respectively. None of the increases in total respiratory resistance by test atmospheres was significant for either the healthy or the asthmatic group.

In healthy subjects exposed to air, the mean maximum flow at 50 percent expired vital capacity decreased 8 percent. Also in healthy subjects, maximum flow at 50 percent of expired vital capacity decreased 6 percent after ozone exposure, and 4 percent after nitrogen dioxide exposure.

As in Phase I, the analyses of subjective symptoms during the exposures showed no consistent changes, although asthmatic subjects again reported more symptoms. In general, asthmatic subjects reported fewer symptoms during Phase II than during Phase I, and healthy subjects reported more symptoms.

Table 6. Physical Characteristics of Asthmatic and Healthy Adolescent Subjects: Phase II

Subject #	Sex	Age	Ht.(cm)	Baseline FEV_1	Medication use	Response to Methacholine*
Asthmatic						
1121	M	19	174	4.20	Albuterol; Theodur	10.0 mg/ml
1129	M	15	176	3.80	Theodur	2.5 mg/ml
1227	F	12	163	2.50	Albuterol	5.0 mg/ml
0216	M	15	166	3.70	Albuterol	2.5 mg/ml
0322	F	15	160	3.20	Albuterol	0.25 mg/ml
0405	M	11	149	2.40	Albuterol	0.25 mg/ml
0215	F	18	157	3.10	Theodur	5.0 mg/ml
0315	F	13	160	3.10	Albuterol; Theodur	0.25 mg/ml
0427	F	15	165	2.60	Theodur	0.25 mg/ml
0520	F	13	165	2.30	Albuterol	2.5 mg/ml
Healthy						
1012	M	18	180	4.90		
1019	F	15	163	3.20		
0416	M	14	172	3.50		
1027	F	15	159	3.20		
1103	F	14	158	3.30		
0214	M	15	180	4.80		
1028	F	14	154	3.20		
0223	F	14	158	3.10		
0302	F	14	160	3.40		
0418	F	17	158	3.50		

* Concentration of methacholine at which FEV_1 decreased by 20% or greater.

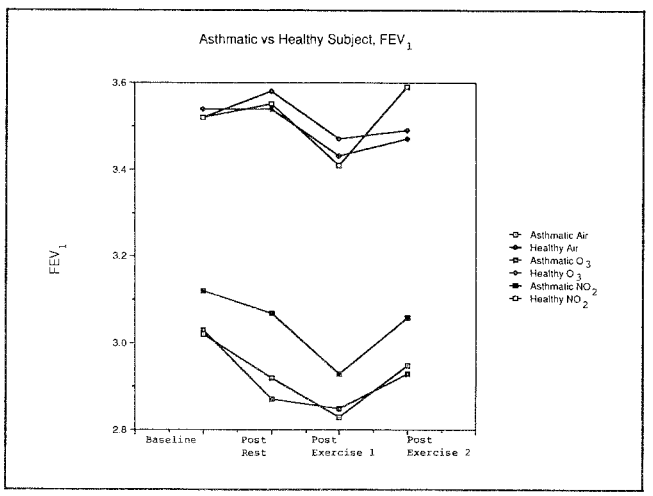

Figure 5: Mean changes in FEV_1 after 30 minutes' exposure at rest and 10 minutes' exposure during moderate exercise. Phase II.

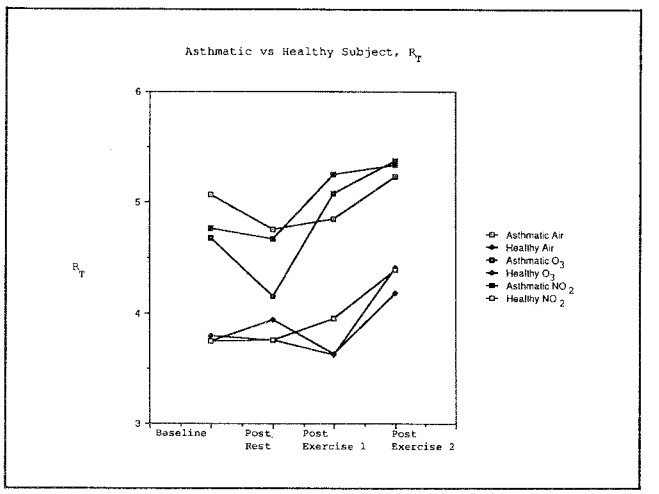

Figure 6: Mean changes in R_T after 30 minutes' exposure at rest and 10 minutes' exposure during moderate exercise. N = 10 in each group. Units for R_T, $cmH_2O/1/sec$. Phase II.

Ambient concentrations of ozone and nitrogen dioxide in the Seattle area during Phase II exposures (August, 1984 through March, 1985) were low. The maximal hourly averages were 0.01 to 0.07 ppm for ozone and 0.03 to 0.07 ppm for nitrogen dioxide. As in Phase I, the subjects were not exposed to significant concentrations of the oxidants outside of our laboratory.

PHASE III

The mean oxidant concentrations generated in Phase III were 178.4 ± 1.17 ppb ozone and 181.1 ± 0.99 ppb nitrogen dioxide for asthmatic subjects, and 179.0 ± 1.15 ppb ozone and 181.1 ± 0.99 ppb nitrogen dioxide for healthy subjects. The physical characteristics of the subjects are given in Table 9. The average ventilation rate during exercise was 41.3 ± 9.3 l/min in the healthy subjects, and 39.4 ± 12.5 l/min in the asthmatic subjects. The means and standard deviations for the pulmonary functional values in the asthmatic and healthy subjects are shown in Tables 10 and 11.

Table 7. Pulmonary Functional Measurements in Asthmatic Adolescent Subjects Before, During, and After Exposure: Phase II (N = 10). Mean ± Standard Deviation.

Functional Measurement	Exposure Atmosphere	Baseline	Post-Rest Exposure	Post-Exercise-Exposure 2 to 3 min. post-exercise	Post-Exercise-Exposure 7 to 8 min. post-exercise
Asthmatic Subjects					
R_T	Air	5.07 ± 1.34	4.74 ± 1.36	4.85 ± 1.49	5.23 ± 1.90
$cmH_2O/l/sec$	O_3	4.68 ± 1.45	4.15 ± 1.42	5.08 ± 1.71	5.38 ± 1.52
	NO_2	4.76 ± 1.58	4.67 ± 1.33	5.25 ± 1.60	5.34 ± 1.61e 2
FRC	Air	2.79 ± 0.63	2.71 ± 0.42	2.74 ± 0.59	2.89 ± 0.69
l	O_3	2.75 ± 0.55	2.71 ± 0.54	2.82 ± 0.65	2.75 ± 0.61
	NO_2	2.70 ± 0.65	2.75 ± 0.74	2.83 ± 0.54	2.70 ± 0.53
$\dot{V}_{max50}$	Air	2.52 ± 0.64	2.52 ± 0.83	2.44 ± 0.69	2.45 ± 0.85
l/sec	O_3	2.57 ± 0.77	2.54 ± 0.71	2.36 ± 0.92	2.40 ± 0.67
	NO_2	2.52 ± 0.59	2.59 ± 0.62	2.20 ± 0.75	2.36 ± 0.73
$\dot{V}_{max75}$	Air	0.99 ± 0.32	0.96 ± 0.42	0.93 ± 0.32	1.00 ± 0.38
l/sec	O_3	1.00 ± 0.37	1.01 ± 0.31	0.88 ± 0.43	0.88 ± 0.29
	NO_2	0.95 ± 0.25	0.99 ± 0.36	0.85 ± 0.35	0.92 ± 0.40
FEV_1	Air	3.02 ± 0.63	2.92 ± 0.60	2.83 ± 0.62	2.95 ± 0.61
l	O_3	3.03 ± 0.62	2.87 ± 0.83	2.85 ± 0.62	2.93 ± 0.64
	NO_2	3.12 ± 0.52	3.07 ± 0.51	2.93 ± 0.74	3.06 ± 0.59
FVC	Air	3.66 ± 0.59	3.61 ± 0.64	3.59 ± 0.58	3.65 ± 0.64
l	O_3	3.56 ± 0.71	3.60 ± 0.66	3.48 ± 0.71	3.56 ± 0.61
	NO_2	3.76 ± 0.58	3.76 ± 0.61	3.68 ± 0.73	3.63 ± 0.65

