

Hhealth risk evaluation of ozone

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Preface

This criteria document on ozone has been prepared at the Institute of Environmental Medicine at the Karolinska Institute in Stockholm at the request of the Swedish Environment Protection Agency. Although each section has a main author, the work has been a joint effort, and all conclusions and recommendations have been thoroughly discussed by the group. The recommendations in this document will serve as a basis for revised air quality standards for ozone in the ambient air in Sweden.

Most of the cited studies report ozone concentrations in parts per billion (ppb) or parts per million (ppm). In this document a practical conversion factor of $1 \text{ ppb} = 2.0 \mu\text{g} \cdot \text{m}^{-3}$ has been used throughout, unless otherwise specified.

Abstract

Bylin G, Cotgreave I, Gustafsson L, Nyberg F, Pershagen G, Sundell J, Victorin K, Zuber A. Health risk evaluation of ozone. *Scand J Work Environ Health* 1996;22 suppl 3:104 p.

Ground-level ozone is formed in complex atmospheric photochemical reactions between nitrogen oxides and hydrocarbons. The levels are highest in summertime and outside city centers. Elevated concentrations of ground-level ozone is mainly due to chemical reactions of emissions of volatile organic compounds and nitrogen oxides. The average concentrations of ground-level ozone occurring in non-urban areas in western Europe range from 40 to 70 $\mu\text{g} \cdot \text{m}^{-3}$. One-hour maximum values may reach 300 $\mu\text{g} \cdot \text{m}^{-3}$ in central Europe and 200 $\mu\text{g} \cdot \text{m}^{-3}$ in Sweden. Numerous studies, ranging from experimental studies with laboratory animals to controlled studies with humans to epidemiologic studies, have been performed on the health effects of ozone. In animal experiments, morphological effects on lung tissue, effects on host defense mechanisms, inflammation, fibrosis and tumors have all been induced by the inhalation of ozone. The lowest concentration tested that has given rise to these effects is 200 $\mu\text{g} \cdot \text{m}^{-3}$. It led to hyperplastic changes, proinflammatory changes, and sensitization to allergens.

In chamber exposure studies of 1- to 6-h duration, respiratory symptoms and pulmonary effects such as lung function decrements, increased airway responsiveness, and inflammatory reactions have all been demonstrated in humans. These effects have occurred even at the lowest concentration tested, 160 $\mu\text{g} \cdot \text{m}^{-3}$ for 6 h. Reduced lung function at 1-h maximum daily values of 160 $\mu\text{g} \cdot \text{m}^{-3}$ has also been found in epidemiologic studies, and some reports indicate effects at even lower levels. Other effects of ozone seen in epidemiologic studies include respiratory symptoms, hospital admissions, and emergency room visits for respiratory conditions, all of which correlated with daily 1-h maximum values of 240 $\mu\text{g} \cdot \text{m}^{-3}$ and lower. From these data on humans, and with the use of a safety factor of 2, a 1-h guideline value of 80 $\mu\text{g} \cdot \text{m}^{-3}$ is recommended. This level is also intended to protect against health effects in the most sensitive sections of the population. The proposed guideline level is currently exceeded and should be regarded as a goal for action to avoid adverse health effects in the future.

The experimental and epidemiologic basis for a risk assessment concerning long-term exposure is much weaker than for short-term exposure, and no explicit long-term guideline value is recommended. However, the proposed short-term guideline would probably offer some protection against potential effects of ozone following long-term exposure.

Ozone and other photochemical oxidants in ambient and indoor air – properties, sources and concentrations

by Jan Sundell & André Zuber

Physical and chemical properties

Ozone (O_3) occurs naturally in the atmosphere as a result of thunderstorms and photochemical reactions. It is also an air pollutant due to emissions of nitrogen oxides and volatile organic compounds. Ozone and other photochemical oxidants all have the common feature that they react rapidly with organic substances. Examples of other photochemical oxidants are peroxyacetyl nitrate, hydrogen peroxide, and hydrogen peroxy radicals. Ozone is the photochemical oxidant that occurs at the highest concentrations in the atmosphere, and it is that which is the most investigated with respect to its environmental impact.

About 90% of the ozone content of the atmosphere is contained in the stratosphere at an altitude of 10 to 50 km. Stratospheric ozone can be transported to the troposphere during sporadic events, in particular in mountainous regions and in connection with weather fronts. At the earth's surface ozone can increase to high concentrations during such events. The stratospheric ozone plays an important role in screening out harmful ultraviolet radiation from the sun. Ozone is also an important greenhouse gas.

The remainder of the earth's ozone, about 10%, is in the troposphere, from the ground up to about 10 km. Ozone contained between the ground and about 1500 m, the *boundary layer*, determines, to a large extent, the concentration of ground-level ozone. The *free troposphere*, between the boundary layer and the stratosphere, acts as a reservoir of ozone, which in turn supplies the boundary layer with ozone.

Ozone is a triatomic allotrope of oxygen. It is an irritating and corrosive, pale-blue gas and is explosive at high concentrations. The characteristic stale smell has given the gas its name from the Greek *o'zo*, to smell. The odor detection threshold for ozone is about $40 \mu\text{g} \cdot \text{m}^{-3}$ (1). The molecular weight of the gas is $47.8992 \text{ g} \cdot \text{mol}^{-1}$ and, at a pressure of 1013 mbar, the pure gas has a density of $2.144 \text{ g} \cdot \text{l}^{-1}$ at 0°C and a boiling point of -111.3°C . At temperatures above about 100°C ozone rapidly decays to oxygen (O_2). The solubility of ozone in water at 0°C is 1.09 g per liter and this value decreases with increasing temperature, being $0.57 \text{ g} \cdot \text{l}^{-1}$ at 20°C and $0.40 \text{ g} \cdot \text{l}^{-1}$ at 30°C (2). In the liquid phase ozone can react directly with biomolecules or indirectly via the production of reactive oxidative molecules, such as the

hydrogen peroxy radical and hydrogen peroxide. [See also the section on the absorption and metabolic fate of ozone in this issue (3).]

