

# Controlled human studies of ozone exposure

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In controlled studies on humans, volunteers are exposed to ozone in an exposure chamber, and possible health effects can be assessed by different methods. The effects of ozone on lung function have been extensively studied, and there are several reports on inflammatory effects on the airways, on airway responsiveness, on mucociliary clearance, and on exercise performance. During recent years there have also been some reports on the effects of ozone exposure on allergen-induced reactions in the airways. The subjects selected for study have comprised both normal subjects and persons purported to be especially sensitive, such as patients with asthma, chronic obstructive lung disease, or heart disease. The duration of the exposure has varied from about 1 to 8 h in the different studies. In some investigations the subjects were exposed while at rest, but in most they were exposed to the gas during exercise, varying from light to very heavy. Most studies were performed with a single exposure, but there are also several reports on the effects of repeated exposures to ozone. Subjects have also been exposed to a combination of ozone and other gaseous pollutants, either as a mixture of the gases or in sequence with the separate gases. In such investigations a special type of controlled exposure experiment is performed in mobile laboratories set up in polluted areas, where the subjects are exposed to the ambient, polluted air (including ozone). Thus controlled experiments can give valuable information on the possible adverse health effects of exposure to ozone.

The objective of this review is to summarize the results reported after short-term exposure of humans to ozone. The effects of ozone are presented in separate sections dealing with lung function, bronchial responsiveness, exercise performance, inflammation in the nose and bronchi, host defense, and effects of ozone mixed with other pollutants, including data from studies in mobile laboratories.

## Lung function measurements

### Forced expiratory volume and vital capacity

When the subject performs a maximal expiratory effort, the forced expiratory volume (FEV) is the volume of gas exhaled from the fully inspired lung over a defined period of time. The time interval is usually 1 s and the index is then termed FEV<sub>1,0</sub>. When the airways are obstructed the FEV<sub>1,0</sub> is decreased. The FEV<sub>3,0</sub>, FEV in 3 s, is some-

times used when patients with severe obstruction are investigated. FEV is measured by spirometry, which is a routine method for lung function measurement with high reproducibility. FEV<sub>1,0</sub> mainly reflects the function of the central airways and is poorly influenced by the degree of expiratory effort.

Another index of forced expiration is the peak expiratory flow rate (PEFR or PEF), which can easily be measured by the subject at home and at work. However, the test is to a considerable extent dependent on the degree of expiratory effort and is thus not as reproducible as FEV.

Vital capacity (VC) is the maximal volume which the subject can expel from the lung when he or she is instructed to continue the exhalation until no more gas can be pressed out. When this maneuver is performed as a forced expiration, the index is termed forced vital capacity (FVC), which is often slightly less than the VC recorded during a more leisurely expiration.

### Airway resistance and lung volume

The resistance of the airways ( $R_{aw}$ ) can be measured with whole-body plethysmography, and an increased  $R_{aw}$  is an early sign of airway obstruction. Airway resistance mainly reflects the function of central airways.

Airway resistance is dependent on the lung volume at which it is measured. In order to correct for this dependence, the term specific airways resistance ( $SR_{aw}$ ; resistance times volume) is used. Airway conductance is the inverted value of resistance and is usually expressed as specific airway conductance ( $SG_{aw}$ ).

A plethysmographic measurement can also yield the lung volume. Thoracic gas volume (TGV) is the volume of the lung. It is commonly measured during tidal breathing, that is, at functional residual capacity (FRC). By adding a maximal inspiration (inspiratory capacity, IC or IVC) to breathing at the FRC, the total lung capacity (TLC) can be calculated.

### Forced expiratory flow and the maximal expiratory flow-volume curve

During a maximal expiration from total lung capacity the maximal flow rate is normally reached rapidly and then the flow declines. From the forced expiratory spirogram, or a registration of maximal expiratory flow together with lung volume, different indices of the flow at defined points during expiration can be derived (eg, forced expiratory flow between 25% and 75% of the vital capacity,

FEF<sub>25-75</sub>, or maximum expiratory flow at 50% of the vital capacity,  $V_{\max 50}$ ).

These measurements of flow at lung volumes less than total lung capacity also reflect small airway function and can give additional information to that gained by FEV<sub>1.0</sub> and FVC. However, these indices are less reproducible and show greater normal variation than do FEV<sub>1.0</sub> and FVC.

Other tests which provide information about small airway function are the closing volume test and the single breath nitrogen test.

### *Breathing pattern and oxygen uptake*

The breathing frequency ( $f$ ) and the volume of a single breath at quiet breathing (tidal volume,  $V_T$ ) can also be affected by the inhalation of air pollutants. Minute ventilation ( $V_E$ ) is the expired volume per minute and the maximum minute ventilation is termed  $V_{E\max}$  or MVV (maximum voluntary ventilation).  $\dot{V}O_2$  denotes oxygen uptake by the body and  $\dot{V}O_{2\max}$  the maximal oxygen uptake.

### *Lung function after single exposure*

#### *Effects of ozone on the lung function of normal subjects*

Results of 24 studies of ozone-induced effects on the lung function of normal humans after a single exposure are shown in table 1. Table 1 is based on comprehensive work performed by the Environmental Protection Agency (EPA) in the United States, in selecting important scientific data (tables 7—1 and 7—9 in reference 1). The studies have been listed in the order of increasing concentration of the gas according to the lowest concentration used in each study. In estimates of exposure dose, the duration of exposure and the minute ventilation must both be taken into account.

Published reports are also available on lung function changes after ozone exposure in experiments mainly designed to study the effects on airway inflammation and airway responsiveness. These studies are not included in table 1, but they will be presented in the text under the corresponding headings.

Furthermore, there are four reports on the effects of pharmacological agents on the ozone-induced responses, including data on lung function changes, and these are presented at the end of the present section. So that the effects of ozone can be judged, comparisons have been made between effects of ozone and filtered air, the subjects functioning as their own controls, unless otherwise stated. In some studies mean pre- and postexposure values within a study group have been compared, and in one study subjects exposed to ozone were compared with a control group exposed to filtered air.

Healthy adult subjects exposed at rest for 2 h to varying concentrations of ozone (2, 3) showed a significant effect on FVC and FEV<sub>1.0</sub> at 1000 and 1500  $\mu\text{g} \cdot \text{m}^{-3}$ , respectively, compared with sham exposure to filtered air. No effects were seen at lower concentrations (500 and 740  $\mu\text{g} \cdot \text{m}^{-3}$ ). Linn et al (4) exposed 24 normal men to ozone concentrations between 160 and 320  $\mu\text{g} \cdot \text{m}^{-3}$  for 2 h, with intermittent exercise, and found no change in lung function. However, a statistically significant, though small, increase in lower respiratory symptoms was noted after 2-h exposure to 320  $\mu\text{g} \cdot \text{m}^{-3}$ .

When highly trained competitive cyclists were exposed to ozone for 1 h during intermittent, heavy exposure, significant decreases in FVC and FEV<sub>1.0</sub> were seen at both 360 and 480  $\mu\text{g} \cdot \text{m}^{-3}$  (5). In addition, the number of reported symptoms, such as shortness of breath, cough and raspy throat, increased significantly at 360 and 480  $\mu\text{g} \cdot \text{m}^{-3}$ . In another study of competitive cyclists performing heavy exercise (6), decreases in FVC and FEV<sub>1.0</sub> were found during exposure to 400  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h. Subjective symptoms such as soreness of the chest and shortness of breath were observed at 240  $\mu\text{g} \cdot \text{m}^{-3}$ , and at 400  $\mu\text{g} \cdot \text{m}^{-3}$  the symptoms were intensified with cough and nausea, the symptoms probably limiting the maximal performance of 13 of the 17 subjects. Similar results showing decrements in spirometric lung function data after 1-h exposure to ozone during heavy exercise have been reported in several studies (7—11).

The effects of ozone on lung function after 1-h exposure with exercise have mostly been detected at exposure concentrations from 400  $\mu\text{g} \cdot \text{m}^{-3}$  and upwards. However, effects are sometimes detected at 320  $\mu\text{g} \cdot \text{m}^{-3}$ . A 25% fall of the spontaneous tidal volume and a 9% decrease in the uptake of ozone in the lower respiratory tract was found (12) after exposure to 800  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h with light exercise on a treadmill.

In a study performed with a 2-h exposure to ozone at concentrations of 0, 200, 300, 400 and 500  $\mu\text{g} \cdot \text{m}^{-3}$  (13), with intermittent, heavy exercise on a treadmill, decreases were seen in FVC, FEV<sub>1.0</sub>, FEF<sub>25-75</sub> and SG<sub>aw</sub>. The threshold for lung function response was between 200 and 300  $\mu\text{g} \cdot \text{m}^{-3}$ . There was also a significant dose-response relationship for cough, nose or throat irritation, and chest discomfort, the symptoms starting at 300  $\mu\text{g} \cdot \text{m}^{-3}$ .

McDonnell et al (14) exposed young, healthy adults for 2.5 h to ozone concentrations ranging between 240 and 800  $\mu\text{g} \cdot \text{m}^{-3}$  and also found decreases in FVC, FEV<sub>1.0</sub> and FEF<sub>25-75</sub> from 240  $\mu\text{g} \cdot \text{m}^{-3}$  upwards. At this concentration, airway resistance was not affected, but at 480  $\mu\text{g} \cdot \text{m}^{-3}$  an increase in SR<sub>aw</sub> was also seen. A significant increase in the reported incidence and severity of cough was observed at all the ozone concentrations.

With longer exposures, lasting from 4 to 8 h, effects on lung function have been found at ozone concentra-

**Table 1.** Effects of controlled human exposure to ozone on the pulmonary function of healthy subjects.<sup>a</sup> (BSA = body surface area, CE = continuous exercise, FEF = forced expiratory flow, VC = vital capacity, FEF<sub>25-75</sub> = forced expiratory flow between 25% and 75% of VC, FEV<sub>1.0</sub> = forced expiratory volume in 1 s, FEV<sub>3.0</sub> = forced expiratory volume in 3 s, FVC = forced VC, IC = inspiratory capacity, IE = intermittent exercise, MVV = maximum voluntary ventilation, NO<sub>2</sub> = nitrogen dioxide, O<sub>2</sub> = oxygen, O<sub>3</sub> = ozone, SG<sub>aw</sub> = specific airway conductance, SO<sub>2</sub> = sulfur dioxide, SR<sub>aw</sub> = specific airway resistance, TLC = total lung capacity, V = ventilation, V<sub>25</sub> = ventilation at 25% VC, V<sub>50%</sub> = ventilation at 50% VC, V<sub>E</sub> = minute ventilation, V<sub>T</sub> = total volume, VO<sub>2</sub> max = maximal oxygen uptake)