		Baseline	20 min. Post-Exp	3 hrs. Post-Exp	4 hrs. Post-Exp	5 hrs. Post-Exp	6 hrs. Post-Exp
Peak Flow	Air	388 ± 72	396 ± 58	420 ± 60	395 ± 53	383 ± 62	389 ± 61
l/sec	O_3	400 ± 90	389 ± 73	Incom data#	Incom data#	415 ± 66	395 ± 50
	NO_2	409 ± 50	402 ± 73	Incom data#	Incom data#	411 ± 57	412 ± 43

Incomplete data

Table 8. Pulmonary Functional Measurements in Healthy Adolescent Subjects Before, During, and After Exposure: Phase II (N = 10). Mean ± Standard Deviation.

Functional Measurement	Exposure Atmosphere	Baseline	Post-Rest Exposure	Post-Exercise-Exposure 2 to 3 min. post-exercise	Post-Exercise-Exposure 7 to 8 min. post-exercise
Healthy Subjects					
RT	Air	3.75 ± 0.90	3.94 ± 1.19	3.63 ± 0.95	4.18 ± 1.04
$cmH_2O/l/sec$	O_3 +	3.80 ± 1.23	3.76 ± 1.00	3.62 ± 1.49	4.41 ± 1.24
	NO_2 +	3.75 ± 1.13	3.76 ± 0.96	3.95 ± 1.39	4.39 ± 1.39
FRC	Air	2.92 ± 0.69	2.92 ± 0.74	2.95 ± 0.68	2.77 ± 0.65
l	O_3 +	2.93 ± 0.72	2.84 ± 0.60	2.79 ± 0.61	2.82 ± 0.68
	NO_2 +	2.96 ± 0.79	2.99 ± 0.85	3.01 ± 0.97	2.95 ± 0.77
$\dot{V}_{max50}$	Air	3.65 ± 0.66	3.64 ± 0.70	3.52 ± 0.66	3.32 ± 0.71
l/sec	O_3 +	3.65 ± 0.64	3.80 ± 0.74	3.49 ± 0.68	3.48 ± 0.69
	NO_2 +	3.58 ± 0.82	3.61 ± 0.77	3.64 ± 0.80	3.60 ± 0.74
$\dot{V}_{max75}$	Air	1.54 ± 0.37	1.58 ± 0.44	1.50 ± 0.45	1.43 ± 0.39
l/sec	O_3 +	1.58 ± 0.44	1.73 ± 0.46	1.58 ± 0.48	1.50 ± 0.40
	NO_2 +	1.53 ± 0.46	1.49 ± 0.49	1.43 ± 0.58	1.59 ± 0.51
FEV_1	Air	3.54 ± 0.65	3.54 ± 0.66	3.43 ± 0.63	3.47 ± 0.68
l	O_3 +	3.52 ± 0.68	3.58 ± 0.69	3.47 ± 0.70	3.49 ± 0.68
	NO_2 +	3.52 ± 0.66	3.55 ± 0.59	3.41 ± 0.59	3.59 ± 0.65
FVC	Air	3.95 ± 0.88	3.92 ± 0.93	3.93 ± 0.79	3.92 ± 0.90
l	O_3 +	3.92 ± 0.82	4.06 ± 0.89	3.95 ± 0.81	3.91 ± 0.85
	NO_2 +	3.99 ± 0.88	3.93 ± 0.79	3.97 ± 0.77	3.96 ± 0.86

		Baseline	20 min. Post-Exp	3 hrs. Post-Exp	4 hrs. Post-Exp	5 hrs. Post-Exp	6 hrs. Post-Exp
Peak Flow	Air	466 ± 82	456 ± 65	436 ± 77	453 ± 58	453 ± 58	446 ± 72
l/sec	O_3 +	456 ± 83	456 ± 68	454 ± 60	452 ± 76	456 ± 74	463 ± 70
	NO_2 +	460 ± 84	443 ± 60	437 ± 52	432 ± 59	435 ± 61	433 ± 60

+ O_3 and NO_2 concentration, 0.12 ppm.

Table 9. Physical Characteristics of Asthmatic and Healthy Adolescent Subjects: Phase III

Subject #	Sex	Age	Ht.(cm)	Baseline FEV_1	Medication use	Response to Methacholine*
Asthmatic						
0405	M	12	152	2.30	Albuterol	2.5 mg/ml
0923	F	12	155	2.00	Albuterol	0.25 mg/ml
1003	M	15	175	3.80	Theodur	2.5 mg/ml
1107	M	12	147	1.70	Theodur; Alupent	0.025 mg/ml
0427	F	16	166	2.80	Theodur	0.25 mg/ml
1206	F	12	153	2.20	Theodur; Albuterol	10.0 mg/ml
1205	M	13	157	2.80	Theodur; Albuterol	10.0 mg/ml
1129	M	16	179	4.50	Theodur	2.5 mg/ml
0216	M	16	181	3.90	Albuterol	2.5 mg/ml
0127	M	18	169	4.60	Slo-Bid	2.5 mg/ml
Healthy						
0418	F	17	158	3.40		
0214	M	16	182	5.10		
1028	F	15	159	3.40		
1012	M	19	180	5.10		
1112	F	16	168	3.20		
1115	F	16	165	3.20		
1116	M	19	182	5.00		
1103	F	15	158	3.70		
0416	M	15	172	3.90		
1019	F	16	163	3.30		

* Concentration of methacholine at which FEV_1 decreased by 20% or greater.