For ozone in ambient air the practical conversion factor for the volume mixing ratio to mass concentration is:

$$1 \text{ ppb} = 2.0 \mu\text{g} \cdot \text{m}^{-3} \text{ (at } 20^\circ\text{C and } 101.3 \text{ kPa).}$$

The general, more precise conversion relationship for other temperatures and pressures is:

$$1 \text{ ppb of ozone} = 1.962 (293/(273+t)) (p/101.3) \mu\text{g} \cdot \text{m}^{-3},$$

where t is the temperature in $^\circ\text{C}$ and p the pressure in kPa.

The temperature at ground level varies within a relatively limited range. Thus temperature has a moderate influence on the conversion factor of 2, about $\pm 10\%$ for a temperature range from -20 to $+35^\circ\text{C}$. However, for sites at high elevation, the reduced pressure must be accounted for. For instance, Mexico City, with an elevation of 2240 m, has a mean pressure that is about 75% of the pressure at sea level, and the conversion factor is therefore close to 1.5. For Albuquerque, New Mexico, at an elevation of 1600 m above sea level, the conversion factor is 1.64 (figure 1).

Formation mechanisms of ozone and photochemical oxidants

Atmospheric ozone is formed primarily through photochemical reactions. Several other processes may also

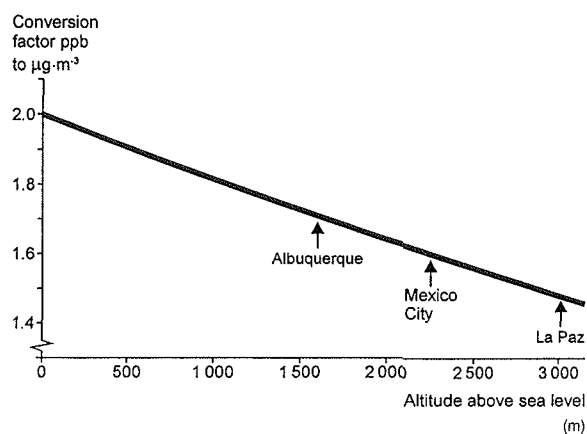
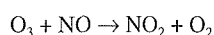
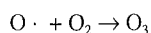


Figure 1. Ozone conversion factor from parts per billion to micrograms per cubic meter as a function of altitude in a standard atmosphere.

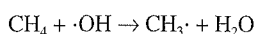
directly create ozone, for instance, electrical discharges and lightning. In the stratosphere ultraviolet solar radiation dissociates molecular oxygen into its atoms, which in turn react with oxygen to form ozone molecules.

Ozone in the troposphere exists, to some extent, due to downward net transport from the stratosphere. However, the major source of tropospheric ozone is the photochemical oxidation of volatile organic compounds in the presence of nitrogen oxides. Volatile organic compounds and nitrogen oxides are *precursors* to photochemical pollution. Oxides of nitrogen play a key role in ozone formation. Nitrogen dioxide (NO_2) is readily dissociated by sunlight, forming nitrogen monoxide (NO) and atomic oxygen (O). The oxygen atom can react with an oxygen molecule to form ozone. However, the ozone molecule can react with nitrogen monoxide, resulting in the destruction of the ozone molecule and the reappearance of nitrogen dioxide:

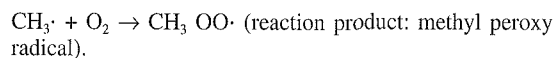


These reactions do not result in net ozone production. In order to obtain net production of ozone, nitrogen monoxide must react with some other oxygen-containing molecule, such as the oxidation intermediates of volatile organic compounds. In particular peroxy radicals react with nitrogen monoxide and compete with ozone for the available nitrogen monoxide. The oxidation of volatile organic compounds often starts with their reaction with hydroxyl radicals, which are formed through photochemical reactions.

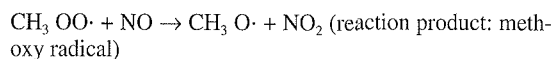
As an example of this reaction mechanism (figure 2), the important steps in methane oxidation are:



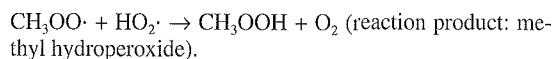
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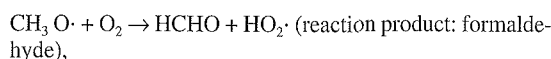
The methyl peroxy radical can react in different ways, the more important of these being:



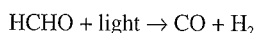
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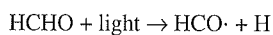
The reaction product from the first of these two reactions, the methoxy radical, reacts to formaldehyde:



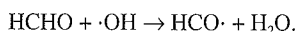
and this reaction is then followed by the following reactions:



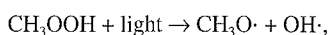
or



or



The hydrocarboxy radical, $\text{HCO}\cdot$, reacts readily with oxygen and also forms carbon monoxide. This carbon monoxide is slowly removed by reaction with the hydroxyl radical and the formation of carbon dioxide. The methyl hydroperoxide is photolyzed:



and the methoxy radical enters into the previously described cycles, or it reacts with hydroxyl radicals:

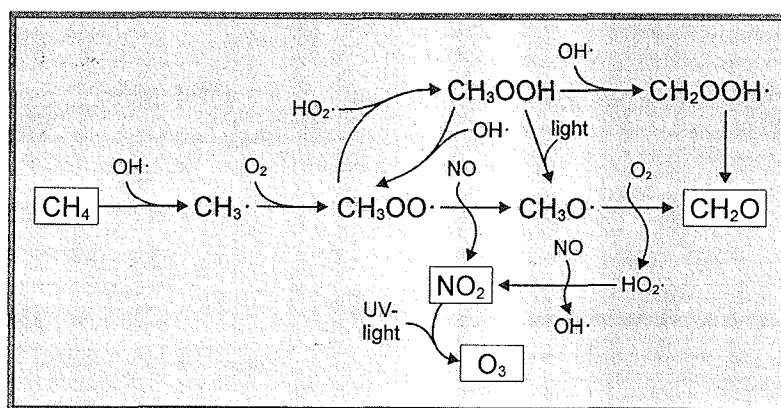
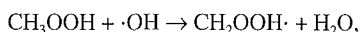
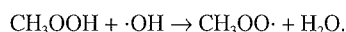
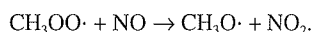


Figure 2. Oxidation cycle of methane as an example of the oxidation of volatile organic compounds and the production of ozone. The compounds in boxes are stable.

where the reaction product undergoes a rapid reaction to formaldehyde. The other important reaction with hydroxyl radicals is:



The reaction scheme for ozone with organic compounds other than methane differs from the one already described, but only in small details. The general characteristic in the oxidation of organic volatile compounds is the production of organic peroxy radicals, $\text{R-CH}_2\text{OO}\cdot$, which act as a supply of oxygen in the reaction with nitrogen monoxide:



The nitrogen dioxide formed is then photolyzed back to nitrogen monoxide and an oxygen atom, the latter rapidly reacting with oxygen to form a new molecule of ozone. The nitrogen monoxide is then freed to react with a new organic peroxy radical and the cycle is repeated.

The number of ozone molecules being formed, as well as the rate of ozone formation, depends strongly on the species of the organic molecule involved in the cycle, the amount of radiation that drives the reactions, the temperature, and, not the least, the amount of nitrogen oxides present.

The general relationship between the concentrations of precursors and the production of photooxidants is not linear. In polluted areas, such as the heavily industrialized areas of northwest Europe, each nitrogen dioxide molecule emitted into the air participates in the generation of 3 to 10 ozone molecules before it is lost from the atmosphere through deposition. Under background conditions, such as those prevailing in remote areas and in the middle troposphere, the concentrations of nitrogen oxides are lower, and each nitrogen oxide molecule emitted into this part of the atmosphere participates in the net generation of 10 to 100 ozone molecules before it is lost from the atmosphere.

However, should the concentration of nitrogen oxides be below about 0.008 to $0.040 \mu\text{g} \cdot \text{m}^{-3}$, the atmospheric chemistry favors a net destruction of ozone. Such low levels of nitrogen oxides are only found over the large oceans and in the upper troposphere.

In the absence of photo-dissociating light, which drives the reactions, ozone formation decreases rapidly.

The major sources of the precursors to photochemical pollution are transport (cars, trucks, air transport and ships), and off-road vehicles and nonroad mobile machinery. In Sweden and other Nordic countries, small-scale wood heating also contributes significantly to the emissions of volatile organic compounds. Emissions of nitrogen monoxide reduce the concentration of ozone in the short-term scale. The rapid reaction, $\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2$, converts ozone within minutes to nitrogen dioxide. The temporary decrease in ozone concentration that fol-

lows from emissions of nitrogen monoxide is generally found in polluted areas, such as in cities and close to industrial areas.

Occurrence of ozone in urban and rural areas

The local ozone concentration depends on the extent of the emissions, local conditions of terrain and meteorology, temperature, and solar radiation.

On the North American continent episodes of elevated ozone concentration have been a major concern for public health and agriculture since the 1960s. During the last two or three decades summertime ozone episodes have also been a persistent feature in Europe. In Sweden, the levels of the emissions, the temperature, and the sunlight conditions differ strongly from the conditions dominating in southern and central Europe and in the United States (such as the Los Angeles area). Locally produced photochemical smog, with ozone concentrations over $240 \mu\text{g} \cdot \text{m}^{-3}$, does not occur in Sweden. Instead, the occurrence of increased ozone concentrations at ground level is mainly due to long-range transport of the pollutant and its precursors.

The levels of ozone are highest in rural areas and are generally higher in southern Sweden as compared with northern Sweden. In urban areas, the discharges of nitrogen monoxide, primarily from cars, react rapidly with ozone and can thus reduce the levels of ozone somewhat. In the larger city centers (Stockholm, Göteborg, Malmö) the levels of ozone are thus 10% to 40% lower than outside the cities. However, the emissions from the urban areas contribute to ozone formation on a regional scale.

There is a general increase in the relative ozone concentration or mixing ratio with altitude. This increase is due to the ozone in the free troposphere, which acts as a reservoir of the gas. In mountainous areas, such as the Alpine region and the Scandinavian mountains, the ozone levels are therefore higher than at lower altitudes.