| Reference                                | Ozone concentration ( $\mu\text{g} \cdot \text{m}^{-3}$ ) | Exposure duration | Activity                                                                                              | Number of subjects                                            | Subjects characteristics               | Observed significant effect(s)                                                                                                                                                                                                                                                                                |
|------------------------------------------|-----------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Healthy adult subjects at rest</i>    |                                                           |                   |                                                                                                       |                                                               |                                        |                                                                                                                                                                                                                                                                                                               |
| Horvath et al, 1979 (3)                  | 0<br>500<br>1000<br>1500                                  | 2 h               | .                                                                                                     | 8 men & 5 women                                               | Healthy adults                         | FVC decreased with 1000 and 1500 $\mu\text{g} \cdot \text{m}^{-3}$ O <sub>3</sub> exposure compared with filtered air                                                                                                                                                                                         |
| Silverman et al, 1976 (2)                | 0<br>740<br>1000<br>1500                                  | 2 h               | .                                                                                                     | 20 men & 8 women                                              | Young healthy adults                   | Decrease in FEV <sub>1.0</sub> , V <sub>25</sub> and V <sub>50</sub> with 1500 $\mu\text{g} \cdot \text{m}^{-3}$ O <sub>3</sub> exposure compared with filtered air                                                                                                                                           |
| <i>Healthy exercising adult subjects</i> |                                                           |                   |                                                                                                       |                                                               |                                        |                                                                                                                                                                                                                                                                                                               |
| Linn et al, 1986 (4)                     | 0<br>160<br>200<br>240<br>280<br>320                      | 2 h               | IE (4×15 min at V <sub>E</sub> =68 l · min <sup>-1</sup> )                                            | 24 men                                                        | Young, healthy adults                  | No significant changes in pulmonary function measurements                                                                                                                                                                                                                                                     |
| Horstman et al, 1990 (15)                | 160<br>200<br>240                                         | 6.6 h             | IE (6×50 min at V <sub>E</sub> =39 l · min <sup>-1</sup> )                                            | 22 men                                                        | Healthy nonsmokers                     | FVC and FEV <sub>1.0</sub> decreased throughout the exposure; FEV <sub>1.0</sub> decrease at end of exposure was 7.0%, 7.0%, and 12.3%, respectively; FEV <sub>1.0</sub> change >15% occurred in 3, 5, and 9 subjects at 160, 200, and 240 $\mu\text{g} \cdot \text{m}^{-3}$ , respectively                   |
| McDonnell et al, 1991 (17)               | 160<br>200                                                | 6.6 h             | IE (6×50 min at V <sub>E</sub> =40 l · min <sup>-1</sup> )                                            | 38 men                                                        | Healthy nonsmokers (mean age=25 years) | FEV <sub>1.0</sub> decreased 8.4% at 160 $\mu\text{g} \cdot \text{m}^{-3}$ and 11.4% at 200 $\mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                                                 |
| Kulle et al, 1985 (13)                   | 0<br>200<br>400<br>500                                    | 2 h               | IE (4×14 min on treadmill at mean V <sub>E</sub> =70 l · min <sup>-1</sup> )                          | 20 men                                                        | Young, healthy nonsmokers              | FVC, FEV <sub>1.0</sub> , FEF <sub>25-75</sub> , SG <sub>aw</sub> , IC, and TLC all decreased with increasing O <sub>3</sub> concentration, and increasing time of exposure; threshold for response was above 200 $\mu\text{g} \cdot \text{m}^{-3}$ but below 300 $\mu\text{g} \cdot \text{m}^{-3}$           |
| Schelegle & Adams, 1986 (5)              | 0<br>240<br>360<br>480                                    | 1 h               | Competitive simulation exposures at mean V <sub>E</sub> =87 l · min <sup>-1</sup>                     | 10 men                                                        | 10 highly trained competitive cyclists | Decrease in FVC and FEV <sub>1.0</sub> for O <sub>3</sub> exposure of 360 and 480 $\mu\text{g} \cdot \text{m}^{-3}$ compared with filtered-air exposure; decrease in exercise time for subjects unable to complete the competitive simulation at 360 and 480 $\mu\text{g} \cdot \text{m}^{-3}$ , respectively |
| McDonnell et al, 1983 (14)               | 0<br>240<br>360<br>480<br>600<br>800                      | 2.5 h             | IE (4×15 min of treadmill exercise at V <sub>E</sub> =65 l · min <sup>-1</sup> )                      | 20 men<br>22 men<br>20 men<br>21 men<br>20 men<br>29 men      | Young, healthy adults                  | Decrease in FVC, FEV <sub>1.0</sub> , and FEF <sub>25-75</sub> at an O <sub>3</sub> level of 240 $\mu\text{g} \cdot \text{m}^{-3}$ , decrease in V <sub>T</sub> and increase in breathing frequency and SR <sub>aw</sub> at 480 $\mu\text{g} \cdot \text{m}^{-3}$                                             |
| McDonnell et al, 1985 (22)               | 240<br>360<br>480<br>600<br>800                           | 2×2.5 h           | IE (4×15 min of treadmill exercise at V <sub>E</sub> =35 l · min <sup>-1</sup> · m <sup>-2</sup> BSA) | 8 men<br>8 men<br>5 men<br>5 men<br>6 men                     | Young, healthy adults                  | Pulmonary function variables SR <sub>aw</sub> and V <sub>E</sub> were not significantly different in repeat exposures, indicating that the response to 360 $\mu\text{g} \cdot \text{m}^{-3}$ or higher is reproducible                                                                                        |
| McDonnell et al, 1993 (23)               | 240<br>360<br>480<br>600<br>800                           | 2×2.5 h           | IE (4×15 min of treadmill exercise at V <sub>E</sub> =35 l · min <sup>-1</sup> · m <sup>-2</sup> BSA) | 290 men                                                       | Young, healthy adults                  | O <sub>3</sub> concentration and age predicted FEV <sub>1.0</sub> decrements (ie, older subjects being less responsive to O <sub>3</sub> )                                                                                                                                                                    |
| Seal et al, 1993 (35)                    | 0<br>240<br>360<br>480<br>600<br>800                      | 2.5 h             | IE (4×15 min of treadmill exercise at V <sub>E</sub> =25 l · min <sup>-1</sup> · m <sup>-2</sup> BSA) | 15—17 each of white men, black men, white women & black women | Young, healthy whites and blacks       | Decreases in FEV <sub>1</sub> for all levels of O <sub>3</sub> increase in SR <sub>aw</sub> with 360 $\mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                                        |
| Gong et al, 1986 (6)                     | 0<br>240<br>400                                           | 1 h               | CE (mean V <sub>E</sub> =89 l · min <sup>-1</sup> )                                                   | 15 men<br>2 men                                               | Highly trained competitive cyclists    | Decrease in V <sub>E</sub> max, VO <sub>2</sub> max, V <sub>T</sub> max, work load, ride time, FVC, and FEV <sub>1.0</sub> with an O <sub>3</sub> exposure of 400 $\mu\text{g} \cdot \text{m}^{-3}$ , but not significant with 240 $\mu\text{g} \cdot \text{m}^{-3}$                                          |

(continued)

Table 1. Continued

| Reference                     | Ozone concentration ( $\mu\text{g} \cdot \text{m}^{-3}$ )                                                                                                                               | Exposure duration | Activity                                                                                                                                              | Number of subjects                                                                   | Subjects characteristics                                                         | Observed significant effect(s)                                                                                                                                                                                                                                                                                                                                                                                     |
|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Folinsbee et al, 1988 (18)    | 240                                                                                                                                                                                     | 6.6 h             | IE (6×50 min at $V_E = 42.6 \text{ l} \cdot \text{min}^{-1}$ )                                                                                        | 10 men                                                                               | Healthy nonsmokers                                                               | $\text{FEV}_{1.0}$ decreased by 13% after 6.6 h; FVC dropped 8.3%                                                                                                                                                                                                                                                                                                                                                  |
| Hazucha et al, 1992 (40)      | 240<br>Varied from 0.0 to 480 (increased by $0.12 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ , then decreased by $0.12 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ ) | 8 h               | IE (8×30 min $V_E = 40 \text{ l} \cdot \text{min}^{-1}$ )                                                                                             | 23 men                                                                               | Healthy nonsmokers                                                               | $240 \mu\text{g} \cdot \text{m}^{-3}$ : $\text{FEV}_{1.0}$ decreased 5% by 6 h and remained at this level through 8 h; variation from 0.0 to $480 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ : $\text{FEV}_{1.0}$ change mirrored $\text{O}_3$ concentration change with a lag time of $\approx 2$ h; maximum decrease of 10.2% after 6 h; $\text{FEV}_{1.0}$ change was reduced in last 2 h of exposure |
| Linn et al, 1994 (20)         | 240                                                                                                                                                                                     | 6.5 h/day         | IE [6×50 min (2 days of exposure) at $V_E = 31 \text{ l} \cdot \text{min}^{-1}$ (asthmatic) and $V_E = 34 \text{ l} \cdot \text{min}^{-1}$ (healthy)] | 15 (8 men & 7 women)<br>30 (13 men & 17 women)                                       | Healthy nonsmokers (age 22—41 years),<br>asthmatics nonsmokers (age 18—50 years) | $\text{FEV}_{1.0}$ decreased 4% (pre- to postexposure) in healthy subjects and in 7.8% asthmatics; responses were generally less on the second day; two healthy and four asthmatics had $\text{FEV}_{1.0}$ decreases of >10%                                                                                                                                                                                       |
| Folinsbee et al, 1994 (19)    | 240                                                                                                                                                                                     | 6.6 h             | IE (6×50 min at $V_E = 38.8 \text{ l} \cdot \text{min}^{-1}$ )                                                                                        | 17 men                                                                               | Healthy nonsmokers                                                               | $\text{FEV}_{1.0}$ changed by -12.8, 8.7, 2.5, 0.6, and +0.2% on days 1—5 of $\text{O}_3$ exposure                                                                                                                                                                                                                                                                                                                 |
| Avol et al, 1984 (10)         | 0<br>320<br>480<br>640                                                                                                                                                                  | 1 h               | CE (mean $V_E = 57 \text{ l} \cdot \text{min}^{-1}$ )                                                                                                 | 42 men & 8 women                                                                     | Competitive bicyclists                                                           | Small decrements in $\text{FEV}_{1.0}$ at $320 \mu\text{g} \cdot \text{m}^{-3}$ with larger decrements at $480 \mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                                                                                                                                    |
| Aris et al, 1993 (15)         | 0<br>400                                                                                                                                                                                | 4 h               | IE (4×50 min cycle ergometry or treadmill running at $V_E = 40 \text{ l} \cdot \text{min}^{-1}$ )                                                     | 11 men<br>3 women (filtered-air exposure); 9 men<br>3 women ( $\text{O}_3$ exposure) | Adult, healthy nonsmokers                                                        | Decrease in FVC, $\text{FEV}_{1.0}$ , $V_T$ , and $\text{SR}_{\text{aw}}$ and increase in breathing frequency                                                                                                                                                                                                                                                                                                      |
| Adams & Schelegle, 1983 (7)   | 0<br>400<br>700                                                                                                                                                                         | 1 h               | CE or competitive simulation (mean $V_E = 77 \text{ l} \cdot \text{min}^{-1}$ )                                                                       | 10 men                                                                               | Well-trained distance runners                                                    | Decrease in FVC, $\text{FEV}_{1.0}$ and $\text{FEF}_{25-75}$ , with 400 and $700 \mu\text{g} \cdot \text{m}^{-3}$ ; $V_T$ decreased and breathing frequency increased with continuous 50-min $\text{O}_3$ exposures; three subjects unable to complete training and competitive protocols at $700 \mu\text{g} \cdot \text{m}^{-3}$                                                                                 |
| Folinsbee et al, 1984 (8)     | 0<br>420                                                                                                                                                                                | 1 h               | CE (75% $\dot{V}\text{O}_2$ max)                                                                                                                      | 6 men & 1 women                                                                      | Well-trained cyclists                                                            | Decrease in FVC, $\text{FEV}_{1.0}$ , $\text{FEF}_{25-75}$ , and MVV with an $\text{O}_3$ level of $420 \mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                                                                                                                                           |
| Folinsbee & Horvath, 1986 (9) | 0<br>500                                                                                                                                                                                | 1 h               | CE (mean $V_E = 63 \text{ l} \cdot \text{min}^{-1}$ )                                                                                                 | 19 men & 7 women                                                                     | Active nonathletes                                                               | FVC, $\text{FEV}_{1.0}$ and MVV all decreased with an $\text{O}_3$ level of $500 \mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                                                                                                                                                                  |
| Hazbun et al, 1993 (91)       | 0<br>500                                                                                                                                                                                | 1 h               | CE cycle ergometer ( $V_E = 30 \text{ l} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ BSA)                                                              | 5 men & 2 women                                                                      | Young, healthy non-smokers                                                       | 12.4% decrease in $\text{FEV}_{1.0}$                                                                                                                                                                                                                                                                                                                                                                               |
| Schelegle et al, 1991 (11)    | 0<br>600                                                                                                                                                                                | 1 h               | CE cycle ergometry (mean $V_E = 60 \text{ l} \cdot \text{min}^{-1}$ )                                                                                 | 5 men                                                                                | Normal                                                                           | Decrease in FVC and $\text{FEV}_{1.0}$ and increase in $\text{SR}_{\text{aw}}$ 1 h post- $\text{O}_3$ exposure                                                                                                                                                                                                                                                                                                     |
| Gerrity et al, 1993 (101)     | 0<br>800                                                                                                                                                                                | 1 h               | CE treadmill exercise; ( $V_E = 20 \text{ l} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ BSA)                                                          | 20 men                                                                               | Young, healthy nonsmokers                                                        | $V_T$ fell by 25%, and $\text{O}_3$ uptake efficiency in the lower respiratory tract fell by 9% during $\text{O}_3$ exposure                                                                                                                                                                                                                                                                                       |

<sup>a</sup> This table is based on tables 7-1 and 7-9 in reference 1.

tions as low as  $160 \mu\text{g} \cdot \text{m}^{-3}$ . Exposure to  $400 \mu\text{g} \cdot \text{m}^{-3}$  caused a 10% decrease in  $\text{FEV}_{1.0}$  in 12 subjects, as compared to a +1% change in 14 subjects exposed to filtered air (15). There was also a significant increase in respiratory symptoms in the ozone-treated group. Horstman et al (16) exposed 22 healthy men for 6.6 h to 160, 200, and  $240 \mu\text{g} \cdot \text{m}^{-3}$  during intermittent, moderate exercise and reported that  $\text{FEV}_{1.0}$  decreased by 7.0%, 7.0%, and 12.3%, respectively. In addition, cough ratings increased as the ozone concentration increased.