Table 10. Pulmonary Functional Measurements in Asthmatic Adolescent Subjects Before, During, and After Exposure: Phase III (N = 10). Mean ± Standard Deviation

Functional Measurement	Exposure Atmosphere +	Baseline	Post-Rest Exposure	Post-Exercise-Exposure 2 to 3 min. post-exercise	Post-Exercise-Exposure 7 to 8 min. post-exercise
R_T $cmH_2O/l/sec$	Air	4.82 ± 1.71	4.54 ± 1.23	5.07 ± 1.42	4.92 ± 1.54
	O_3 +	4.65 ± 1.25	4.59 ± 1.52	5.14 ± 1.58	5.36 ± 2.12
	NO_2 +	4.64 ± 1.37	4.52 ± 1.13	5.18 ± 1.93	4.81 ± 1.93
FRC l	Air	2.92 ± 0.68	2.76 ± 0.74	2.96 ± 0.62	2.73 ± 0.72
	O_3 +	2.73 ± 0.74	2.67 ± 0.74	2.79 ± 0.70	2.80 ± 0.68
	NO_2 +	2.68 ± 0.59	2.68 ± 0.76	2.89 ± 0.69	2.52 ± 0.65
$\dot{V}_{max50}$ l/sec	Air	2.77 ± 1.15	2.84 ± 1.06	2.64 ± 1.03	2.63 ± 1.01
	O_3 +	2.88 ± 1.00	2.92 ± 0.99	2.65 ± 1.01	2.71 ± 1.01
	NO_2 +	2.81 ± 1.14	2.84 ± 1.09	2.60 ± 1.20	2.71 ± 1.15
$\dot{V}_{max75}$ l/sec	Air	1.13 ± 0.52	1.15 ± 0.47	1.09 ± 0.47	1.09 ± 0.45
	O_3 +	1.23 ± 0.44	1.20 ± 0.41	1.03 ± 0.45	1.13 ± 0.47
	NO_2 +	1.13 ± 0.52	1.15 ± 0.52	1.02 ± 0.57	1.13 ± 0.57
FEV_1 l	Air	3.07 ± 1.10	3.09 ± 1.07	3.03 ± 1.02	3.05 ± 1.02
	O_3 +	3.06 ± 0.99	3.07 ± 0.95	2.95 ± 0.95	3.03 ± 0.99
	NO_2 +	3.01 ± 1.01	2.96 ± 1.03	2.91 ± 1.01	2.96 ± 1.03
FVC l	Air	3.88 ± 1.14	3.90 ± 1.13	3.97 ± 1.16	3.89 ± 1.11
	O_3 +	3.82 ± 1.20	3.76 ± 1.12	3.76 ± 1.13	3.74 ± 1.11
	NO_2 +	3.83 ± 1.19	3.77 ± 1.28	3.92 ± 1.21	3.86 ± 1.24

		Baseline	20 min. Post-Exp	3 hrs. Post-Exp	4 hrs. Post-Exp	5 hrs. Post-Exp	6 hrs. Post-Exp
Peak Flow l/sec	Air	445 ± 95	413 ± 114	433 ± 106	423 ± 100	385 ± 110	388 ± 109
	O_3	395 ± 126	394 ± 128	441 ± 132	441 ± 140	418 ± 151	412 ± 138
	NO_2	413 ± 119	418 ± 140	444 ± 140	433 ± 148	431 ± 136	446 ± 140

\+ O_3 and NO_2 concentration, 0.18 ppm.

Table 11. Pulmonary Functional Measurements in Healthy Adolescent Subjects Before, During, and After Exposure: Phase III (N = 10). Mean ± Standard Deviation.

Functional Measurement	Exposure Atmosphere +	Baseline	Post-Rest Exposure	Post-Exercise-Exposure 2 to 3 min. post-exercise	Post-Exercise-Exposure 7 to 8 min. post-exercise
R_T	Air	3.57 ± 0.95	3.63 ± 1.08	3.76 ± 1.27	3.94 ± 1.10
$cmH_2O/l/sec$	O_3 +	3.69 ± 0.70	4.06 ± 0.95	3.83 ± 0.81	4.72 ± 1.24
	NO_2 +	4.08 ± 1.34	3.81 ± 0.90	4.07 ± 1.41	4.15 ± 0.98
FRC	Air	3.16 ± 0.95	3.00 ± 0.80	3.02 ± 0.80	3.01 ± 0.78
l	O_3 +	3.01 ± 0.74	2.98 ± 0.72	2.91 ± 0.68	3.00 ± 0.82
	NO_2 +	3.08 ± 0.90	3.18 ± 0.92	3.14 ± 0.85	2.89 ± 0.87
$\dot{V}_{max50}$	Air	4.41 ± 0.81	4.36 ± 0.67	4.18 ± 0.73	4.25 ± 0.78
l/sec	O_3 +	4.35 ± 0.92	4.37 ± 0.94	4.27 ± 0.91	4.34 ± 0.91
	NO_2 +	4.33 ± 0.80	4.40 ± 0.77	4.29 ± 0.71	4.23 ± 0.90
$\dot{V}_{max75}$	Air	1.99 ± 0.45	1.96 ± 0.41	1.82 ± 0.39	1.90 ± 0.43
l/sec	O_3 +	2.04 ± 0.49	2.04 ± 0.52	1.92 ± 0.54	1.89 ± 0.52
	NO_2 +	1.97 ± 0.39	1.98 ± 0.35	1.85 ± 0.35	1.86 ± 0.43
FEV_1	Air	3.92 ± 0.82	3.88 ± 0.76	3.81 ± 0.75	3.86 ± 0.75
l	O_3 +	3.89 ± 0.78	3.88 ± 0.79	3.83 ± 0.78	3.84 ± 0.75
	NO_2 +	3.86 ± 0.75	3.89 ± 0.73	3.80 ± 0.67	3.82 ± 0.76
FVC	Air	4.60 ± 1.15	4.57 ± 1.11	4.50 ± 1.03	4.54 ± 1.05
l	O_3 +	4.54 ± 1.04	4.55 ± 1.05	4.50 ± 1.04	4.54 ± 1.04
	NO_2 +	4.53 ± 1.08	4.56 ± 1.07	4.53 ± 0.99	4.59 ± 1.08

		Baseline	20 min. Post-Exp	3 hrs. Post-Exp	4 hrs. Post-Exp	5 hrs. Post-Exp	6 hrs. Post-Exp
Peak Flow	Air	491 ± 64	486 ± 47	478 ± 66	484 ± 51	496 ± 55	487 ± 52
l/sec	O_3	493 ± 92	478 ± 76	499 ± 51	470 ± 81	480 ± 72	483 ± 69
	NO_2	485 ± 65	489 ± 58	493 ± 46	482 ± 53	488 ± 49	500 ± 47

+ O_3 and NO_2 concentration, 0.18 ppm.

Changes in FEV_1 and total respiratory resistance during exposures are shown in Figures 7 and 8.

The analysis of covariance on total respiratory resistance values yielded a significant F ratio for the interaction of test atmospheres (air, ozone, nitrogen dioxide) and conditions (PR,PE1,PE2). These changes also are shown in Tables 12 and 13 and in Figures 9, 10 and 11. From Figure 11, it is clear that the ozone exposure was responsible for the significant interaction. After adjusting for baseline, the post-exercise-exposure 2 value increased 16 percent over the post-rest value (15 percent for healthy subjects and 32 percent for asthmatic subjects). There was no evidence that healthy and asthmatic subjects differed in this response. Nine of ten asthmatic subjects, and all the healthy subjects, experienced increased R_T values after ozone exposure. Also, although not significant, graphical analysis suggested the possibility of a dose-related trend in the increases in total respiratory resistance (Figure 12). However, it should be noted that some of the subjects were different in the three Phases.

The F ratio based on FEV_1 values for conditions was significant (Table 13). In the asthmatic subjects, FEV_1 decreased an average of 1 percent after air exposure, 4 percent after ozone exposure, and 3 percent after nitrogen dioxide exposure. In healthy subjects, FEV_1 decreased an average of 3 percent after air exposure, 2 percent after ozone exposure, and 2 percent after nitrogen dioxide exposure.