Annual and daily variations

The atmospheric levels of ozone are generally higher during summer than winter due to the lower photochemical activity in the absence of sunlight. In addition the uptake of ozone by vegetation and soils is relatively slow in winter, and the levels are therefore similar for nighttime and daytime conditions. In wintertime, the daily (24 h) mean ozone concentrations in rural areas are about $60 \mu\text{g} \cdot \text{m}^{-3}$ in southern Sweden and about $50 \mu\text{g} \cdot \text{m}^{-3}$ in northern Sweden. In cities traffic emissions of nitrogen monoxide react with ozone and form nitrogen dioxide and thus reduce the levels of ozone somewhat, particu-

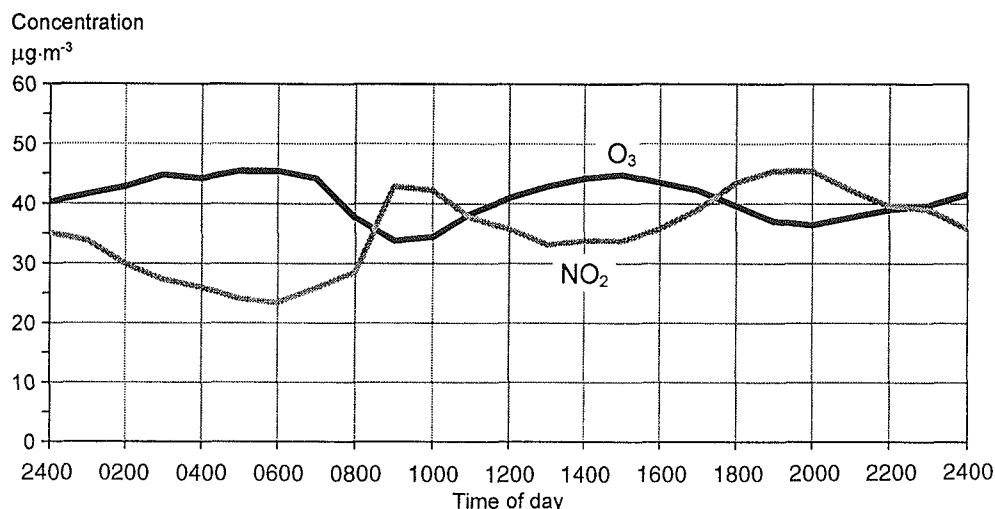


Figure 3. Ozone (O_3) and nitrogen dioxide (NO_2) in central Göteborg. Courtesy of the monitoring network of the Göteborg Municipal Authority.

larly during rush hours. On the average, the nitrogen dioxide concentration in cities is low during the early morning hours, peaks around 0900, decreases somewhat until the evening rush hour sets in, and then peaks again around 1900. In these urban areas the ozone levels may be about 10% to 40% lower than those in the surrounding rural areas (figure 3).

During the summer the increased photochemical production of ozone, increased vertical transport, and increased uptake by vegetation give large differences between daytime and nighttime ozone concentrations. In rural areas of southern Sweden the ozone levels are typically below $50 \mu\text{g} \cdot \text{m}^{-3}$ during nighttime and increase to about 80 to $90 \mu\text{g} \cdot \text{m}^{-3}$ during daytime. In northern Sweden the concentration of ozone is about 10 to $15 \mu\text{g} \cdot \text{m}^{-3}$ lower. In high-concentration episodes during spring and summer the levels can increase to above $180 \mu\text{g} \cdot \text{m}^{-3}$ in southern Sweden during the daytime but stay at relatively low levels during the nighttime. This behavior

during high-concentration episodes reflects the importance of vertical transport, which is stronger during daytime and ceases during nighttime, in particular under the influence of high-pressure weather conditions with clear skies.

Episodes with high concentrations of ozone

Episodes with high concentrations of ground-level ozone occur mainly during the spring and summer. Their main cause is the transport of polluted air containing high concentrations of ozone and ozone precursors from other countries in northwest Europe (4). Ozone concentrations during these episodes can increase to 150 to $200 \mu\text{g} \cdot \text{m}^{-3}$ for a period of several days. In southern and central Sweden, $120 \mu\text{g} \cdot \text{m}^{-3}$ is, on the average, exceeded 130 h per year and $150 \mu\text{g} \cdot \text{m}^{-3}$ is exceeded 12 h per year. In northern Sweden the levels of ozone during ozone episodes are lower, $120 \mu\text{g} \cdot \text{m}^{-3}$ being exceeded 18 h per year on the average (5).

Local meteorological conditions are important for ozone episodes. For instance, in land- and sea-breeze circulation, pollutants such as nitrogen oxides, volatile organic compounds, and ozone can return repeatedly to the area of the emissions and hence accumulate higher and higher concentrations of pollutants. This is one of the causes of the high levels of ozone in the Mediterranean area during the summer, as well as the high levels of Los Angeles photochemical smog (table 1).

Ozone background level

Another aspect of photochemical pollution is the background level of ozone. The background level is somewhat loosely defined as the level excluding the episodic events with high concentrations of ozone. For practical purposes, the long-term average ozone concentration has been used in Sweden to quantify the background levels.

Table 1. Ozone level at the rural monitoring station Aspveten, 100 km south of Stockholm. The number of hours above ozone concentrations of $120 \mu\text{g} \cdot \text{m}^{-3}$ and $150 \mu\text{g} \cdot \text{m}^{-3}$ and the 24-h average concentrations for summer (April to September) and winter (October to March) are given.