McDonnell et al (17) studied young, healthy men exposed to 160 and  $200 \mu\text{g} \cdot \text{m}^{-3}$ , again during moderately heavy exercise performed intermittently. The effects were similar to those reported by Horstman et al (16) with an 8.4% reduction in  $\text{FEV}_{1.0}$  at  $160 \mu\text{g} \cdot \text{m}^{-3}$  and a 11.4% reduction at  $200 \mu\text{g} \cdot \text{m}^{-3}$ . There was also a significant increase in respiratory symptoms.

In a study of 10 men exposed for 6.6 h to  $240 \mu\text{g} \cdot \text{m}^{-3}$  (18), a 13% decrease in  $\text{FEV}_{1.0}$  was found, and FVC dropped by 8.3%. Similarly, in another study (19) with a similar design, a 13% decline in  $\text{FEV}_{1.0}$  was found.

A milder response to an ozone concentration of  $240 \mu\text{g} \cdot \text{m}^{-3}$  was found in 15 normal subjects exposed for 6.5 h, with intermittent exercise (20). The mean decrease in  $\text{FEV}_{1.0}$  in the group (4%) was about one-third of that reported by Horstman and others (16). The authors speculated that this attenuation of response was due to adaptation to ozone among the subjects of their study, who were all living in the Los Angeles area.

*Reproducibility of ozone effects on lung function.* The human responses to ozone have been shown to be reproducible (21, 22). McDonnell et al (22) studied small groups of young, healthy adults exposed to 240, 360, 480, 600, and  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2.5 h. The subjects were exposed on two separate occasions to at least one of the five concentrations in order to study the reproducibility of the response. The authors concluded that the individual responses to ozone, as assessed by FVC and  $\text{FEV}_{1.0}$ , were highly reproducible over periods as long as 10 months. However, responses measured by  $\text{FEF}_{25-75}$  were somewhat less reproducible. These observations of high reproducibility of lung function response were related to ozone levels equal to or greater than  $360 \mu\text{g} \cdot \text{m}^{-3}$ , whereas the reproducibility at  $240 \mu\text{g} \cdot \text{m}^{-3}$  was diminished. The reproducibility of symptoms such as cough and shortness of breath were also statistically significant, although lower than for the spirometric data. In a study by Gliner and his co-workers (21) the effects of ozone ( $400 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h) on lung function were reproducible for the subjects especially sensitive to ozone, as well as for subjects with normal sensitivity.

The effects of ozone on pulmonary function seem not to be dependent on the route of breathing. Similar results concerning spirometric data and symptoms have been

reported in studies comparing oral, nasal, and oronasal breathing (23, 24).

*Age-dependency of ozone effects on lung function.* The effect of age was assessed in a sample of 290 white male volunteers, 18 to 32 years of age, who were exposed to one of six concentrations of ozone between 0 and  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h with intermittent exercise (25). Age was a significant predictor of response, the older subjects being less responsive to ozone. However, the effect of age in the entire population has been somewhat difficult to assess, as most subjects so far tested have been young adults, and very few studies have been performed with children and subjects older than 50 years. McDonnell et al (26) exposed 23 boys, between 8 and 11 years of age, to  $240 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h during heavy intermittent exercise and found small decrements in  $\text{FEV}_{1.0}$ . The magnitude of this  $\text{FEV}_{1.0}$  change was similar to that shown under comparable exposure conditions with adults by the same investigators. Small decrements in  $\text{FEV}_{1.0}$  seemed to persist 16 to 20 h after exposure to ozone. There was no significant increase in cough due to the ozone exposure.

In another study involving children, 33 boys and 33 girls with a mean age of 9.4 years were exposed to outdoor ambient air drawn into an environmental chamber in a mobile laboratory. The ozone concentration during exposure averaged  $220 \mu\text{g} \cdot \text{m}^{-3}$ , and the children were exposed for 1 h during light exercise (27). A correlation analysis between ozone concentration and  $\text{FEV}_{1.0}$  showed a significant negative correlation. In addition, adolescents and adults had previously been investigated by the same research group (10, 28), and the findings had been similar for the children.

Eight women and eight men, aged between 51 and 76 years, were exposed for 2 h to an ozone concentration of  $900 \mu\text{g} \cdot \text{m}^{-3}$  (29) during light, intermittent exercise, and the results suggested that older persons may be less responsive to ozone than young ones.

*Effects of gender on ozone-involved effects on lung function.* There are several reports suggesting that women might be more responsive to ozone than men (21, 30, 31). On the other hand, Adams et al (32) found no significant difference between male and female responses to the gas. Most studies involving both male and female subjects have not been designed to elucidate possible gender differences and have often only included a few female subjects. However, during recent years there have been some studies specifically addressing the question of a possible gender difference in ozone responsiveness. Messineo & Adams (33) exposed two groups of 14 young women, differing significantly in lung volume, to ozone concentrations of 360 and  $600 \mu\text{g} \cdot \text{m}^{-3}$  during moderate intermittent exercise. The objective of the study was to test an hypothesis that the previously observed difference

in response between men and women was due to a difference in lung size (31). However, both groups elicited similar change in pulmonary function and symptoms. Notably, the  $FEV_{1.0}$  decrease to  $600 \mu\text{g} \cdot \text{m}^{-3}$  ( $-24\%$ ) was nearly double that observed for young adult men exposed to the same total inhaled ozone dose in a study published by the same investigators.

An enhanced response to ozone occurred in nine women during the follicular phase of the menstrual cycle (34). The subjects were exposed to  $600 \mu\text{g} \cdot \text{m}^{-3}$  during 1 h, both during the follicular and the midluteal menstrual phases. The decrease in  $FEV_{1.0}$  was significantly greater during the follicular phase ( $18.1\%$  versus  $13.1\%$ ), and the authors suggested that the difference could be related to the antiinflammatory effects of increased systemic progesterone levels during the luteal phase.

In a large study, 372 subjects, white and black, men and women, participated in a study designed to determine whether gender or ethnic differences in the response to ozone exist (35). The subjects were exposed for 2.33 h, during intermittent light exercise, to five different concentrations of ozone from 240 up to  $800 \mu\text{g} \cdot \text{m}^{-3}$ . There were no statistically significant differences in the response to ozone among the individual groups in this extensive study.

A methodological problem in these studies comparing ozone effects among men and women is that ventilation must be normalized, and it is not clear if it should be done according to lung size, body size, or some other parameter (35).

*Relationship between ozone dose and lung function change.* The relationship between ozone dose and airway response has been analyzed by several authors (36–40). In 1987 Hazucha analyzed a data-base comprising 24 published studies on the effects of ozone on lung function involving 299 subjects (36) and found a very strong negative association between functional response and ozone concentration. A quadratic, nonlinear regression curve was constructed of the results predicting the decrease in lung function within high confidence limits. Indeed, ozone concentration exerted a greater influence on functional change than did ventilation. It was not possible, however, to define a threshold concentration below which no pulmonary function change was elicited.

Hazucha et al (40) have tested the hypothesis that, regardless of the pattern of concentration and the exposure rate, the same exposure dose will induce the same spirometric response. In this study 23 subjects were exposed for 8 h with intermittent exercise to air, an ozone concentration of  $240 \mu\text{g} \cdot \text{m}^{-3}$  steady-state, or ozone in a triangular exposure pattern giving the same total dose as in the steady-state design (concentration increasing from 0 to  $480 \mu\text{g} \cdot \text{m}^{-3}$  over the first 4 h and then decreasing back to 0 by 8 h). Similar exposure doses over the first

4 h elicited responses of similar magnitude, regarding both subjective symptoms and spirometry. However, during the second 4-h period of exposure the lung function changes were significantly larger in the triangular ozone profile than in the steady-state profile. The  $FEV_{1.0}$  change during the experiment with a variable exposure concentration mirrored the ozone concentration with a lag of about 2 h. The authors concluded that the product of concentration  $\times$  time  $\times$  ventilation is not a sufficient index of exposure when ozone concentration varies, which is a frequent event in the ambient atmosphere. The ozone concentration in the exposure dose product may need to be weighted more heavily than time of exposure and ventilation.

Kleinman (37) derived a dose-response relationship for the effect of ozone on pulmonary function using a concept of internal thoracic dose. The dose model could be used to compare results from clinical studies in which subjects were exposed under disparate conditions of exercise, exposure concentration, and duration. On the basis of the dose of ozone (micrograms of ozone per kilogram of body mass) children, between 8 and 15 years of age and adults were equally sensitive to the gas. Children under the age of 6 years, on the other hand, received a greater dose to the respiratory tract tissues than older children and adults according to this model.

Kriebel & Smith described a pharmacological model expressing the quantitative relationship between short-term exposure to ozone and pulmonary airway resistance (38). The model was validated with data from chamber studies, and the authors claimed that their model is superior to the "effective dose" model, which uses the product of ozone concentration, minute ventilation, and exposure duration.

Using  $FEV_{1.0}$  data from 21 men exposed to 0, 160, 200, and  $240 \mu\text{g} \cdot \text{m}^{-3}$  during exercise, Larsen et al (39) derived a lognormal multiple linear regression model to predict the  $FEV_{1.0}$  decrease as a function of ozone concentration and exposure duration (at a given ventilation of  $38.8 \text{ l} \cdot \text{min}^{-1}$ ). The model predicted that ozone concentrations of 160, 200, and  $240 \mu\text{g} \cdot \text{m}^{-3}$  would decrease  $FEV_{1.0}$  by 9%, 15% and 20%, respectively, during an 8-h workday of heavy manual labor.

*Relationship between ozone-induced symptoms and lung function change.* In most of the studies that have already been presented on the effects of ozone on lung function in normal subjects, there have been statistically significant associations between the change in spirometric results and the occurrence of respiratory symptoms such as cough, substernal pain, and shortness of breath. Among the symptoms, shortness of breath provided the closest correlation with functional change in a study of 64 healthy men exposed to  $700 \mu\text{g} \cdot \text{m}^{-3}$  with intermittent exercise for 130 to 150 min (41). Shortness of breath and

cough were the symptoms most closely associated with decrements in FEV<sub>1.0</sub> in another study (42), although the associations were weak.

In an attempt to predict respiratory symptoms induced by ozone from pulmonary function changes, Ostro et al (43) reanalyzed data from four ozone chamber studies (4, 10, 13, 14). Logistic regression models were used to determine the quantitative relationship between changes in FEV<sub>1.0</sub> and the probability of a lower respiratory symptom. The authors estimated that a 10% decrease in FEV<sub>1.0</sub> is associated with a 15% increase in the probability of any lower respiratory symptom and a 6% increase in the risk of a moderate or severe lower respiratory symptom. These estimations suggest that the associations between respiratory symptoms and spirometric changes are rather weak, notwithstanding their statistical significance. The occurrence of symptoms is not a reliable predictor of functional response.