None of the other pulmonary function parameters showed significant F ratios. However, measures of maximal flow at 50 and 75 percent of expired vital capacity were reduced from baseline values in asthmatic subjects exposed to ozone. Also, maximal flow at 75 percent expired vital capacity was decreased over baseline values in healthy subjects exposed to ozone.

After Phase III exposures, subjects' symptom scores showed no consistent changes within or between groups among the test atmospheres, although healthy subjects reported more total symptoms than did asthmatic subjects.

Ambient concentrations of ozone and nitrogen dioxide during Phase III (September, 1985 through March, 1986) of this experiment also were low, except during December 1985, when the Seattle area experienced unhealthful air due to an inversion. Our research was curtailed for approximately two weeks. The maximal ambient hourly averages, during periods of experimentation, were 0.03 to 0.04 ppm for ozone; the averages were 0.07 to 0.08 for nitrogen dioxide.

Table 12. Adjusted Cell Means from the Analysis of Covariance of FEV_1 and R_T for Adolescent Subjects.

		Adjusted Cell Means For FEV_1 (1)					
		Phase I		Phase II		Phase III	
		Healthy	Asthmatic	Healthy	Asthmatic	Healthy	Asthmatic
Air	PR +	3.27	3.17	3.33	3.24	3.44	3.49
	PE1	3.25*	3.21*	3.23	3.14	3.36	3.42
	PE2			3.25	3.25	3.41	3.40
	PE3			3.27	3.25	3.39	3.44
NO_2	PR	3.18	3.17	3.37	3.26	3.51	3.48
	PE1	3.17	3.14	3.23	3.11	3.41	3.38
	PE2			3.40	3.21	3.43	3.41
	PE3			3.38	3.25	3.44	3.44
O_3	PR	3.25	3.18	3.39	3.28	3.47	3.50
	PE1	3.29	3.13	3.28	3.18	3.42	3.38
	PE2			3.39	3.20	3.42	3.44
	PE3			3.29	3.27	3.42	3.44
		Adjusted Cell Means For R_T ($cmH_20/1/sec.$)					
		Phase I		Phase II		Phase III	
		Healthy	Asthmatic	Healthy	Asthmatic	Healthy	Asthmatic
Air	PR	3.92	4.15	4.35	4.16	4.14	4.11
	PE1	4.18*	3.72*	4.04	4.26	4.27	4.65
	PE2			4.59	4.61	4.45	4.49
NO_2	PR	4.07	3.97	4.17	4.31	3.93	4.42
	PE1	4.18	3.76	4.36	4.89	4.19	4.83
	PE2			4.80	4.98	4.27	4.48
O_3	PR	3.90	3.70	4.13	3.87	4.48	4.28
	PE1	4.12	3.54	4.00	4.64	4.24	4.83
	PE2			4.71	5.11	5.13	5.66

*Second rest period

\+ PR = Post-Rest

PE1 = Post-Exercise Exposure 1 recording (2 to 3 min. post-exercise)

PE2 = Post-Exercise Exposure 2 recording (7 to 8 min. post-exercise)

PE3 = Post-Exercise Exposure 3 recording (12 to 13 min. post-exercise)

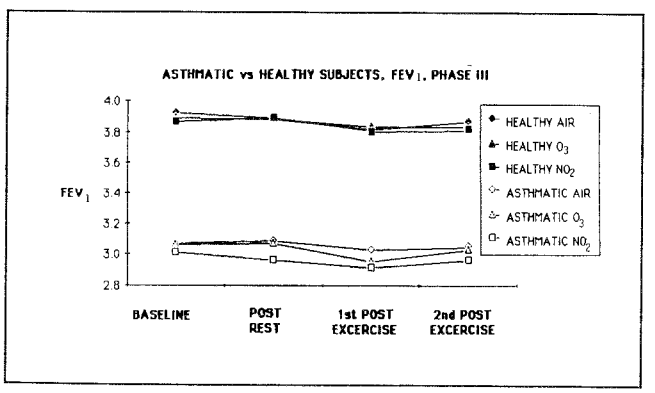

Figure 7: Mean changes in FEV_1 after 30 minutes' exposure at rest and 10 minutes' exposure during moderate exercise. Phase III.

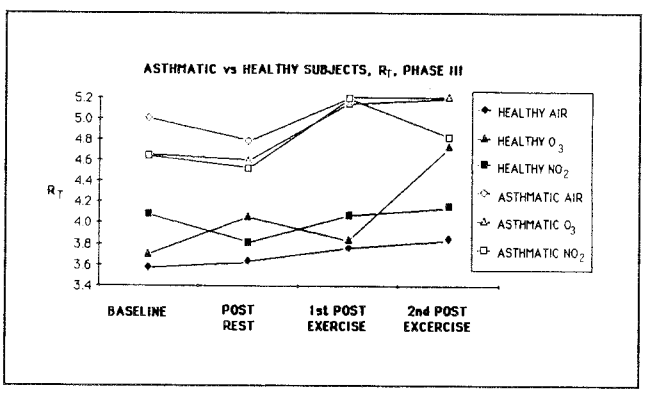

Figure 8: Mean changes in R_T after 30 minutes' exposure at rest and 10 minutes' exposure during moderate exercise. Units for R_T, $cmH_2O/1/sec$. Phase III.

Table 13. Analysis of Covariance of FEV_1 and R_T using Baseline Values as Covariates.
Groups = Asthmatic or Healthy; Atmospheres = Air, O_3, or NO_2; Conditions = Recording periods, post-rest 1 or 2 and post-exercise-exposure 1 or 2.

SUMMARY OF ANALYSES OF COVARIANCE OF FEV_1

SOURCE OF VARIATION	PHASE I D.F.#	PHASE I M.S.+	PHASE II D.F.	PHASE II M.S.	PHASE II D.F.	PHASE II M.S.
Group	1	.11321*	1	.31631	1	.00494
Baseline	1	69.94859	1	81.20906	1	172.29045
ERROR I	16	.01815	16	.08386	17	.01411
Atmosphere	2	.00728	2	.03977	2	.00363
Atmos x Group	2	.02105	2	.01058	2	.01690
Baseline	1	.36405	1	.19236	1	.61190
ERROR II	33	.02165	33	.02866	35	.03576
Conditions	1	.00033	3	.14753**	3	.07540**
Cond x Group	1	.00130	3	.01135	3	.00262
ERROR III	17	.00605	51	.02048	54	.00870
Atmos x Cond	2	.00270	6	.00961	6	.00076
Atmos x Cond x Grp	2	.01264	6	.01334	6	.00453
ERROR IV	34	.00676	102	.01186	108	.00521

SUMMARY OF ANALYSES OF COVARIANCE OF R_T

SOURCE OF VARIATION	PHASE I D.F.	PHASE I M.S.	PHASE II D.F.	PHASE II M.S.	PHASE II D.F.	PHASE II M.S.
Group	1	2.55876	1	.08934	1	.01195
Baseline	1	94.01553	1	219.90161	1	202.11973
ERROR I	16	.83182	16	1.96329	17	1.36128
Atmosphere	2	.15097	2	.54176	2	1.63127
Atmos x Group	2	.35014	2	.02869	2	.15532
Baseline	1	12.25873	1	1.56647	1	9.75483
ERROR II	33	.71514	33	.55596	35	.54936
Conditions	1	.03571	2	5.98167**	2	2.73325*
Cond x Groups	1	1.54884*	2	1.15748	2	1.08646
ERROR III	17	.33619	34	.52545	36	.55476
Atmos x Cond	2	.02821	4	.55911	4	.72012*
Atmos x Cond x Grp	2	.10406	4	.15290	4	.14769
ERROR IV	34	.22321	68	.24352	72	.27176