Year	Hours above $120 \mu\text{g} \cdot \text{m}^{-3}$	Hours above $150 \mu\text{g} \cdot \text{m}^{-3}$	Winter mean ($\mu\text{g} \cdot \text{m}^{-3}$)	Summer mean ($\mu\text{g} \cdot \text{m}^{-3}$)
1985	338	98	·	79
1986	1	0	49	53
1987	33	0	52	56
1988	302	23	43	72
1989	189	24	51	73
1990	117	0	49	67
1991	19	0	45	65
1992	153	9	47	72
1993	49	1	52	62
1994	87	7	51	69
Mean 1985— 1994	128	16	49	69

In regions with more frequent episodes, such as southern Europe, the basis for the calculation of the background level may be different.

In Europe, the background level has increased during the last century. Ozone observations made at the Montsouris Observatory outside Paris during the late 19th century showed a background annual mean ozone concentration of about $20 \mu\text{g} \cdot \text{m}^{-3}$. Currently, ozone background levels are above $50 \mu\text{g} \cdot \text{m}^{-3}$ (6). The measurement of ozone in Sweden has a shorter record; ozone levels have been regularly monitored since 1985. Again, the trend is towards higher ozone concentrations (5). For the protection of plants, for example, crops and forests, the average ozone levels during summer days are important, since physiological activities such as gas exchange and photosynthesis are concentrated to this period. The period used to calculate the average concentration of ozone over the vegetation season is also of relevance for the protection of human health since a large portion of our outdoor activities takes place during that season. Over the years 1987 to 1989 the mean ozone concentration during the period April to September, between 0900 and 1600, was about $85 \mu\text{g} \cdot \text{m}^{-3}$ in southern and central Sweden and about $65 \mu\text{g} \cdot \text{m}^{-3}$ in northern Sweden. For the period 1985 to 1990 the trend in ozone levels over the vegetation season varied from unchanged, or even somewhat decreasing levels in the south, to a slowly increasing level in central Sweden. At the Aspvreten station 100 km south of Stockholm the average increase has been 3% to 4% annually since 1985 (5). The elevated levels of ozone during the growing season in central Sweden may be caused by the generally increased emissions of nitrogen oxides in Europe. The relative stability or decreasing trend of ozone levels in southern Sweden may partly be due to a saturation effect in the photochemical ozone production. The saturation effect is expressed as a disproportionately small change in the ozone levels when emissions of ozone precursors are changed. Such saturation effects are known to occur in some parts of northwest Europe, where the concentrations of pollutants are so high that no more or even less ozone is formed close to the emissions (7).

However, due to the short history of ozone monitoring in Sweden, it cannot be ruled out that the trend in ozone levels is due to the interannual variability in weather patterns (5).

Relationship between short- and long-term averages

Levels of air pollutants are monitored during shorter or longer averaging times, and it is possible to derive a frequency distribution from any time series. The conversion between short-term and long-term values is generally expressed as a transfer function. A widely used transfer is the log-normal frequency distribution, which is adequate for pollutants such as nitrogen dioxide but less

useful for ozone. The frequency distribution of ozone concentration in rural areas is closer to a normal frequency function. For ozone, the annual mean value is about $65 \mu\text{g} \cdot \text{m}^{-3}$ in southern and central Sweden, and the mean standard deviation is about $35 \mu\text{g} \cdot \text{m}^{-3}$. For urban areas the distribution is far from normal. To our knowledge, no general transfer functions for short-term and long-term ozone concentrations representative of human exposure currently exist that can convert 1- or 8-h average ozone concentrations to the annual or summertime averages (figure 4).

Ozone indoors

Since human beings spend a large portion of their time indoors, quantitative knowledge on the indoor pollution from ozone is important to assess health impact. In contrast to the major interest regarding ozone in outdoor air, the interest in determining ozone concentrations and related health effects in nonindustrial indoor air has been limited, mainly because the major source of indoor ozone is outdoor ozone and, as ozone quickly reacts with indoor surfaces, indoor concentrations are normally lower than those outdoors (8).

Major sources of indoor ozone, besides outdoor air, are electrical discharges from appliances, such as photo-

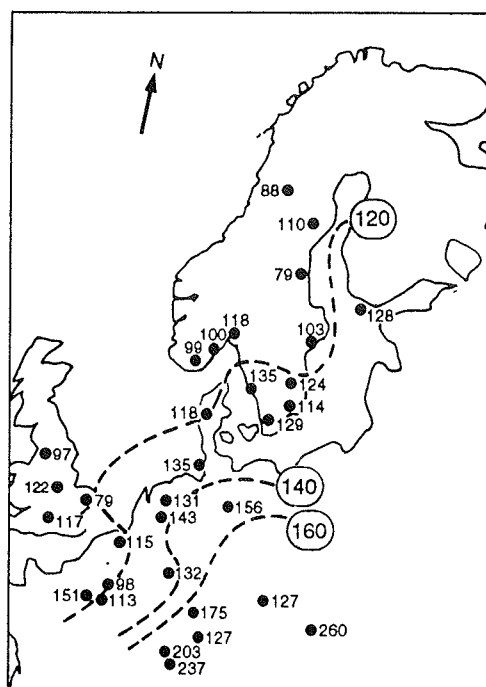


Figure 4. Distribution of ozone above Europe in April to September 1986, 98th percentile for ozone concentration (expressed in micrograms per cubic meter) (3).

copiers, printers, electrical filters, ionizers, and ozonizers. Ultraviolet light is also a source of ozone.