*Effects of pharmacological agents on ozone-induced changes in lung function.* To elucidate the possible mechanisms for the ozone-induced effects on lung function, attempts have been made to block the responses with different pharmacological agents.

Indomethacin, an antiinflammatory agent and inhibitor of the cyclooxygenation of arachidonic acid to prostaglandins, was given to 14 subjects for 5 d before exposure to an ozone concentration of 700  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h, and the results were compared with those of groups receiving a placebo and no medication (44). There was a significant effect of indomethacin in decreasing the change in both FVC and FEV<sub>1.0</sub>, when the gas conditions were compared with both the placebo and no drug ones. A similar effect of indomethacin was reported by Ying et al (45) in a study comparing the effects of indomethacin, placebo, and no medication on ozone-induced alterations to lung function. Seven subjects were exposed to 800  $\mu\text{g} \cdot \text{m}^{-3}$  for 2 h, and the decreases in FEV<sub>1.0</sub> were -20.7%, -19%, and -4.8% for the no medication, placebo, and indomethacin group, respectively. Indomethacin did not attenuate the ozone-induced effect on bronchial responsiveness in the latter study, and the authors concluded that ozone-induced effects on lung function and bronchial responsiveness occur through different mechanisms.

McKenzie et al (46) attempted to inhibit the lung function changes and the pulmonary discomfort due to ozone by pretreating subjects with salbutamol, a  $\beta_2$ -stimulating agent possessing bronchodilatory properties. No effect of salbutamol was seen on either FVC or FEV<sub>1.0</sub>, and the subjective symptoms were also unaffected in a pilot study of only three subjects exposed to 680  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h. In another study (47) with a larger sample of subjects, 15 competitive cyclists were exposed to 420  $\mu\text{g} \cdot \text{m}^{-3}$  while exercising heavily, and the effects of

pretreatment with salbutamol were compared with those of a placebo. Salbutamol did not prevent ozone-induced respiratory symptoms or a decrease in FVC, FEV<sub>1.0</sub>, or FEF<sub>25-75</sub>. The authors concluded that the contribution of  $\beta$ -adrenergic mechanisms to the acute airway responses to ozone appears to be minimal.

Pretreatment with atropine, a blocker of muscarinic receptors in the parasympathetic nervous system, prevented the increase in airway resistance in eight subjects exposed to 800  $\mu\text{g} \cdot \text{m}^{-3}$  for 2 h (48). Atropine partially blocked the decrease in FEF<sub>25-75</sub>, but did not affect FVC, changes in respiratory rate and tidal volume, or reported respiratory symptoms. The results suggested that the increase in airway resistance, and to some extent also the decrease in expiratory flow, during ozone exposure is mediated by a parasympathetic mechanism and that changes in other variables are mediated, at least partially, by mechanisms not dependent on muscarinic receptors in the parasympathetic nervous system.

Lidocaine, an anesthetic agent, was sprayed into the upper airway in 11 subjects after they had been exposed to an ozone concentration of 1000  $\mu\text{g} \cdot \text{m}^{-3}$  for 2 h (49). The ozone exposure caused a 17% decrease in FVC, which was associated with a marked decrease in inspiratory capacity without an increase in residual volume. This decrease in FVC was immediately reversed towards control values after the spraying of lidocaine. Hazucha et al concluded that ozone stimulates lidocaine-sensitive receptors in the larynx and trachea and leads to an involuntary inhibition of full inspiration, a reduction in FVC, and a concomitant decrease in maximal expiratory flow rates.

The results of these studies with indomethacin, atropine, and lidocaine suggest that ozone exerts its acute effects on the airways via different mechanisms, including the release of bronchoconstricting agents, such as prostaglandins, the stimulation of afferent bronchial nerves with reflex responses via the parasympathetic system, and the stimulation of pain receptors in the upper airways.

#### *Lung function in subjects with airway or heart disease*

The effects of ozone exposure on subjects with allergic rhinitis, asthma, chronic obstructive pulmonary disease, and heart disease are summarized in table 2. The table is slightly modified from table 7—2 in the EPA report (1).

*Allergic rhinitis.* McDonnell et al (50) exposed 26 non-asthmatic subjects with allergic rhinitis to an ozone concentration of 360  $\mu\text{g} \cdot \text{m}^{-3}$  for 2 h with intermittent exercise. The subjects showed significant increases in respiratory symptoms, airway responsiveness to histamine and SR<sub>aw</sub>, and also decreases in FVC and FEV<sub>1.0</sub> after the exposure, as compared with exposure to filtered air. However, the responses, with the exception of airway

**Table 2.** Effects of controlled human exposure to ozone on the pulmonary function of subjects with preexisting disease.<sup>a</sup> (BSA = body surface area, CE = continuous exercise, FEF<sub>25-75</sub> = forced expiratory flow between 25% and 75%, FEV<sub>1.0</sub> = forced expiratory volume in 1 s, FEV<sub>3.0</sub> = forced expiratory volume in 3 s, FVC = forced vital capacity, IC = inspiratory capacity, IE = intermittent exercise, NO<sub>2</sub> = nitrogen dioxide, O<sub>2</sub> = oxygen, O<sub>3</sub> = ozone, PD = provocative dose, SO<sub>2</sub> = sulfur dioxide, SR<sub>aw</sub> = specific airway resistance, V = ventilation, V<sub>E</sub> = minute ventilation, Vmax<sub>50</sub> = maximum expiratory flow at 50% of the vital capacity)

| Reference                              | Ozone concentration (µg · m <sup>-3</sup> ) | Exposure duration | Activity                                                                                                                                             | Number and gender of subjects                                   | Subjects characteristics                                                                                                                        | Observed effect(s)                                                                                                                                                                                                                                                                                                                |
|----------------------------------------|---------------------------------------------|-------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Subjects with allergic rhinitis</i> |                                             |                   |                                                                                                                                                      |                                                                 |                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
| McDonnell et al, 1987 (50)             | 0<br>360                                    | 2 h               | IE (4×15 min)                                                                                                                                        | 26 men                                                          | History of allergic rhinitis                                                                                                                    | Increased respiratory symptoms, SR <sub>aw</sub> , and reactivity to histamine and decreased FVC, FVC <sub>1.0</sub> and FEF <sub>25-75</sub>                                                                                                                                                                                     |
| Bascom et al, 1990 (51)                | 0<br>1000                                   | 4 h               | Rest                                                                                                                                                 | 6 men<br>6 women                                                | History of seasonal allergic rhinitis; acute response to nasal challenge with antigen                                                           | Increase in upper and lower respiratory symptom scores, cell influx, epithelial cells; no effect on acute allergic response to nasal antigen challenge between O <sub>3</sub> and filtered air exposure                                                                                                                           |
| <i>Adult subjects with asthma</i>      |                                             |                   |                                                                                                                                                      |                                                                 |                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
| Weymer et al, 1994 (56)                | 0<br>200<br>500                             | 2 h               | Light IE (2×15 min on treadmill, V <sub>E</sub> = 10–20 l · min <sup>-1</sup> )                                                                      | 7 men                                                           | Stable mild asthmatics with FEV <sub>1.0</sub> >70% and increased methacholine responsiveness                                                   | No significant differences in FEV <sub>1.0</sub> or postexposure exercise challenge                                                                                                                                                                                                                                               |
| Koenig et al, 1990 (81)                | 0<br>240                                    | 0.75 h            | IE (V <sub>E</sub> = 30 l · min <sup>-1</sup> followed by 15 min of exercise while inhaling SO <sub>2</sub> (300 µg · m <sup>-3</sup> ))             | 8 men & 5 women (asthmatics) ages 12 to 18 years                | Asthmatics classified on basis of positive clinical history and methacholine challenge; asymptomatic at time of study                           | Filtered air followed by SO <sub>2</sub> or O <sub>3</sub> alone did not cause significant changes in pulmonary function; O <sub>3</sub> followed by SO <sub>2</sub> resulted in a significant decrease in FEV <sub>1.0</sub> (8%) and Vmax <sub>50</sub> (15%) and a significant increase in total respiratory resistance (19%)  |
| McBride et al, 1994 (54)               | 0<br>210<br>480                             | 1.5 h             | IE (V <sub>E</sub> = 25 l · min <sup>-1</sup> )                                                                                                      | 4 men, 4 women (nonasthmatics); 5 men & 5 women (asthmatics)    | Physician-diagnosed asthma confirmed with metacholine challenge test, all nonsmokers and asymptomatic at time of study; nine were atopic        | No significant changes in pulmonary and nasal function measurements in either asthmatics or nonasthmatics; significant increase in nasal lavage white cell count and epithelial cell count following O <sub>3</sub> exposure in asthmatics only                                                                                   |
| Linn et al, 1994 (20)                  | 0<br>240                                    | 6.5 h/day         | IE [6×50 min (2 days of exposure) and V <sub>E</sub> = 31 l · min <sup>-1</sup> (asthmatic) and V <sub>E</sub> = 34 l · min <sup>-1</sup> (healthy)] | 8 men & 7 women (nonasthmatics); 13 men & 17 women (asthmatics) | Asthmatics classified on basis of positive clinical history, previous physician diagnosis, and low PD <sub>20</sub> ; mild to severe asthmatics | FEV <sub>1.0</sub> decreased 8.6% in asthmatics and 1.7% in nonasthmatics, with difference between the two groups not being significant                                                                                                                                                                                           |
| Molfino et al, 1991 (82)               | 0<br>240                                    | 1 h               | Rest                                                                                                                                                 | 4 men & 3 women                                                 | Mild, stable asthma                                                                                                                             | Increase in bronchial responsiveness to allergen; no change in baseline lung function                                                                                                                                                                                                                                             |
| Linn et al, 1978 (53)                  | 0<br>400                                    | 2 h               | IE                                                                                                                                                   | 20 men & 2 women                                                | Physician diagnosed asthma; 6 smokers, 9 ex-smokers, 7 nonsmokers                                                                               | No significant changes in pulmonary function measurements; significant blood biochemical changes                                                                                                                                                                                                                                  |
| Silverman, 1979 (55)                   | 0<br>500                                    | 2 h               | Rest                                                                                                                                                 | 5 men & 12 women                                                | Nonsmoking asthmatics selected from a clinical practice                                                                                         | No significant changes in pulmonary function measurements                                                                                                                                                                                                                                                                         |
| Kreit et al, 1989 (52)                 | 0<br>800                                    | 2 h               | IE (4×15 min cycle ergometry)                                                                                                                        | 4 men & 5 women (nonasthmatics); 4 men & 5 women (asthmatics)   | Asthmatics as diagnosed by a physician; history of chest tightness and wheezing                                                                 | Decrease in FVC and IC with O <sub>3</sub> in asthmatics; increase in airway responsiveness to methacholine in asthmatics with O <sub>3</sub> and filtered air; asthmatic subjects had significantly greater decreases in FEV <sub>1.0</sub> and FEF <sub>25-75</sub> with O <sub>3</sub> exposure than did nonasthmatic subjects |
| <i>Adolescent subjects with asthma</i> |                                             |                   |                                                                                                                                                      |                                                                 |                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                   |
| Koenig et al, 1985 (57)                | 0<br>240                                    | 1 h               | Rest                                                                                                                                                 | 4 men & 6 women (nonasthmatics); 4 men & 6 women (asthmatics)   | Asthmatics had a history of atopic extrinsic asthma and exercise-induced bronchospasm                                                           | No consistent significant changes in pulmonary functional parameters in either group or between groups                                                                                                                                                                                                                            |

(continued)