*p < 0.05 **p < 0.001 #Degrees of freedom +Mean square

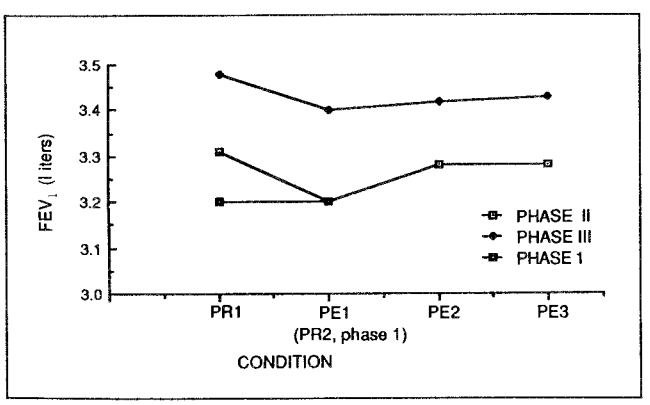

Figure 9. FEV_1 combined for groups and test atmospheres to the same baseline value within a phase.

COMBINED ANALYSIS

The analyses of covariance and the graphs (Figures 9 through 12) show no significant evidence of a differential effect in responses to pollutants between asthmatic and healthy subjects. There also is no evidence of a significant differential dose-response effect. However, at PE2, graphical analysis revealed a pattern in response after ozone exposure which was consistent with a linear trend in total respiratory resistance response to doses (Figure 12). It should be noted that different subjects participated in the different dose exposures, especially in the case of the asthmatic subjects where four new subjects were recruited for Phase II and six new subjects for Phase III.

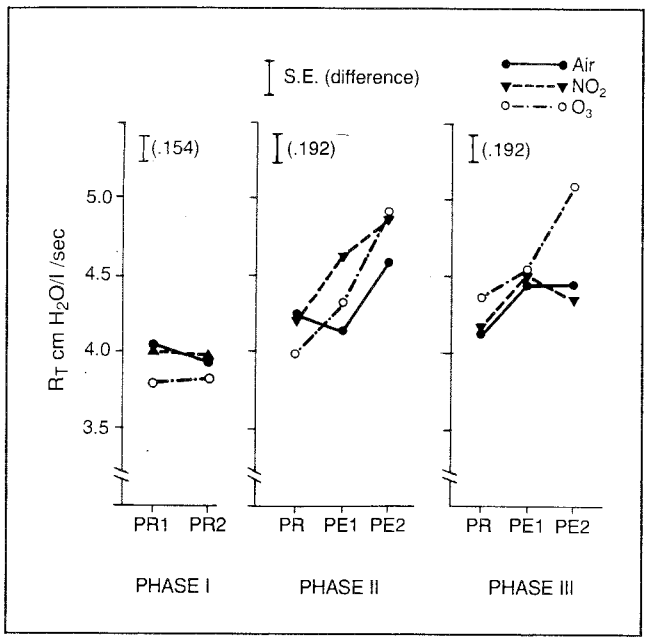

Figure 10. R_T by conditions during exposure (PR, PE1, PE2) and atmospheres (air, NO_2, and ozone) and phase. Vertical bars represent standard errors of the difference between means under the same condition.

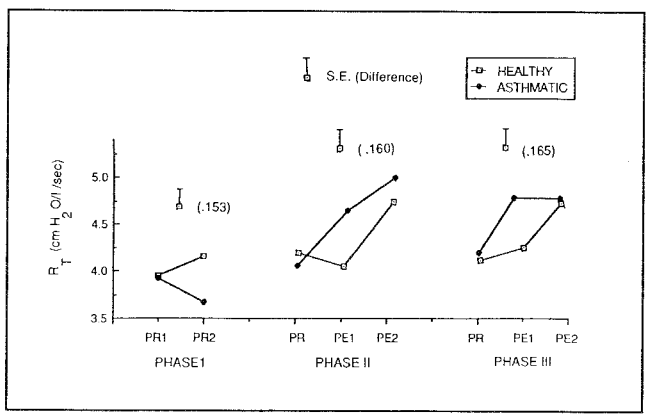

Figure 11. R_T by group and conditions during exposure (PR, PE1, PE2). Vertical bars represent standard error of the difference between means under the same condition.

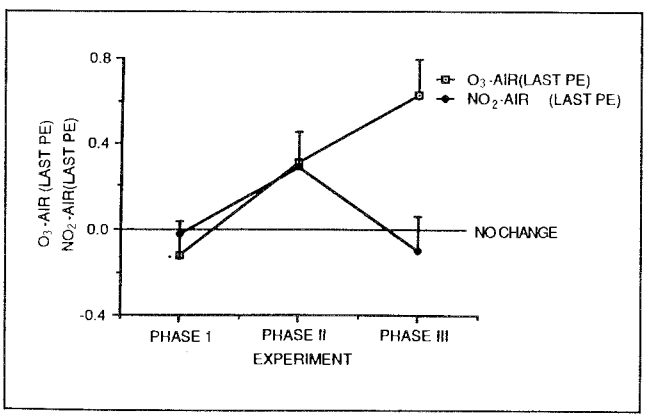

Figure 12. R_T for ozone-air or nitrogen dioxide-air adjusted to the same baseline. A vertical bar represents the standard error of estimated effect.

Figure 9 indicates a pattern in FEV_1 response which is similar in Phases II and III under the four experimental conditions. Table 13 indicates highly significant differences among these conditions. Figure 11 compares total respiratory resistance changes between the healthy and asthmatic groups of subjects.

Figure 10 portrays the atmosphere by condition interaction. Table 13 indicates that this interaction is significant in Phase III ($p < 0.05$). The vertical bars on the graph represent the standard error of the difference between two means within the same condition. Thus, in Phase III, the ozone exposure at PE2 is significantly higher than the average of nitrogen dioxide and air. In Phase II, the overall F test for this interaction is not significant and the pattern is not similar to that in Phase III.

Figure 12 shows the dose-related change in total respiratory resistance for ozone-air exposure.

DISCUSSION

The most important conclusion of this study is that no group-by-test-atmosphere interactions were significant, which indicates that in this study, asthmatic subjects were no more responsive to ozone or nitrogen dioxide than were healthy subjects.

Phase I of this study demonstrated that one hour of inhalation by subjects at rest, of either 0.12 ppm ozone or 0.12 ppm nitrogen dioxide, had no adverse effects in either healthy or asthmatic adolescents. The decreases in FEV_1 and increases in total respiratory resistance for all conditions seen in both asthmatic and healthy subjects during Phase II probably reflect, to some degree, an effect of exercise, since such changes were seen after the subjects were exposed to the air test atmosphere. The asthmatic subjects did show a prolonged decrease in FEV_1 after exposure to 0.12 ppm ozone during exercise (Table 4). In the healthy subjects, moderate exercise had a slight bronchoconstrictive effect. This was demonstrated by both the decrease in mean FEV_1 after exercise during exposure to air and by the increases in mean total respiratory resistance after exercise during exposure to all three test atmospheres. The exercise level was chosen to avoid exercise-induced bronchospasm in the asthmatic subjects; the healthy subjects were screened so that none had exercise-induced bronchospasm. Nevertheless, a small exercise-induced change in pulmonary function was evident in both Phases II and III. This is surprising considering the low-to-moderate levels of exercise used.