Hansen & Andersen (9) measured the ozone emission from 69 photocopying machines. The source strength, during continuous copying, varied between 0 to $1350 \mu\text{g} \cdot \text{min}^{-1}$, with most ($N = 57$) copiers emitting $< 500 \mu\text{g} \cdot \text{min}^{-1}$. The same laboratory measured ozone emissions from a laser printer. With a new ozone-filter the emission rate was $58 \mu\text{g} \cdot \text{min}^{-1}$, with an old filter it was $292 \mu\text{g} \cdot \text{min}^{-1}$, and without a filter it was $910 \mu\text{g} \cdot \text{min}^{-1}$ (10). In conclusion, they, as well as Olander (11) in a review of the literature, found that, in small rooms with normal ventilation, small printers will hardly cause ozone concentrations to rise above $100 \mu\text{g} \cdot \text{m}^{-3}$.

Wolkoff et al (12) studied human reactions to office machines in climate chambers with 60 recirculations of chamber air per hour and 50% fresh air exchange per hour. Thirty healthy females were exposed in groups of five for 6 h in an office environment with three personal computers, one laser printer, and one photocopier. As a control, the subjects were exposed to an office environment without office machinery. The laser printer chosen had an ozone emission of $113 \mu\text{g} \cdot \text{min}^{-1}$, and that of the copying machine was $215 \mu\text{g} \cdot \text{min}^{-1}$ at continuous operation. The peak ozone levels were $52 \mu\text{g} \cdot \text{m}^{-3}$ in the room containing the office machinery and $< 2 \mu\text{g} \cdot \text{m}^{-3}$ in the control chamber. The mean atmospheric formaldehyde levels were three times higher in the chamber containing the office machinery ($95 \pm 11 \mu\text{g} \cdot \text{m}^{-3}$) than in the control chamber, whereas the concentrations of volatile organic compounds and particulate matter were almost the same. The authors found significantly greater ($P = 0.015$) epithelial damage in the conjunctiva of the eye, as well as subjective eye irritation, and worsened immediate perception of air quality in the presence of the office machinery. The study was not conclusive regarding which agent(s) caused the adverse health effects, but ozone, per se, as well as products from the ozonolysis of volatile organic compounds, were suspected.

Ozone-generating devices are used indoors for deodorization after fires or after hotel room residence by smoking guests, that is, in rooms that are not occupied. However, they are also used in occupied rooms for the modification of room air with focus on the biocidal and deodorizing properties of ozone. The Food and Drug Administration (FDA) in the United States has claimed that, in order to be effective in such respects, the air concentration of ozone has to be much higher than can be safely tolerated by humans, and it labels any device which generates an ozone concentration greater than $100 \mu\text{g} \cdot \text{m}^{-3}$ in room air as "adulterated and misbranded" (13, 14).

Kissel (15) has calculated outlet-air ozone concentrations from ozone generators to be in the range of 400 to

$4000 \mu\text{g} \cdot \text{m}^{-3}$ and resulting room air concentrations to be in the range of 80 to $800 \mu\text{g} \cdot \text{m}^{-3}$, assuming air exchange rates of 30% and 200%, respectively, and ozone half-lives of between 30 and 8 min.

Indoor-outdoor relationships

The half-life of ozone in office rooms is reported to be in the range of 9 to 26 min, and in residences it is between 8 and 21 min (16–19). It depends on the types of surfaces in the room, with high decomposition rates for organic materials such as cotton, wool and neoprene and low rates for inorganic materials such as aluminum and glass (19). Accordingly, the surface-to-volume ratio is also related to the decomposition rate (18). The rate also depends on the humidity of the air, and it increases five-fold over the range of 5% to 55% relative humidity (20).

Extended, simultaneous measurements of ozone indoors and outdoors have been performed by Weschler et al (21) in a telephone switching office in Burbank, California, in the United States. The office had no internal ozone source. During maximum ventilation the concentration of ozone was roughly 65% of the outdoor value, while at minimum ventilation it was typically 30%. Stock & Venso (22) simultaneously measured indoor and outdoor concentrations of ozone in and around six homes in El Paso and four in Houston in the United States. The hourly average indoor ozone concentrations ranged from 2 to $50 \mu\text{g} \cdot \text{m}^{-3}$, the indoor to outdoor ratios being between 0.45 to 0.67 in El Paso and 0.05 to 0.12 in Houston. The variations in ratios were explained by the type of ventilation or cooling used in each dwelling. High ratios (El Paso) were associated with evaporative cooling, using high outdoor airflow rates, and low ratios (Houston) were associated with refrigerative cooling, using low outdoor airflow rates.

If there is no indoor source of ozone, the indoor-to-outdoor ratio is typically reported to be in the range of 0.1 to 0.8 (8, 23), with low values at low ventilation rates and high values at high ventilation rates. The influence of indoor surfaces, surface-to-volume ratio, and the relative humidity of indoor air is, in most studies, not obvious, as the variability of such aspects in most studies is low. Seemingly, as judged from reported continuous measurements, the indoor-to-outdoor ratio is relatively stable during varying outdoor concentrations.

In the case of Sweden, there is a lack of measurements of indoor ozone. However, the ventilation rates are generally in the lower range of the values determined in the United States, which indicate that the indoor-to-outdoor ratio should be low in Swedish dwellings (possibly between 0.1 to 0.5), the lowest values being measured in single family houses and higher values in buildings such as offices or schools (possibly between 0.3 to 0.8), on the assumption that there is no internal sources.

With indoor sources, such as copying machines or laser printers, indoor concentrations can exceed those outdoors.