Table 2. Continued

| Reference                                                  | Ozone concentration ( $\mu\text{g} \cdot \text{m}^{-3}$ ) | Exposure duration | Activity                                                                               | Number and gender of subjects                                 | Subjects characteristics                                                                                                       | Observed effect(s)                                                                                                                                                                                                                                              |
|------------------------------------------------------------|-----------------------------------------------------------|-------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Koenig et al, 1988 (59)                                    | 0<br>240                                                  | 1 h               | IE (2×15 min of treadmill walking at mean $V_E=32.5 \text{ l} \cdot \text{min}^{-1}$ ) | 5 men & 8 women (nonasthmatics); 9 men & 3 women (asthmatics) | Asthmatics selected from a clinical practice and had exercise-induced bronchospasm                                             | Decrease in maximal flow at 50% of FVC in asthmatics with $\text{O}_3$ exposure; no significant changes with combined $\text{O}_3\text{-NO}_2$ exposure                                                                                                         |
| Koenig et al, 1987 (58)                                    | 0<br>240<br>360                                           | 40 min            | IE (1×10 min of treadmill walking at mean $V_E=32.5 \text{ l} \cdot \text{min}^{-1}$ ) | 4 men & 9 women (nonasthmatics); 8 men & 8 women (asthmatics) | Asthmatics had allergic asthma, positive responses to methacholine, and exercise-induced bronchospasm                          | Decrease in $\text{FEV}_{1.0}$ and increase in total respiratory resistance in nonasthmatic and asthmatics with an $\text{O}_3$ level of 240 and $360 \mu\text{g} \cdot \text{m}^{-3}$ exposure; no consistent differences between nonasthmatics and asthmatics |
| <i>Subjects with chronic obstructive pulmonary disease</i> |                                                           |                   |                                                                                        |                                                               |                                                                                                                                |                                                                                                                                                                                                                                                                 |
| Linn et al, 1982 (60)                                      | 0<br>240                                                  | 1 h               | IE (2×15 min of light bicycle ergometry)                                               | 18 men & 7 women                                              | 8 smokers, 14 ex-smokers, 3 non-smokers; $\text{FEV}_{1.0}/\text{FVC} = 32\text{--}66\%$                                       | No significant changes in pulmonary function measurements; small significant decrease in arterial $\text{O}_2$ saturation                                                                                                                                       |
| Linn et al, 1983 (61)                                      | 0<br>360<br>500                                           | 1 h               | IE (2×15 min of light bicycle ergometry)                                               | 15 men & 13 women                                             | 15 smokers, 11 ex-smokers, 2 nonsmokers; $\text{FEV}_{1.0}/\text{FVC} = 36\text{--}75\%$                                       | No significant changes in pulmonary function measurements; no significant change in arterial $\text{O}_2$ saturation                                                                                                                                            |
| Solic et al, 1982 (63)                                     | 0<br>400                                                  | 2 h               | IE (4×7.5 min of light treadmill running)                                              | 13 men                                                        | 8 smokers, 4 ex-smokers, 1 non-smoker; productive cough; $\text{FEV}_{1.0}/\text{FVC} = 46\text{--}70\%$                       | No significant changes in pulmonary function measurements; small significant decrease in arterial $\text{O}_2$ saturation                                                                                                                                       |
| Kehrl et al, 1985 (62)                                     | 0<br>600                                                  | 2 h               | IE (4×7.5 min of light treadmill running)                                              | 13 men                                                        | 9 smokers, 4 non-smokers; $\text{FEV}_{1.0}/\text{FVC} = 37\text{--}65\%$                                                      | No significant changes in pulmonary function measurements or arterial $\text{O}_2$ saturation                                                                                                                                                                   |
| Kulle et al, 1984 (64)                                     | 0<br>820                                                  | 3 h daily         | (1×15 min of light bicycle ergometry during each exposure for 5 days)                  | 17 men & 3 women                                              | All smokers; productive cough; $\text{FEV}_{1.0}/\text{FVC} = 56\text{--}82\%$ $\text{FEV}_{3.0}/\text{FVC} = 75\text{--}93\%$ | Decrease in FVC and $\text{FEV}_3$ with an $\text{O}_3$ exposure of $820 \mu\text{g} \cdot \text{m}^{-3}$                                                                                                                                                       |
| <i>Subjects with heart disease</i>                         |                                                           |                   |                                                                                        |                                                               |                                                                                                                                |                                                                                                                                                                                                                                                                 |
| Superko et al, 1984 (65)                                   | 0<br>400<br>600                                           | 40 min            | CE treadmill walking                                                                   | 6 men                                                         | Coronary heart disease with angina pectoris                                                                                    | No significant changes in pulmonary function measurements, exercise ventilatory pattern, oxygen uptake, or cardiovascular parameters                                                                                                                            |

<sup>a</sup> This table is based on table 7-2 in reference 1.

resistance, were not greater than those seen in normal subjects previously studied by the same group under similar exposure conditions. The subjects with allergic rhinitis had a small but significantly greater increase in  $\text{SR}_{\text{aw}}$ .

Twelve subjects with allergic rhinitis were exposed to an ozone concentration of  $1000 \mu\text{g} \cdot \text{m}^{-3}$  at rest for 4 h by Bascom et al (51). Exposure to ozone caused an increase in symptoms from both the upper and lower airways, and in nasal lavage neutrophils, eosinophils, mononuclear cells, and albumin. A nasal allergen challenge was performed after the exposure, but the acute response to the allergen was not affected by ozone.

**Asthma.** Kreit et al (52) exposed nine asthmatic and nine normal adult subjects to an ozone concentration of  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h during intermittent exercise and re-

ported significantly greater responses in airway resistance,  $\text{FEV}_{1.0}$ , and  $\text{FEF}_{25\text{--}75}$  of the asthmatic subjects. The changes in FVC, subjective symptoms, and bronchial responsiveness to methacholine were similar in both groups. The authors concluded that a high dose of ozone produces greater airway obstruction in asthmatic than in normal subjects although symptoms, lung volume, and airway responsiveness seem to be equally affected. These influences on spirometric data, with different effects on indices of lung volume and expiratory flow, are consistent with the results of other studies suggesting that ozone-induced effects on FVC and forced expiratory flow are mediated via different mechanisms (48).

However, there are several studies on adult or adolescent asthmatic subjects showing no effect of ozone exposure for 1 to 2 h at concentrations between 200 and  $500 \mu\text{g} \cdot \text{m}^{-3}$  on lung function (53–57). In other studies

an effect of ozone (240 and 360  $\mu\text{g} \cdot \text{m}^{-3}$ ) on lung function was reported, but there were no significant differences between normal and asthmatic subjects (58, 59). Prolonged exposure (6.5 h) to 240  $\mu\text{g} \cdot \text{m}^{-3}$  during moderate intermittent exercise caused a progressive decrease in FEV<sub>1.0</sub> in 30 subjects with asthma, starting from the third hour of exposure (20). The ozone-induced decrease in FEV<sub>1.0</sub> among the asthmatic subjects, whether expressed in liters or in percentage of preexposure FEV<sub>1.0</sub>, did not significantly differ from the FEV<sub>1.0</sub> loss observed in 15 normal subjects in the same study. However, the authors considered that the clinical implication might be more important for the asthmatic subjects, whose average FEV<sub>1.0</sub> was already depressed by their disease and the effects of exercise.

**Chronic obstructive pulmonary disease.** There are four reports (60–63) based on subjects with chronic obstructive pulmonary disease and exposed to ozone concentrations between 240 and 600  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 to 2 h with light intermittent exercise, and no changes in pulmonary function were observed. Solic et al (63) found, however, a small but consistent and statistically significant reduction in oxygen saturation in arterial blood (SaO<sub>2</sub>) (95.3% and 94.8% after filtered air and ozone, respectively). At a higher concentration, 820  $\mu\text{g} \cdot \text{m}^{-3}$ , Kulle et al (63) found small (<3%) but statistically significant decreases in FVC and FEV<sub>3.0</sub> after a 3-h exposure to ozone with light exercise.

**Ischemic heart disease.** Superko et al (65) exposed six male volunteers to an ozone concentration of 400 and 600  $\mu\text{g} \cdot \text{m}^{-3}$  during continuous treadmill walking at work loads simulating their regularly prescribed training regimen. Ozone exposure did not affect the onset of angina pain, the shape of the electrocardiogram, or the pulmonary function measurements. The authors concluded that patients with angina seem to be no more susceptible to ozone than clinically normal subjects, but caution was advised in generalizing the observations from this small study to other patients. It was noted, however, that the intensity of the exercise and the duration of exposure was limited due to symptoms of angina pectoris among these patients, and therefore the total dose of ozone was lower than in studies with normal subjects at similar ozone concentrations.

### Summary

In summary, data from human studies with single exposures to steady-state levels of ozone show that ozone causes respiratory irritation and affects lung function in a dose-dependent manner. The dose of ozone reaching the airways depends on the ozone concentration in inhaled air, the duration of exposure and the minute ventilation of the subject. In the studies with long exposure times

(about 6 h) for exercising subjects with high ventilation, a significant increase in respiratory symptoms and a decrease in lung function has been documented at 160  $\mu\text{g} \cdot \text{m}^{-3}$ . When normal subjects are exposed at rest for 2 h, a decrease in lung function has been reported at ozone concentrations of about 1000  $\mu\text{g} \cdot \text{m}^{-3}$  and above.

It seems as if exposure to short peak levels of ozone may give greater responses than exposure to the same dose of ozone in a steady-state design, but there are no established models for predicting the effects of short peaks of ozone on lung function.

Indeed, there is considerable variation in sensitivity to ozone among normal persons. Young subjects seem to be more sensitive than elderly subjects, and women might be more sensitive than men, but there is no conclusive evidence for this gender effect yet.

The effects of ozone on lung function in subjects with asthma have been of the same order of magnitude as those in normal subjects. However, although the lung function decrease seems to be similar in normal persons and asthmatics, the clinical importance may be greater for persons with airway disease, as their lung function is already compromised. In subjects with chronic obstructive lung disease a decrease in arterial oxygen saturation seems to be a more sensitive parameter for ozone-induced effects than are pulmonary function changes.

The mechanisms for the ozone-induced effects on lung function are poorly understood. There seem to be different mechanisms involved, including sensory irritation of the upper airways causing involuntary inhibition of deep breaths, bronchial constriction due to stimulation of vagal nerves, and the release of arachidonic acid metabolites from epithelial cells or other cells in the lower airways.

### Lung function after repeated exposure

The results of the chamber studies that have already been presented deal with the effects of a single exposure to ozone, but in reality people are often exposed to ambient levels of ozone day after day. The effects of repeated exposures to ozone have also been investigated in chamber studies. Data on changes in FEV<sub>1.0</sub> from 13 studies are summarized in table 3, which is slightly modified from an EPA report (1). Please note that the table presents only results from exposure to one ozone concentration on each row, which means that results of a study including more than one ozone concentration is presented on several rows. The results from the study by Kulle et al (66) are presented with data observed among male and female subjects separated. In the study of Gliner et al (21) the data for a subgroup of nine especially sensitive subjects are shown separately. These studies with re-