After exercise-exposure to 0.18 ppm ozone in Phase III of this study, the average total respiratory resistance in both healthy and asthmatic subjects increased significantly based on analysis of covariance (15 percent and 32 percent, respectively). The magnitude of the increase in total pulmonary resistance did not differ significantly between the two groups of adolescents. In both groups, the ozone-induced increase

was greater than the small increase seen after exposure to air, which is probably the effect of exercise. The change in FEV_1 after exercise-exposure to all test atmospheres in healthy and asthmatic subjects is interpreted to be a response to exercise alone, similar to that seen in Phase II.

It is possible that asthmatic subjects, due to their illness, have learned to be more aware of respiratory symptoms than healthy individuals are. In any case, during Phase I, asthmatic subjects reported more symptoms than the healthy subjects did. The asthmatic subjects reported 1.6 times more symptoms after ozone exposures, and two times more symptoms after nitrogen dioxide exposures, than after air exposure. Healthy subjects reported essentially the same number of symptoms after exposure to the three test atmospheres. Two subjects did guess correctly when they breathed ozone. One of these was a healthy subject who reported a sore throat and felt more tired the day after ozone exposure. One asthmatic subject reported feeling nauseated; his peak flow values were reduced from 500 l/s to 350 l/s four hours after ozone exposure. One healthy subject had an upper respiratory infection and flu-like symptoms three days after nitrogen dioxide exposure.

Symptom rating scores associated with the different test atmospheres did not differ significantly in Phase II. As was true in Phase I of this study, asthmatic subjects reported approximately twice the number of symptoms that the healthy subjects reported. However, in Phase II, asthmatic subjects showed no trend toward more symptoms after ozone or nitrogen dioxide exposures.

In Phase III, healthy subjects reported more symptoms than asthmatic subjects did. One healthy subject was responsible for 20 to 30 percent of the total symptoms reported by her group. The lack of symptom correlation with increases in total respiratory resistance during Phase III may be due to diffuse changes in the lung, which were not sufficient to cause shortness of breath or trigger irritant cough receptors.

This study indicates that both healthy and asthmatic adolescent subjects at rest do not show pulmonary functional changes after short-term exposures to either 0.12 ppm ozone or nitrogen dioxide, and that asthmatic and healthy subjects show insignificant changes after exposure to 0.12 ppm ozone or nitrogen dioxide during moderate exercise. These data do indicate that total respiratory resistance increased in both healthy and asthmatic adolescent subjects after exercise-exposure to 0.18 ppm ozone. It is important to note that all the asthmatic subjects in this study were asymptomatic at the time of study, and were in a carefully monitored clinical care program. The fact that, in spite of this, they showed significant increases in total respiratory resistance, indicates that appropriate pharmacological management is not always sufficient to protect asthmatic subjects from air pollutant-induced pulmonary effects during exercise.

The indication of a dose-response effect seen in Figure 12 needs to be viewed cautiously. Subjects were not assigned to dose on a random basis and different subjects participated in the three phases.

The fact that significant changes were seen in total respiratory resistance values, but not in FEV_1 values, after 0.18 ppm ozone, is consistent with the conclusion of McDonnell and co-workers (1983). These authors suggested that analysis of the dose-response curves derived from ozone exposures in healthy adult subjects indicated a sigmoid-shaped curve for FEV_1 values, but a curve with no plateau for specific airway resistance values. The authors further concluded that their results suggested that ozone may have effects mediated by more than one mechanism. A more recent article from this same group (McDonnell et al., 1985) showed that atropine sulfate abolished ozone-induced changes in airway resistance, but only partially blocked forced expiratory flow changes. This result also suggested the existence of more than one mechanism for ozone-induced pulmonary function changes. The results from the present study, showing a lack of correlation between total respiratory resistance and FEV_1 changes, are consistent with these conclusions.

Although other investigators have reported changes in lung function after similar exposures in adult subjects, the present study is, to the best of our knowledge, the first that compares the effects of oxidant exposures in healthy and asthmatic adolescent subjects. Since the results of Phase I were published, two other articles reported the response to oxidant air pollution in healthy adolescent subjects. Avol and co-workers (1985) exposed healthy volunteers 12 to 15 years of age (46 male, 13 female) to either purified air or ambient air that contained approximately 0.14 ppm ozone during one hour of vigorous exercise. The mean FEV_1 of the group decreased 4 percent after ambient exposure ($p < 0.01$), but remained the same after purified air exposure. Forced expiratory volume and peak expiratory flow also decreased significantly after ambient exposure. In addition, McDonnell and co-workers (1985) reported significant decreases in FEV_1 in a group of 23 healthy boys (8 to 11 years of age) exposed to 0.12 ppm ozone during vigorous exercise. Exposure to clean air during exercise did not decrease pulmonary function significantly.

Both the Avol and the McDonnell articles describe their exercise as vigorous; we describe our exercise as moderate. However, the reported ventilatory rates are similar. In the study done by Avol and co-workers, the average ventilatory rate was 35.1 liters/minute for boys and 24.6 liters/minute for girls. In the McDonnell study, the average ventilatory rate was 33.0 liters/minute. In Phase II of our study, the average ventilatory rate was 32.3 liters/minute for healthy subjects, and 32.9 liters/minute for asthmatic subjects. It appears that the exercise level was very similar in the three studies. However, the duration of exercise differed among the studies. In the Avol study, subjects exercised for one hour continuously. In the

McDonnell study subjects were exposed for 2.5 hours; during the first two hours of exposure, they exercised 15 minutes.

Changes in bronchial reactivity were not assessed in Phase I of this study; this might have been a useful addition. Therefore, in Phase II, a standard methacholine challenge test was used to assess changes in non-specific bronchial reactivity after subjects were exposed to air, ozone, and nitrogen dioxide. Unfortunately, healthy subjects responded to 5 and 10 mg/ml of methacholine after exposures to air, whereas they had not responded to 25 mg/ml methacholine during the screening process. It may be that inhalation via a mouthpiece during moderate exercise affects bronchial reactivity, even when the test atmosphere is clean air. This would be consistent with our conclusion that the changes in pulmonary function after air exposures in the healthy subjects were due to the effects of exercise. The enhanced response to methacholine after air exposures made it impossible to use the methacholine challenge to assess the additional effects of ozone or nitrogen dioxide.

We conclude that 60 minutes of exposure at rest to 0.12 ppm of either ozone or nitrogen dioxide did not induce consistent pulmonary functional changes in our subjects. The addition of ten minutes of exercise-exposure produced small consistent changes in both FEV_1 and total respiratory resistance in healthy and asthmatic subjects. However, pollutant-induced changes did not differ from changes that followed air exposures. Increasing the concentration of ozone to 0.18 ppm produced consistent significant changes in the total respiratory resistance in both groups of subjects.

Probably the most important finding of this study is that asthmatic adolescents are no more sensitive to inhaled oxidant pollutants at the concentration which is the current primary standard for ozone than are healthy adolescent subjects. However, these data are limited by the small number of subjects studied.