Personal exposure

Liu et al (24) have simultaneously measured the ozone concentration at stationary ambient monitoring sites in and around 23 homes, as well as the personal exposure of 23 children during 12-h episodes (passive samplers during daytime). The mean concentrations ranged from 21 (indoor, nighttime) to 113 $\mu\text{g} \cdot \text{m}^{-3}$ (outdoor, daytime). The outdoor air concentrations measured at domestic sites and at a stationary ambient monitoring site were highly correlated ($r = 0.81$, $P < 0.01$), even though the concentrations at the domestic sites were, on the average, typically 20% lower than at the stationary ambient monitoring site. In regions with high populations and traffic densities, the outdoor ozone concentrations were lower than in more rural areas. Daytime personal exposure was correlated with both outdoor ($r = 0.41$, $P < 0.01$), at the domestic site, and indoor ($r = 0.55$, $P < 0.01$) concentrations of the gas. There was a highly significant correlation between the indoor and outdoor concentration of ozone at individual domestic sites ($r = 0.56$, $P < 0.01$), the indoor-to-outdoor ratio ranging from 0.07 to 1.16 (mean 0.45, SD 0.23), and there were large differences in this ratio between homes, possibly due to variations in ventilation rates and dissimilar housing materials (walls, furniture, drapes, and books). The variability in personal exposure was explained, to 76%, by exposures indoors and outdoors (at the domestic site) for those persons who stayed at or near their home for at least 95% of the monitoring period ($N = 14$). For the total population ($N = 23$) 40% of the variability in personal exposure could be, at most, explained by the indoor and outdoor domestic concentrations, leaving 60% to be explained by exposure when absent from or near the home.

Monn et al (25) used passive sampling of ozone during four workdays (and nights) to assess the concentrations of ozone indoors and outdoors at the home, as well as the personal exposure. Twenty-six adults were included in the study, which was repeated three times. Over the sampling period, the average outdoor concentration of ozone was 52 (SD 11) $\mu\text{g} \cdot \text{m}^{-3}$, indoor levels were 8 (SD 6) $\mu\text{g} \cdot \text{m}^{-3}$, and the personal exposure was 22 (SD 7.6) $\mu\text{g} \cdot \text{m}^{-3}$. The indoor-to-outdoor ratio was 0.15 (SD 0.12). The personal-to-indoor ratio was 2.75 (SD 2.5) and the personal-to-outdoor ratio was 0.42 (SD 0.33). A high degree of correlation ($r = 0.75$) between the personal exposure and the indoor concentration was found for nonworkers, while the correlation was weaker for part-time ($P = 0.32$) and full-time workers ($P = 0.28$).

Due to the relatively long time spent indoors, total exposure is largely dependent on indoor levels. Variation in indoor ozone concentrations can be large between buildings in the same area, mainly due to differences in ventilation rates. In addition, the variation in personal exposure can be large due to the time spent at home, outdoors, and at work. Thus there are large variations in exposure between people mostly staying at home, such as housewives or small children, and people who spend time in day nurseries, schools, or workplaces, even if they are living in the same building or area. Office workers may have an elevated exposure to ozone from photocopiers or laser printers. Studies involving different categories of people, people living in different types of housing, or people living in different climatic regions (with different ventilation rates) should consider these differences. For instance, it has been shown that buildings in northern Sweden have a mean ventilation rate which is 20% lower than those in southern Sweden, with the same type of ventilation. It is also known that single-family homes have a significantly lower ventilation rate than multi-apartment buildings (26).

Indirect effects of indoor ozone

Ozone can play a role in indoor air chemistry by producing compounds damaging to health, such as aldehydes and free radicals. Thus measurements of ozone concentrations indoors, and without assessment of ozone-related compounds, can underestimate the impact of indoor ozone on human health. Indoor ozone reacts with volatile organic compounds, the result being aldehydes, organic acids, and free radicals (12, 27, 28). Weschler et al (28) measured volatile organic compounds in both the absence and presence of ozone (range 60 to 100 $\mu\text{g} \cdot \text{m}^{-3}$) in a stainless-steel room with fresh carpets. The concentration of carpet emissions, especially unsaturated volatile organic compounds, significantly decreased in the presence of ozone, while concentrations of aldehydes significantly increased. The "loss" of volatile organic compounds, other than aldehydes, possibly due to such reactions, has been significantly associated with an increased prevalence of symptoms of the sick building syndrome. The most important symptoms are the induction of headache, tiredness, perturbations to mucous membrane function, and skin symptoms (12, 29).

Some techniques to determine the concentration of ozone in ambient air

Wet-chemical techniques

Earlier wet-chemical techniques for measuring oxidants suffered from positive interference from other photochemical oxidants, such as peroxyacetyl nitrate, nitrogen

dioxide, and peroxides. The neutrally buffered potassium iodide method suffers also from negative interference from sulfur dioxide. The response to ozone and other interfering gases depends on the type of detector, pretreatment of the sample, and corrections through independent measurements of the interfering gases. The true ozone concentration is generally in the range of 80% to 110% of the total oxidant concentration, as measured with this method. For carefully made measurements, the errors can be substantially lower (30). The improved potassium iodide wet-chemical method acidically buffered with boric acid suffered less from interference from other pollutants.

Optical techniques

Two main techniques are currently being used to determine the concentration of ozone in air at stationary sites such as monitoring stations in urban or rural environments. The *chemiluminescent technique* involves the measurement of light emitted from chemical reactions. For ozone measurements, ethene is added to the sample of air, and the reaction of ozone and ethene generates light which is measured with a light-sensitive detector. The method is sensitive to temperature changes and needs to be re-calibrated often in order to guarantee a high degree of accuracy. The measurement is a point measurement.