**Table 3.** Changes in forced expiratory lung volume in 1 s (FEV<sub>1.0</sub>) after repeated daily exposure to ozone.<sup>a</sup> (CE = continuous exercise, IE = intermittent exercise V<sub>E</sub> = minute ventilation, O<sub>3</sub> = ozone)

| References                 | Ozone concentration (µg · m <sup>-3</sup> ) | Exposure duration and activity <sup>b</sup> | Number of subjects           | Percent change in FEV <sub>1.0</sub> on consecutive exposure days |        |       |        |       |
|----------------------------|---------------------------------------------|---------------------------------------------|------------------------------|-------------------------------------------------------------------|--------|-------|--------|-------|
|                            |                                             |                                             |                              | First                                                             | Second | Third | Fourth | Fifth |
| Folinsbee et al, 1994 (19) | 240                                         | 6.6 h, IE (40)                              | 17 men                       | -12.79                                                            | -8.73  | -2.54 | -0.6   | +0.2  |
| Folinsbee et al, 1980 (71) | 400                                         | 2 h, IE (30)                                | 10 men                       | +1.4                                                              | +2.7   | -1.6  | —      | —     |
| Gliner et al, 1983 (21)    | 400                                         | 2 h, IE (18 and 30)                         | 8 men & 13 women             | -3.0                                                              | -4.5   | -1.1  | —      | —     |
| Gliner et al, 1983 (21)    | 400                                         | 2 h, IE (18 and 30)                         | 9 <sup>c</sup>               | -8.7                                                              | -10.1  | -3.2  | —      | —     |
| Brookes et al, 1989 (68)   | 400                                         | 1 h, CE (60)                                | 15 men                       | -5.0                                                              | -7.8   | —     | —      | —     |
| Folinsbee et al, 1986 (9)  | 500                                         | 1 h, CE (63)                                | 4 men & 2 women              | -20.2                                                             | -34.8  | —     | —      | —     |
|                            |                                             |                                             | 5 men & 2 women              | -18.8                                                             | —      | -22.3 | —      | —     |
| Folinsbee et al, 1980 (71) | 700                                         | 2 h, IE (30)                                | 10 men                       | -5.3                                                              | -5.0   | -2.2  | —      | —     |
| Foxcroft & Adams 1986 (72) | 700                                         | 1 h, CE (60)                                | 8 men                        | -31                                                               | -41    | -33   | -25    | —     |
| Schonfeld et al, 1989 (69) | 700                                         | 1 h, CE (60)                                | 10 men                       | -16.1                                                             | -30.4  | —     | —      | —     |
|                            |                                             |                                             | 10 men                       | -14.4                                                             | —      | -20.6 | —      | —     |
| Brookes et al, 1989 (68)   | 700                                         | 1 h, CE (60)                                | 15 men                       | -15.9                                                             | -24.6  | —     | —      | —     |
| Kulle et al, 1982 (66)     | 800                                         | 3 h, IE (4—5 × resting)                     | 13 men                       | -9.2                                                              | -10.8  | -5.3  | -0.7   | -1.0  |
| Kulle et al, 1982 (66)     | 800                                         | 3 h, IE (4—5 × resting)                     | 11 men                       | -8.8                                                              | -12.9  | -4.1  | -3.0   | -1.6  |
| Horvath et al, 1981 (75)   | 840                                         | 2 h, IE (30)                                | 24 men                       | -21.1                                                             | -26.4  | -18.0 | -6.3   | -2.3  |
| Bedi et al, 1985 (67)      | 900                                         | 2 h, IE (27)                                | 1 man & 5 women              | -13.3                                                             | —      | -22.8 | —      | —     |
| Bedi et al, 1989 (70)      | 900                                         | 2 h, IE (27)                                | 10 men & 6 women             | -5.8                                                              | -5.6   | -1.9  | —      | —     |
| Linn et al, 1982 (74)      | 940                                         | 2 h, IE (3 × resting)                       | 8 men & 3 women <sup>d</sup> | -11.4                                                             | -22.9  | -11.9 | -4.3   | —     |
| Folinsbee et al, 1980 (71) | 1000                                        | 2 h, IE (30)                                | 8 men                        | -8.7                                                              | -16.5  | -3.5  | —      | —     |
| Hackney et al, 1977 (73)   | 1000                                        | 2.5 h, IE (2 × resting)                     | 6                            | -2.7                                                              | -4.9   | -2.4  | -0.7   | —     |

<sup>a</sup> This table is based on table 7-7 in reference 1.<sup>b</sup> Exposure duration and intensity of IE or CE were variable; V<sub>E</sub> (number in parentheses) given in liters per minute or as a multiple of resting ventilation.<sup>c</sup> Subjects especially sensitive on prior exposure to 820 µg · m<sup>-3</sup> O<sub>3</sub> as evidenced by a decrease in FEV<sub>1.0</sub> of more than 20%. These nine subjects were a subset of the total group of 21 persons used in this study.<sup>d</sup> Seven subjects completed entire experiments.

peated exposures have included two (9, 67—69), three (21, 70, 71), four (72—74), or five exposure days (19, 66, 75), and the subjects in each case were normal adults. As can be seen from table 3, the ozone-induced changes in spirometry are generally attenuated with repetitive exposures. However, at higher exposure doses, the response may be enhanced on the second exposure day. Symptoms induced by ozone, such as cough and chest discomfort, are also, like the spirometric changes, attenuated with repeated ozone exposures. The attenuation of response in spirometry which develops after repeated exposures persists for some days, but after one week normal responsiveness is restored (66).

### Airway responsiveness

Airway responsiveness is the disposition of the airways to react to inhaled irritating substances with cough, bronchoconstriction, and increased mucus production. Persistently increased airway responsiveness, or bronchial hyperresponsiveness, is a cardinal feature of asthma, and the degree of bronchial responsiveness is fairly well correlated to the severity of asthma. Nonspecific airway responsiveness can be assessed by different airway chal-

lenges (eg, methacholine, histamine, or cold air). The dose of provoking agent causing a defined bronchoconstriction, measured by FEV<sub>1.0</sub>, PEF, or SR<sub>aw</sub>, is determined in a bronchial challenge test. In the same way, the responsiveness to allergen, often called specific airway responsiveness, can be measured after the inhalation of nebulized allergen.

For a summary of results of studies of ozone-induced effects on airway responsiveness see table 4. Seven of the studies on nonspecific airway responsiveness were performed with normal subjects and three with asthmatic subjects.

#### Airway responsiveness in normal subjects

Eight normal subjects were exposed to a high concentration of ozone, 1200 µg · m<sup>-3</sup>, for 2 h at rest, and a marked increase in airway responsiveness to histamine was observed immediately after the exposure (76). The effect had only partially vanished one week after exposure. Holtzman et al (77), exposing 16 subjects to the same ozone concentration for 2 h with intermittent exercise, also found an ozone-induced increase in airway responsiveness. In this study the increased responsiveness had resolved 24 h after exposure.

The exposure of normal subjects to 800 µg · m<sup>-3</sup> for 1 to 3 h with intermittent exercise also caused increased

**Table 4.** Changes in airway responsiveness following ozone exposures.<sup>a</sup> (BSA = body surface area, FEV<sub>1,0</sub> = forced expiratory volume in 1 s, FVC = forced vital capacity, IE = intermittent exercise, O<sub>3</sub> = ozone, PC<sub>100SRaw</sub> = provocative concentration<sub>100SRaw</sub>, R<sub>aw</sub> = airway resistance, SO<sub>2</sub> = sulfur dioxide, SR<sub>aw</sub> = specific airway resistance, V<sub>E</sub> = minute ventilation, Vmax<sub>50</sub> = maximum expiratory flow at 50% of the vital capacity)

| Reference                  | Ozone concentration (μg · m <sup>-3</sup> )                                                                  | Exposure duration                                           | Activity                                                                                            | Number and gender of subjects | Subject characteristics                                                                 | Observed effect(s)                                                                                                                                                                                                                                                                                                                                                            |
|----------------------------|--------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|-------------------------------|-----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Normal subjects</i>     |                                                                                                              |                                                             |                                                                                                     |                               |                                                                                         |                                                                                                                                                                                                                                                                                                                                                                               |
| Horstman et al, 1990 (16)  | 0<br>160<br>200<br>240                                                                                       | 6.6 h                                                       | IE at ≈ 39 l · min <sup>-1</sup>                                                                    | 22 men                        | Healthy nonsmokers 18—32 years old                                                      | 33%, 47%, and 55% decreases in cumulative dose of methacholine required to produce a 100% increase in SR <sub>aw</sub> after exposure to O <sub>3</sub> at 160, 200 and 240 μg · m <sup>-3</sup> , respectively                                                                                                                                                               |
| Gong et al, 1986 (6)       | 0<br>240<br>400                                                                                              | 1 h                                                         | 1 h at V <sub>E</sub> = 89 l · min <sup>-1</sup> followed by 3—4 min at ≈ 150 l · min <sup>-1</sup> | 15 men & 2 women              | Elite cyclists                                                                          | Greater than 20% increase in histamine responsiveness in one subject at an O <sub>3</sub> level of 240 μg · m <sup>-3</sup> and in 9 subjects at 400 μg · m <sup>-3</sup>                                                                                                                                                                                                     |
| Folinsbee et al, 1988 (18) | 0<br>240                                                                                                     | 6.6 h                                                       | IE at ≈ 25 l · min <sup>-1</sup> · m <sup>-2</sup> BSA                                              | 10 men                        | Nonsmokers 18—33 years old                                                              | Approximate doubling of mean methacholine responsiveness after exposure; on an individual basis, no relationship between O <sub>3</sub> -induced changes in airway responsiveness and FEV <sub>1,0</sub> or FVC                                                                                                                                                               |
| Dimeo et al, 1981 (78)     | 400                                                                                                          | 2 h                                                         | IE at 2× resting                                                                                    | 12 men                        | Nonsmokers 21—32 years old                                                              | 110% increase in the change in SR <sub>aw</sub> to a 10-breath histamine aerosol challenge after exposure to O <sub>3</sub> at 800 μg · m <sup>-3</sup> , but no change at 400 μg · m <sup>-3</sup> ; progressive adaptation of this effect over 3-day exposure                                                                                                               |
|                            | 800                                                                                                          | 2 h                                                         | IE at 2× resting                                                                                    | 7 women                       |                                                                                         |                                                                                                                                                                                                                                                                                                                                                                               |
|                            | 800                                                                                                          | 2 h/d for 3 d                                               |                                                                                                     |                               |                                                                                         |                                                                                                                                                                                                                                                                                                                                                                               |
| Kulle et al, 1982 (66)     | 0<br>800                                                                                                     | 3 h/d for 5 d in a row                                      |                                                                                                     | 13 men<br>11 women            | Nonsmokers                                                                              | Enhanced response to methacholine after the first 3 days, but this response normalized by day 5                                                                                                                                                                                                                                                                               |
| Golden et al, 1978 (76)    | 0<br>1200                                                                                                    | 2 h                                                         | Rest                                                                                                | 5 men & 3 women               | Nonsmokers 22—30 years old                                                              | 300% increase in histamine-induced change in R <sub>aw</sub> 5 min after O <sub>3</sub> exposure; 84% increase and 50% increase 24 h and 1 week after exposure (P>0.05); two subjects had an increased response to histamine 1 week after exposure                                                                                                                            |
| Holtzman et al, 1979 (77)  | 0<br>1200                                                                                                    | 2 h                                                         | IE at 2× resting                                                                                    | 11 men & 5 women              | 9 atopic & 7 nonatopic, nonsmokers                                                      | Ten-breath methacholine or histamine challenge increased SR <sub>aw</sub> ≥150% in 16 nonasthmatics after O <sub>3</sub> . The increased responsiveness resolved after 24 h                                                                                                                                                                                                   |
| <i>Asthmatic subjects</i>  |                                                                                                              |                                                             |                                                                                                     |                               |                                                                                         |                                                                                                                                                                                                                                                                                                                                                                               |
| Koenig et al, 1990 (81)    | 240 O <sub>3</sub> -300 SO <sub>2</sub><br>240 O <sub>3</sub> -240 O <sub>3</sub><br>air-300 SO <sub>2</sub> | 45 min in first atmosphere and 5 min in second              | IE                                                                                                  | 8 men & 5 women               | Asthmatic, 12—18 years old                                                              | Greater declines in FEV <sub>1,0</sub> and Vmax <sub>50</sub> and greater increase in respiratory resistance after O <sub>3</sub> -SO <sub>2</sub> than after O <sub>3</sub> -O <sub>3</sub> or air-SO <sub>2</sub>                                                                                                                                                           |
| Molfino et al, 1991 (82)   | Air-antigen<br>240 O <sub>3</sub> -antigen                                                                   | 1 h                                                         | Rest                                                                                                | 4 men & 3 women               | Asthmatic, 21—64 years old                                                              | Increased bronchoconstrictor response to inhaled ragweed or grass after O <sub>3</sub> exposure compared with air                                                                                                                                                                                                                                                             |
| Kreit et al, 1989 (52)     | 0<br>800                                                                                                     | 2 h with IE at V <sub>E</sub> = 53—55 l · min <sup>-1</sup> |                                                                                                     | 8 men & 10 women              | 9 asthmatics (5 women & 4 men) and 9 healthy persons (5 women & 4 men), 18—34 years old | Decreased PC <sub>100SRaw</sub> from 33 mg · ml <sup>-1</sup> to 8.5 mg · ml <sup>-1</sup> in healthy subjects after O <sub>3</sub> ; PC <sub>100SRaw</sub> fell from 0.52 mg · ml <sup>-1</sup> to 0.19 mg · ml <sup>-1</sup> in asthmatic subjects after exposure to O <sub>3</sub> and from 0.48 mg · ml <sup>-1</sup> to 0.27 mg · ml <sup>-1</sup> after exposure to air |

<sup>a</sup> This table is based on table 7-10 in reference 1.

nonspecific airway responsiveness (52, 78—80). An attenuation of the change in airway responsiveness was seen on the fifth day in 24 subjects exposed to 800 μg · m<sup>-3</sup> for 3 h a day for 5 d in a row (66).