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SYMPTOM RATING SCALE

Phone: Jane Koenig 543-4383
HSB Room F 551
Emergency 24-hour phone: 527-1200

Subject number _____

Exposure date _____

Do you regularly take vitamins? _____

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 = no effect
1 = small effect
2 = small-moderate effect
3 = moderate effect
4 = moderate-large effect
5 = large effect

	During exposure	Following exposure *(Remainder of same day)*	Following Day
Cough			
Chest pain or burning			
Dyspnea (shortness of breath)			
Fatigue			
Headache			
Unusual taste or smell			
Sore throat			
Nasal discharge			
Wheezing			
Dizziness			

Vitalograph readings:

Before exposure _____

15 minutes post-exposure _____

6:30 _____

7:30 _____

8:30 _____

9:30 _____

APPENDIX B

Subjects who dropped out after Phases I and II.

Phase I		**Reason subject did not enter Phase II.**
Asthmatic	**0402:**	Subject had participated in an SO_2 study prior to Phase I and said she was too busy to participate anymore - felt she had done her share.
	0316:	Came for one visit in Phase II. Had been unreliable in terms of keeping appointments in Phase I. After missed appointments and lack of reaching him on phone, gave up.
	0406:	Mother simply said subject decided not to participate in Phase II. There was no mention of illness.
	0418:	Subject was too busy, and since her baselines were a little inconsistent in Phase I (one day had to be rescheduled), I didn't try to encourage her.
Phase I Healthy	**1116:**	Subject graduated from high school and was busy at University. Actually, when I was short of subjects, I encouraged him to come back for Phase III.

Phase II		**Reason subject did not enter Phase III.**
Asthmatic	**0215:**	Graduated from high school and enrolled in college out of area.
	0315:	Apparently moved. Dr. Pierson had no forwarding address.
	1121:	Exceeded the age range.
	1227:	Actually dropped out after two visits in Phase II. Responsible for NO_2 missing data. Apparently found experiment boring. No mention of illness.
	0322:	Mother said subject was too busy. Very cooperative subject who was featured in a press account of our lab. We did have some trouble scheduling her in Phase I and II due to illness, but she and her mother were very supportive and I'm certain her mother would have stated if adverse effect was a cause for discontinuing.
	0520:	Subject was visiting relatives out of state. Mother repeatedly said she was due back, but time dictated that we find another subject.
Phase II Healthy	**0302:**	Mother stated subject had experienced more respiratory illness than usual during Phase II, and stated she did not want her daughter to participate in Phase III.
	1027:	Subject was involved in sports and working after school, and said she didn't have time to participate.
	0223:	Subject was dropped because of her inability to perform reproducible FEV_1 maneuvers.

HEALTH REVIEW COMMITTEE'S REPORT

TECHNICAL EVALUATION

GOALS AND OBJECTIVES

The primary goal of this study was to determine if asthmatic adolescents differ from healthy adolescents in their sensitivity to ozone and nitrogen dioxide at concentrations near the ambient air quality standards.

Sensitivity to exposure to air, ozone, or nitrogen dioxide was assessed by acute pulmonary responses on three separate days at least one week apart. Pulmonary responses measured before, during, and after exposure included forced expiratory volume in one second (FEV_1), peak flow, total respiratory resistance (R_T), thoracic gas volume at functional residual capacity (FRC), maximal flow at 50% and 75% of expired vital capacity ($\dot{V}_{max\ 50}$ and $\dot{V}_{max\ 75}$), breathing room air, and breathing helium and oxygen. Symptoms were recorded at the end of exposure, at 8:30 p.m. on the evening of that day, and on the following day.

The study was conducted over a three-year period in three phases; ten healthy and ten asthmatic adolescent subjects were studied in each phase. Exposures within each phase were administered in random order by a mouthpiece: in Phase I for two 30-minute periods at rest with a brief interval for testing between them; in Phases II and III for 30 minutes at rest, followed by ten minutes at moderate exercise. In Phases I and II, the test exposures were 0.12 ppm ozone and 0.12 ppm nitrogen dioxide, and in Phase III, they were 0.18 ppm ozone and 0.18 ppm nitrogen dioxide. Pulmonary function tests were carried out directly before exposure, between the rest periods, between rest and exercise, and at the end of the tests. Responses to filtered air were measured in all three phases. The measurements of pulmonary function were made by a technician blind to the nature of the exposure.

SUMMARY OF THE INVESTIGATORS' CONCLUSIONS

The major supportable conclusions of the study are:

1) No difference in pulmonary function responses were detected between asymptomatic, treated adolescent asthmatics and healthy adolescents exposed to ozone or nitrogen dioxide under the conditions of these studies.
2) An increase in total respiratory resistance in both asthmatic and healthy adolescent subjects was observed after exposure to 0.18 ppm ozone.

In addition to these conclusions, Koenig et al. suggested several others with which we have some disagreement. A more detailed discussion can be found in the sections that follow.

1) The authors propose that 0.12 ppm ozone elicits a small increase in total respiratory resistance in both healthy and adolescent subjects. However, the results show that the increase is not significantly different from those that occurred with exposures to air and nitrogen dioxide.
2) The investigators suggest that a dose-related increase in response occurs in asthmatic and healthy adolescents after exposure to 0.18 ppm compared with 0.12 ppm ozone. We believe that this observation should be regarded as a hypothesis for testing in a study that is designed for this purpose, and that has adequate power to detect a difference of the expected magnitude.

TECHNICAL REVIEW

The investigators are experienced in testing potentially sensitive individuals exposed to air pollutants in the laboratory, and have published in this area. The work reported here, in essence, represents three separate studies conducted over a three-year period. Valid comparisons can be made between the asthmatic and healthy subjects within each phase. However, the design and conduct of this study had several weaknesses. Comparisons between phases are not appropriate, since the asthmatic populations in each of the phases are different because of the high drop-out rate among the asthmatic subjects. We acknowledge the difficulties in recruiting and maintaining asthmatic subjects. Indeed, of the twenty different asthmatic subjects who took part in the entire study, only three participated in all three phases. For comparison between phases, it is desirable that either all subjects continue through all phases, or a new group be randomly selected for each phase. There is, otherwise, a possibility of selection bias, because subjects who either choose to continue, or who are selected to continue by the investigators, may differ from the subjects who do not continue.

The variation of exposure conditions between each phase of the study further complicates any comparisons between phases. The exposure conditions in each phase were varied for a unique set of two parameters taken from a set of the following three variables: time of exposure, amount of exposure, and exposure at rest compared to exercise. We believe that it would have been desirable to design a single experiment with varying doses of the pollutants administered in a double-blind, randomized fashion. Subjects should have been treated in a uniform manner during periods of rest and exercise. We acknowledge, however, that issues of safety and feasibility, and the use of results from earlier phases to select exposures for later phases, contributed to the investigators' decisions on their study design.

Pulmonary function was measured according to a standardized protocol. Information on outdoor levels of ozone and nitrogen dioxide were recorded at the time of each experiment. The investigators made a large number of observations on few subjects, although only two of the pulmonary function measurements were discussed in detail. These are the forced expiratory volume, FEV_1 (the volume of gas exhaled in one second by a forced expiration from full inspiration), and total

respiratory resistance, R_T (the resistance of the airways and chest wall to movement of air during quiet breathing). Some tests did not yield useful data; for example, ear oximeter signals were not clear during exercise and, therefore, there are no data reported on oxygen saturation. In addition, control subjects responded to methacholine challenge following experimental exposure to air, but they did not do so during screening in a different setting. Therefore, there were no data on bronchial reactivity. Moreover, the cutpoints for distinguishing responders and non-responders to exercise and methacholine challenge, which used well-established standards, are nevertheless arbitrary, so that there may be small differences between the two groups of subjects. However, there is no doubt that the asthmatic subjects had clinically significant disease, and the control subjects did not.