In another study 9 of 17 elite cyclists, exposed to 400 μg · m<sup>-3</sup> for 1 h with very heavy exercise, experienced a significantly increased responsiveness to inhaled histamine after exposure (6).

Upon extension of the exposure period to 6.6 h, ozone has been reported to increase airway responsiveness to methacholine in normal subjects even at concentrations of 160 and 240  $\mu\text{g} \cdot \text{m}^{-3}$  (16, 18). Horstman et al (16) studied 22 subjects at three ozone concentrations (160, 200 and 240  $\mu\text{g} \cdot \text{m}^{-3}$ ) and found a 33%, 47%, and 55% decrease in the dose of methacholine required to produce a 100% increase in  $\text{SR}_{\text{aw}}$  as compared with filtered air. Folinsbee et al (18) also exposed 10 subjects to 240  $\mu\text{g} \cdot \text{m}^{-3}$ , and the methacholine responsiveness was approximately doubled after the inhalation of ozone.

### *Airway responsiveness in asthmatic subjects*

Nine normal and nine asthmatic subjects were exposed to an ozone concentration of 800  $\mu\text{g} \cdot \text{m}^{-3}$  (52) and the airway responsiveness was shown to be significantly increased in both groups. The change was of the same order of magnitude among both normal subjects and asthmatics.

Koenig et al (81) used sulfur dioxide as a bronchoconstricting agent (instead of histamine or methacholine) in a study of 13 asthmatic subjects, aged 12 to 18 years, who were exposed to an ozone concentration of 240  $\mu\text{g} \cdot \text{m}^{-3}$  for 45 min and then exposed to sulfur dioxide for 15 min. A greater decline in  $\text{FEV}_{1.0}$  was seen after the combined ozone and sulfur dioxide exposure than after ozone plus ozone or air plus sulfur dioxide.

Seven subjects with asthma who were allergic to ragweed or grass took part in a study (82) to investigate whether exposure to ozone affects the bronchial responsiveness to allergen. The subjects were exposed to 240  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h at rest, followed by a bronchial challenge with allergen.  $\text{FEV}_{1.0}$  was not affected by ozone exposure, but the airway responsiveness to inhaled allergen was significantly increased. The authors concluded that low doses of ozone, similar to those commonly occurring in urban areas, can increase the bronchial responsiveness to allergen in allergic asthmatic subjects.

This conclusion was supported by a recent report (83) on 24 subjects with allergic asthma who were exposed to 500  $\mu\text{g} \cdot \text{m}^{-3}$  for 3 h with intermittent exercise, followed by an allergen bronchial provocation test 3 h later with pollen, house dust mite, or horse allergen. The responsiveness to allergen was significantly increased after exposure to ozone as compared with a sham exposure.

However, no effect of exposure to ozone at 240 and 400  $\mu\text{g} \cdot \text{m}^{-3}$ , was seen on bronchial responsiveness to allergen in 15 and 10 asthmatic subjects, respectively, in two recently published, preliminary reports (82, 85). The reasons for these diverging results are not known, but differences in sensitivity to ozone among subjects have been proposed as an important factor.

### *Summary*

To sum up, exposure to ozone causes an increase in nonspecific airway responsiveness in both normal and asthmatic subjects. However, there seems not to be any significant difference in sensitivity between the two groups. An effect of ozone on airway responsiveness in normal subjects has been observed at 160  $\mu\text{g} \cdot \text{m}^{-3}$  after 6-h exposure with intermittent exercise. For asthmatics, the lowest level of ozone used in a study on nonspecific airway responsiveness was 240  $\mu\text{g} \cdot \text{m}^{-3}$ , which produced increased airway responsiveness. The ozone-induced effects on airway responsiveness are, like the spirometric changes and occurrence of symptoms, also attenuated by repeated exposures. However, the attenuation of responsiveness seems to be a slower process than that for pulmonary function and symptoms.

It is likely that ozone also increases airway responsiveness to inhaled allergens in subjects with allergic asthma, but, due to conflicting results in experiments of similar design, no firm conclusion can be drawn for the time being.

### *Exercise performance*

The effects of ozone on the capacity for performing heavy exercise have been examined in several chamber studies and mostly with highly trained competitive cyclists or distance runners as subjects. The end points of study in these experiments have been maximal oxygen uptake ( $\dot{V}\text{O}_{2\text{max}}$ ) or the ability to complete strenuous, continuous exercise protocols.

A 6% reduction in  $\dot{V}\text{O}_{2\text{max}}$  was observed by Foxcroft & Adams (72) in eight subjects exposed to 700  $\mu\text{g} \cdot \text{m}^{-3}$  during heavy, continuous exercise for 50 min. Gong et al (6) found a 16% decrease in the  $\dot{V}\text{O}_{2\text{max}}$  of 17 endurance cyclists exposed to 400  $\mu\text{g} \cdot \text{m}^{-3}$  for 1 h during very heavy exercise. In both studies ozone reduced performance time and pulmonary function and gave marked subjective symptoms of respiratory discomfort.

Decreased exercise performance, measured as exercise time or the number of subjects unable to complete a competitive protocol, have been shown in several studies (5–7, 72, 86). Already at 120 to 140  $\mu\text{g} \cdot \text{m}^{-3}$ , Linder et al (86) observed a small reduction in the maximum performance time of 12 healthy men and 12 healthy women and also an increase in respiratory symptoms during ozone exposure.

The symptoms caused by exposure to ozone during high-intensity exercise are laryngeal or tracheal irritation and chest soreness and tightness upon taking a deep breath. These symptoms can be an important factor limiting exercise performance but other, still undefined mechanisms cannot be excluded.

## ***Inflammatory reactions in the airways***

There are several studies on ozone-induced cellular and biochemical changes in the upper or lower airways related to inflammation, damage to tissues, repair processes, and defense against microorganisms. These studies are based on measurements of nasal, bronchial and bronchoalveolar lavage (BAL) constituents or biopsies from the nose and bronchi.

### ***Inflammatory reactions in the bronchi***

Exposure to an ozone concentration of  $800 \mu\text{g} \cdot \text{m}^{-3}$  has resulted in significant increases in polymorphonuclear leukocytes, prostaglandins  $E_2$ , and  $F_2$  alpha and thromboxane  $B_2$  (80) in BAL fluid from a sample of 10 subjects. The results strongly indicate an inflammatory reaction.

Devlin & Koren (87) observed that macrophages, harvested from the bronchi 18 h after the exposure of eight normal subjects to the same ozone concentration, exhibited changes in the rate of synthesis of 123 of a total of nearly 900 cellular proteins.

Eleven subjects exposed to  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h with intermittent heavy exercise had significant increases in polymorphonuclear leukocytes, albumin, immunoglobulin G, prostaglandin  $E_2$ , plasminogen activator, elastase, complement C3a, and fibronectin (88) in the BAL fluid 18 h after exposure. These findings indicate an inflammatory reaction in the bronchi, with leakage of proteins from the circulation and tissue injury and repair. In a similar experiment Koren et al (89) performed a BAL 1 h after exposure to the gas and found significant increases in polymorphonuclear leukocytes, protein, prostaglandin  $E_2$ , thromboxane  $B_2$ , interleukin 6, lactate dehydrogenase, alpha-1 antitrypsin, and tissue factor, as compared with the results from subjects exposed to filtered air. The capacity of alveolar macrophages to phagocytose yeast was also reduced by ozone exposure. The coagulation factors called tissue factor and factor VII were also shown to be increased in BAL fluid, and macrophages in the BAL fluid had an elevated level of the tissue factor messenger RNA (ribonucleic acid) in subjects exposed to  $800 \mu\text{g} \cdot \text{m}^{-3}$  (90).

In order to elucidate the time-course of ozone-induced inflammatory changes, Schelegle et al (11) exposed five subjects to  $600 \mu\text{g} \cdot \text{m}^{-3}$  for 1 h during heavy continuous exercise on three separate days and then performed a BAL 1, 6, and 24 h after the exposure. Polymorphonuclear leukocytes were significantly elevated (+4%) already 1 h after the exposure, but the peak (+17%) was not seen until 6 h after the exposure.

An increased concentration of substance P was seen in the BAL fluid from seven subjects exposed to  $500 \mu\text{g} \cdot \text{m}^{-3}$  for 1 h with heavy exercise (91); this finding suggests that ozone affects the neuropeptide regulating system in the airways. Aris et al (15) performed

isolated lavage of the left mainstem bronchus and forceps biopsy of the mucosa in a group of 14 healthy subjects exposed to  $400 \mu\text{g} \cdot \text{m}^{-3}$  for 4 h. BAL was also performed. The objective of the study was to determine whether ozone causes inflammation of the proximal airway in addition to inflammation in the distal lung, which had previously been documented in human experiments. Total cell counts, neutrophilic polymorphonuclear leukocytes, lactate dehydrogenase, and interleukin-8 were all increased in the proximal airway lavage fluid, and the biopsy material showed that ozone induced a significant increase in interstitial neutrophils in the airway wall. The results of the BAL were similar to those obtained in previous studies. The authors concluded that ozone caused both central airway inflammation and inflammatory changes in the distal lung.

Exposure to  $400 \mu\text{g} \cdot \text{m}^{-3}$  with intermittent exercise for 6 h caused an increased number of polymorphonuclear leukocytes, as well as increased levels of interleukin-8 and interleukin-6, in BAL fluid from five asthmatic subjects (92). However, five normal subjects exposed to the same ozone concentration did not exhibit any significant changes in leukocyte numbers or in interleukin release. The study suggests that asthmatic subjects are more sensitive to ozone in developing neutrophilia in the bronchi.

Exposure to ozone for 6.6 h during heavy exercise at ozone levels which can commonly occur in ambient air, 160 and  $200 \mu\text{g} \cdot \text{m}^{-3}$ , caused significant changes in the composition of the BAL fluid in a group of 18 healthy subjects (93). The BAL fluid of subjects exposed to  $200 \mu\text{g} \cdot \text{m}^{-3}$  had significant increases in neutrophilic polymorphonuclear leukocytes, protein, prostaglandin  $E_2$ , fibronectin, interleukin-6, and lactate dehydrogenase, as compared with the values of BAL fluid from the same subjects when exposed to filtered air. Exposure to  $160 \mu\text{g} \cdot \text{m}^{-3}$  resulted in similar, but smaller, increases in neutrophilic leukocytes and all mediators except protein and fibronectin. In addition, there was a decrease in the ability of the macrophages to phagocytose yeast via the complement receptor at both ozone concentrations.

### ***Inflammatory reactions in the upper airways***

Twelve subjects with allergic rhinitis were exposed for 4 h at rest to  $1000 \mu\text{g} \cdot \text{m}^{-3}$  (51), and the gas was shown to cause a significant increase in upper and lower respiratory symptoms, a 7-fold increase in nasal lavage neutrophils, a 20-fold increase in eosinophils, and a 10-fold increase in mononuclear cells. There was also a significant increase in the nasal lavage albumin concentration on the ozone exposure day, indicating a leakage from the circulation.

Graham et al (94) found increased levels of polymorphonuclear leukocytes in nasal lavage fluid from 21 sub-

jects exposed to an ozone concentration of  $1000 \mu\text{g} \cdot \text{m}^{-3}$  at rest, as compared with a group of 20 subjects exposed to filtered air. Increased numbers of polymorphonuclear leukocytes were also observed in nasal lavage fluid both immediately and 22 h after exposure to  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h with intermittent heavy exercise (95). Tryptase, a marker of mast cell degranulation, was also increased immediately after exposure, but not after 22 h (96). The effects of a 90-min exposure to 240 or  $480 \mu\text{g} \cdot \text{m}^{-3}$  on the upper airways of 10 asthmatic and 8 nonasthmatic subjects were studied by McBride et al (54) by means of nasal lavage. In the asthmatic group significant increases in leukocytes were seen both immediately and 24 h after exposure, as was an increase in epithelial cells immediately after exposure. A significant correlation between neutrophilic leukocyte counts and interleukin-8 was observed among the asthmatic subjects after exposure to  $480 \mu\text{g} \cdot \text{m}^{-3}$ . No significant changes were seen in the nonasthmatic group. The authors concluded that asthmatics seem to be more sensitive within the upper airways to the acute inflammatory effects of ozone than are nonasthmatic persons.

The effect of exposure for 2 h to  $800 \mu\text{g} \cdot \text{m}^{-3}$  on nasal inflammation was examined in 11 allergic asthmatics who were sensitive to house dust mite (97). The allergen-induced eosinophilic inflammation was augmented by ozone. Less allergen was also required to provoke nasal symptoms after ozone exposure.

## Host defense

### Host defense against virus

Henderson et al (98) inoculated 24 subjects with type 39 rhinovirus, and half the subjects were then exposed to an ozone concentration of  $600 \mu\text{g} \cdot \text{m}^{-3}$  and half to filtered air, for 6 h during five consecutive days. No differences in virus titers, polymorphonuclear cells, or interferon gamma in the nasal lavage fluid were found between subjects exposed to ozone and air. The authors concluded that exposure to ozone at this concentration and with this exposure pattern seemed not to have any adverse effect on the immune response to rhinovirus infection.

### Airway epithelial permeability

Kehrl et al (99) showed that exposure to  $800 \mu\text{g} \cdot \text{m}^{-3}$  for 2 h caused radiolabeled DTPA (diethylese triamine pentacetic acid) particles inhaled 75 min after exposure to be taken up by the bloodstream from the lung; the result indicating increased epithelial permeability.

### Mucociliary clearance

There are two studies on the effects of ozone on the mucociliary clearance of inhaled particles. Foster et al

(100) found a dose-dependent increase in clearance 2 h after the exposure of subjects to 400 and  $800 \mu\text{g} \cdot \text{m}^{-3}$ . On the other hand, Gerrity et al (101) found no difference between air-exposed and ( $800 \mu\text{g} \cdot \text{m}^{-3}$ ) ozone-exposed persons on the clearance rate of particles 2 to 5 h after exposure or the next morning. The discrepancy between these two studies can be explained by differences in their respective experimental designs (particles inhaled before exposure in one study and inhaled after in the other), and further studies are needed in order to define the relationship between ozone exposure and the function of mucociliary clearance.

## Combination with other pollutants

### Effects of ozone mixed with other pollutants

There are about a dozen published reports on the respiratory effects of exposure to ozone in combination with other major air pollutants such as PAN (peroxyacetyl nitrate), sulfur dioxide, nitrogen dioxide, sulfuric acid, nitric acid, and particles. A common finding is that the effects of the mixtures are not significantly different from the effects of ozone alone.

Drechsler-Parks et al exposed healthy adults to ozone ( $900 \mu\text{g} \cdot \text{m}^{-3}$ ) and PAN (102) and to the same concentration of ozone and PAN together with nitrogen dioxide (103) and found no difference between these combined exposures and ozone alone. The combined exposure of ozone ( $970 \mu\text{g} \cdot \text{m}^{-3}$ ) and PAN ( $1400 \mu\text{g} \cdot \text{m}^{-3}$ ) has also induced decrements in FVC and  $\text{FEV}_{1.0}$  that were about 10% greater than those observed after exposure to ozone alone (104).

Mixtures of ozone and nitrogen dioxide also gave similar effects to those of ozone alone (32, 105, 106). Kagawa (107) exposed seven subjects to mixtures of sulfur dioxide, nitrogen dioxide, and ozone (0.15 ppm of each), and the pulmonary effects were not significantly different from those seen after exposure to ozone alone.

Aris et al (108) have also examined the effects of a simultaneous exposure to nitric acid and ozone on BAL and proximal airway lavage parameters, as well as on bronchial biopsy specimens, and the data did not suggest any potentiating effect of nitric acid on the inflammatory response elicited by ozone.

In another study combined exposure to ozone and sulfur dioxide or sulfuric acid gave the same result as exposure to ozone alone (109, 110).

### Effects of sequential exposure to ozone and other pollutants

A sequential exposure model involving initial exposure to nitric acid fog, aqueous fog, or filtered air for 2 h and then, after a 1-h break, exposure to ozone for 3 h gave

a surprising 26% decrease in  $FEV_{1.0}$  after air followed by ozone and a significantly smaller reduction after both the aqueous (17%) and nitric acid (18%) fog (111). Inhalation of fog, regardless of whether it contained nitric acid or not, seemed to attenuate the effect of ozone, an observation which remained unexplained by the authors.

Using a sequential exposure model with 2-h exposure to a nitrogen dioxide concentration of  $1200 \mu\text{g} \cdot \text{m}^{-3}$  and 3 h later to an ozone concentration of  $600 \mu\text{g} \cdot \text{m}^{-3}$ , Hazucha et al (112) found that preexposure to nitrogen dioxide significantly enhanced ozone-induced changes in spirometric parameters. Furthermore, the airway responsiveness to methacholine was significantly greater after exposure to nitrogen dioxide and ozone, as compared with exposure to air and ozone, with a change in the provocative dose of methacholine from 5.6 to  $1.7 \text{ mg} \cdot \text{mL}^{-1}$ . Exposure to nitrogen dioxide itself did not affect the spirometric parameters at all. The authors concluded that nitrogen dioxide seems to have a delayed and latent effect on the airways, and this effect enhances those of a subsequent ozone exposure.

Koenig et al (81) exposed 13 allergic asthmatic adolescents sequentially to an ozone concentration of  $240 \mu\text{g} \cdot \text{m}^{-3}$  and a sulfur dioxide concentration of  $300 \mu\text{g} \cdot \text{m}^{-3}$  and found greater decrements in  $FEV_{1.0}$  than after ozone followed by ozone and air followed by ozone.

#### *Effects of ozone in ambient air studied in mobile laboratories*

A special form of chamber study is the field studies conducted using a mobile laboratory. In the mobile laboratory subjects can be exposed to ambient air, filtered air, or filtered air containing artificially generated ozone (or any other pollutant) at a concentration similar to those measured in the ambient environment. There has been a series of investigations with mobile laboratories from the Los Angeles area published during the 1980s (10, 27, 29, 111). The ambient ozone levels varied between 220 and  $320 \mu\text{g} \cdot \text{m}^{-3}$  among the different studies, and the exposure duration was, in all the studies, set at 1 h. At  $220 \mu\text{g} \cdot \text{m}^{-3}$  (1 h) no effect on spirometry or symptoms were found in 66 healthy children (8 to 11 years of age), but at 290, 300 and  $320 \mu\text{g} \cdot \text{m}^{-3}$  a decrease in  $FEV_{1.0}$  of about -5% was observed among the adolescents and adults.

#### **Summary and concluding remarks**

Controlled human exposure studies have provided comprehensive data on the acute effects of ozone on health at concentrations from 160 up to about  $1500 \mu\text{g} \cdot \text{m}^{-3}$ . The effects investigated in these chamber studies have included lung function decrements, changes in airway responsiveness to both nonspecific stimuli and allergens,

inflammatory effects on the upper and lower airways, respiratory epithelial permeability, mucociliary clearance, and exercise performance.

There is a negative relationship between the inhaled dose of ozone and lung function. The dose of ozone depends on the ozone concentration in inhaled air, tidal ventilation, and duration of exposure. Ozone concentration has a greater influence than time and ventilation on this exposure dose product.

Significant decrements in lung function, as well as the appearance of respiratory symptoms, occur after exposure to a steady ozone concentration of  $160 \mu\text{g} \cdot \text{m}^{-3}$ , provided that the exposure time is prolonged (about 6 h) and the subjects are performing moderate exercise.

If the subjects are resting and the exposure time is shortened, higher ozone concentrations can be endured without symptoms and lung function decrements. Ozone affects lung function in resting subjects exposed for a short time (2 h) at about  $1000 \mu\text{g} \cdot \text{m}^{-3}$ .

Almost all studies are performed with a continuous exposure to a steady concentration of ozone, whereas the natural exposure pattern to ozone is often variable, with a peak ozone concentration reached around noon or some hours later. There is only one published study comparing the effects of one and the same dose of ozone delivered by different exposure patterns. The results of this study suggested that a triangular exposure pattern, with a short peak in the middle, had greater effects on lung function than steady-state exposure.

The sensitivity to ozone varies greatly between people. The effects of a single exposure to ozone is, on the other hand, fairly well reproducible within the same person. The sensitivity to ozone seems to decline with age. Women might be more sensitive than men, but there is no conclusive evidence for this.

Ozone seems to elicit effects on the pulmonary function of patients with asthma and chronic obstructive pulmonary disease that are similar to those of normal subjects; this phenomenon contrasts strongly to the effects of other pollutants such as sulfur dioxide and nitrogen dioxide. However, some recent reports on inflammatory changes, both in the upper and lower airways, suggest an increased sensitivity to ozone in asthmatics. Furthermore, patients with asthma and chronic obstructive pulmonary disease often have reduced lung function, and, therefore, even if the ozone-induced decrement in lung function among these groups is comparable to that in normal subjects, the clinical importance of the change is much greater for the patients.

One study has reported ozone-induced increases in nonspecific airway responsiveness for healthy subjects exposed to  $160 \mu\text{g} \cdot \text{m}^{-3}$  for 6 h during exercise. In addition several other studies have reported similar findings for both normal subjects and asthmatic subjects using higher ozone concentrations.

Exposure to an ozone concentration of  $240 \mu\text{g} \cdot \text{m}^{-3}$  for 1 h at rest and to  $500 \mu\text{g} \cdot \text{m}^{-3}$  for 3 h with intermittent exercise enhanced the bronchial responsiveness to inhaled allergen in subjects with allergic asthma in two published studies. These findings might indicate that ozone can potentiate the effects of airborne allergens, which should be an effect of great importance. However, as there are also two preliminary reports on no effects of exposure to ozone at 240 and  $400 \mu\text{g} \cdot \text{m}^{-3}$ , no firm conclusion can yet be drawn.

Changes in BAL fluid indicating inflammatory reactions, tissue damage and repair, and increased endothelial permeability have also been found in one study with normal subjects after 6-h exposure during exercise to an ozone concentration of  $160 \mu\text{g} \cdot \text{m}^{-3}$ , as well in several other studies with higher ozone concentrations.

Repeated exposure to ozone day after day elicits an attenuation of symptoms and lung function responses, with the exception of the second exposure day, when an increased effect on lung function has sometimes been reported. Airway responsiveness appears to attenuate with repeated exposures to the gas but more slowly than the spirometric responses. Airway inflammation also diminishes but some markers of inflammation have not been shown to attenuate.

This review of human exposure studies shows that ozone can induce acute airway responses with subjective symptoms, lung function decrements, increased airway responsiveness, and inflammatory reactions in the airways at ambient concentrations of  $160 \mu\text{g} \cdot \text{m}^{-3}$  and upwards in a dose-dependent manner. A reduction of performance during heavy exercise has been observed in several studies at about  $400 \mu\text{g} \cdot \text{m}^{-3}$ . These acute effects are judged as adverse health effects per se. It is possible that repeated exposures to ozone at concentrations causing acute effects can also lead to chronic respiratory effects, but that question cannot be answered from data obtained in short-term human exposure studies.

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