DATA ANALYSIS

The experimental design was one in which subjects were measured repeatedly within each of three phases. The results obtained from asthmatic and healthy subjects from each phase were analyzed, and comparisons were made between changes in the pulmonary function from exposure to pollutants and changes from exposure to clean air. Comparisons among study conditions within any phase were performed using computed summary statistics and repeated measures of analysis of covariance. Comparisons that involved different groups of individuals were adjusted by using the baseline response as a covariate. This adjustment requires a fairly strong assumption of parallelism. Although the level of response may differ, the same functional response is expected. Examination of the data for compatibility with this assumption could have been more extensive.

Another issue is the test of the condition by group interaction. There is a concern as to whether the response profile is the same for the asthmatic group as it is for the control group. The response profile does not require adjustment for differences that might have been present at the beginning of the experiment. The interaction test was significant at $p = 0.05$ in one of six tests of total respiratory resistance. The probability value is valid for a single comparison. Multiple comparisons require a more stringent p value before significance is attained because of the compounding effect of experimental variation and true variation. Adjusting the significance level for multiple testing gives a more accurate significance probability of less then or equal to 0.10 (Bonferoni inequality equation) for interactions found in total respiratory resistance in Phase I.

INTERPRETATION OF RESULTS

The most striking findings were that, as expected, the mean levels of FEV_1 were lower, and the levels of R_T were higher, in asthmatic than in healthy subjects, both at baseline and following all exposures. Exercise had an adverse effect on FEV_1, whereas exposure to ozone or nitrogen dioxide did not. Exercise also had a significant adverse effect on R_T. There was a significant interaction between exercise and exposure, which was due to an increased response to ozone as compared to air or nitrogen dioxide at the second post-exercise measurement. The response to exercise among asthmatic subjects is not surprising because exercise-induced bronchospasm was a requirement for their inclusion in the study, whereas response to exercise was a reason for excluding healthy control subjects. It should be noted, however, that the responses to exercise in asthmatic and control groups were not significantly different. (No significant "group x conditions interactions" were detected in Phases II and III, as shown in Table 14.)

The authors suggest that there is a dose-response relationship with ozone. However, it should be noted that exercise ventilation was greater in Phase III than in Phase II, and some different subjects were studied in the two phases. This difference could account for the postulated dose-related effect. In addition, the ability of the investigators to detect increased sensitivity among asthmatic subjects to oxidant air pollutants was limited because of the nature of the sample group; the asthmatic subjects used in the study all were asymptomatic and on medication until shortly before testing.

A further problem arises from the selection of adolescents for the sample group. The investigators' rationale for choosing adolescent subjects was based on previous observations of this age group during its exposure to sulfur dioxide (Koenig et al. 1981).† However, this group experiences a phase of rapid growth associated with substantial changes in pulmonary function over a short period of time. This is illustrated in the study of asthmatic subject 1129, whose baseline FEV_1 in Phases I, II, and III ranged from 3.2 to 3.8 to 4.5 litres per second. This subject grew in height from 164cm to 179cm during the course of the study.

The frequency with which both groups of subjects reported respiratory symptoms was described. Opposite trends over the three phases were noted, with a decline in symptom scores during the course of the study among asthmatic and an increase among healthy subjects. In Phases I and II, the average numbers of lower respiratory symptoms reported were higher for asthmatic than for healthy subjects, whereas in Phase III this was reversed, with healthy subjects reporting more symptoms then asthmatic subjects. To what extent this trend was determined by the substantial drop-out rate among asthmatic subjects is not known; the composition of the healthy group changed less and is unlikely to account for the increasing symptom score in this group.

ATTAINMENT OF STUDY OBJECTIVES

This study adds to the body of existing knowledge of the effects of exposure to oxidant pollutants on normal and asthmatic young people. Three observations were made concerning the relative sensitivity of asthmatic adolescents compared to healthy adolescents.

1) Slight but non-significant differences in pulmonary function responses between the two groups were observed in Phase 1 only, but not in Phase II or III. In Phase I,

† See References on page 27.

after pollutant exposure, the R_T increased in healthy subjects but decreased in asthmatic subjects. There is no apparent biologic reason for this pattern of response.

2) Total R_T increased in both groups of subjects in Phases II and III, but it was associated with the addition of exercise to the protocol, and occurred during exposure to air and pollutants. Decreases in FEV_1 after exposure to pollutants at both rest and during exercise were similar to those after exposure to air in both groups of subjects.

3) Both groups of subjects experienced an increase in R_T following exercise and exposure to 0.18 ppm ozone, and this response was different from that following exposure to air or nitrogen dioxide. Thus, this study suggests that exposure to 0.18 ppm ozone has an equally deleterious effect on asthmatic and healthy adolescents.

RECOMMENDATIONS

Although the results of this study have been largely negative, some of the major problems in this type of research are clarified, and will provide useful guidance for future studies. Koenig et al. (1981; 1982; 1983)† have previously reported clinical studies that showed that sulfur dioxide significantly enhanced adverse effects in adolescent asthmatics compared to healthy individuals. The study reported here indicates that this is not the case for ozone and nitrogen dioxide. Indeed, there are some serious disadvantages in using this age group because they undergo a phase of rapid growth during which pulmonary function changes over a short period of time. Thus, responses to varying levels of exposure would be difficult to separate from the changing pulmonary function parameters associated with growth and aging.

When small differences in response are being investigated, sample size becomes very important to obtain significant results. These studies lacked the power either to detect slight differences between responses of asthmatic and healthy subjects or to measure dose effects in either group. When it is not possible to increase sample size, experimental conditions should be altered to provide a greater measurable response. This could be done either by longer exposures at rest or during repeated periods of exercise, or by increasing the level of exposure. The problems of significance are compounded by the intrinsic variability in the responses of healthy and asthmatic people to oxidant pollutant exposure.

New information on healthy subjects (McDonnell et al., 1983)†, as well as a great deal of existing information on asthmatic subjects (Bromberg, 1987)†, indicates that there is a wide intra- and inter-individual variability in levels of pulmonary function, and in response to exposure and exercise. Bronchial reactivity should be measured before, during, and after exposure because other investigators have reported increased bronchial reactivity to methacholine challenge or cold air after exposure to nitrogen dioxide and ozone. More studies on healthy and asthmatic subjects are desirable to measure variability. The frequency and magnitude of exaggerated responses should be recorded, and it should be determined if certain asthmatic subjects always respond adversely because of their disease or its treatment.

CONCLUSIONS

Asthmatic subjects represent an appropriate model of a susceptible population. However, asthma is a heterogeneous condition, and inferences about the sensitivity of affected persons, compared with healthy control subjects, require the study of larger numbers of subjects with a wider range of severity of manifestations than were included in this study. The new data provided by this study have little impact on risk assessment or public policy. However, exploratory studies like this one can provide guidance to the future development of studies that use larger numbers of subjects, and thus can more adequately define the presence or absence of a significant health effect.

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† See References on this page.

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