The *light absorption technique* exploits the fact that ozone absorbs ultraviolet and visible radiation. Two instruments have been developed using light absorption, the ultraviolet absorption instrument, and the differential optical absorption spectrometer. The ultraviolet absorption instrument measures absorption at a single wavelength. The light source is a small mercury lamp, and the radiation is absorbed by ozone over a distance of 10 to 50 cm in a chamber, which is ventilated with ambient air. This instrument measures the concentration at one point in time. The differential optical absorption spectrometer (DOAS) exploits the fact that ozone has an absorption that varies with wavelength. The instrument consists of a light source that gives continuous radiation in the visible and the ultraviolet spectral areas and a spectrometer for dividing the light into different wavelengths. Measurements are made directly in the atmosphere, over distances ranging from several hundred meters up to about 1 km. The assayed concentration is expressed as the mean concentration along the light path.

Ozone dosimeters

There is a growing need to determine the exposure of humans to ozone as they move in different environments. Passive personal samplers or diffusion samplers are now coming into use to determine the ozone exposure of individual subjects. The advantage of the sampler

is that it follows the individual person as he or she is exposed to different environments. The sampler consists of an impregnated filter that absorbs ozone; the filter is then analyzed with respect to its chemical composition (31–33). Hitherto, such samplers have been used in Sweden to monitor the ozone exposure of workers in relation to paper bleaching with the gas. However, there is substantial interference from other components, for example, hydrogen peroxide. These difficulties are not expected to be encountered in other, less contaminated environments. In ambient air the combined effect of lack of accuracy and lack of precision of the passive samplers gives an uncertainty of 10% to 20%. The sampling time for the passive samplers is generally a few days to a few weeks. Improved samplers with forced flow have reduced the sampling time to only a few hours (31).

Current air quality guidelines and standards

World Health Organization

Several guidelines exist for ozone, depending on the purpose for their implementation. The primary objective of the guidelines is to provide a basis for protecting public health from the adverse effects of ozone in air pollution. The World Health Organization (WHO) has recommended the following guidelines for ozone in ambient air (34): (i) the mean value for 1 h should not exceed 150 to 200 $\mu\text{g} \cdot \text{m}^{-3}$; (ii) the mean value over 8 h should not exceed 100 to 120 $\mu\text{g} \cdot \text{m}^{-3}$. For the protection of vegetation WHO also recommends the following guidelines (34): (i) the mean value for 1 h should not exceed 200 $\mu\text{g} \cdot \text{m}^{-3}$, (ii) the mean value for 24 h should not exceed 65 $\mu\text{g} \cdot \text{m}^{-3}$, and (iii) the mean value over the growing season (100 d) should not exceed 60 $\mu\text{g} \cdot \text{m}^{-3}$. New guidelines for air pollutants have recently been prepared within WHO (35): (i) for ozone the mean value over 8 h should not exceed 120 $\mu\text{g} \cdot \text{m}^{-3}$. It is noted in the WHO document that the new guideline for ozone does not provide a margin of safety for protection from all acute health effects in the most sensitive members of the population.

United Nations Economic Commission for Europe

Within the framework of the Convention on Long-Range Trans-boundary Air Pollutants, held under the auspices of the United Nations Economic Commission for Europe (UNECE), the guidelines for ozone exposure have been related to critical loads and levels (36). The critical levels are based on effects on crops and natural ecosystems, for which there is a scientific basis for determining damage. Below these critical levels no visible damage or growth reduction could be determined on crops (table 2).

In a later review of current data the ECE Working Group on Critical Loads and Levels has proposed the use of cumulative doses above a certain threshold value. The threshold value is $80 \mu\text{g} \cdot \text{m}^{-3}$, and the cumulative dose, calculated as the number of hours times the concentration above the threshold level (in units of micrograms per cubic meter per hour), is then related to the damage. The workshop, held in Bern, in 1993 concluded that (37) (i) for crops cumulative doses of $5200 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$ and $10\,600 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$ give a 5% and 10% relative yield reduction, respectively and (ii) forest trees are less sensitive. A 10% reduction of plant biomass growth was found for oak with a cumulative dose of $12\,000 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$, for birch with a cumulative dose of $18\,000 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$, and for Norway spruce and Scots pine with a cumulative dose of $24\,000 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$. The cumulative doses for forests should not exceed $20\,000 \mu\text{g} \cdot \text{m}^{-3} \cdot \text{h}$.

United States air quality standards

The National Ambient Air Quality Standard for ozone (dating from 1979) is $235 \mu\text{g} \cdot \text{m}^{-3}$ (1-h value) in the United States. A new document on air quality criteria is now under preparation that may result in a new air quality standard for ozone.

European Union

The European Union (EU) has a directive for ozone (38) to inform and warn the public when the levels exceed certain threshold values: (i) a population information threshold of $180 \mu\text{g} \cdot \text{m}^{-3}$ for the mean value over 1 h and (ii) a population warning threshold of $360 \mu\text{g} \cdot \text{m}^{-3}$ for the mean value over 1 h. The EU also recommends a health protection threshold of $110 \mu\text{g} \cdot \text{m}^{-3}$ over 8 h and vegetation protection thresholds of $200 \mu\text{g} \cdot \text{m}^{-3}$ for the mean value over 1 h and $65 \mu\text{g} \cdot \text{m}^{-3}$ for the mean value over 24 h.

Sweden

The Swedish Environmental Protection Agency has guidelines or target values for ozone based on the same criteria documents as those used by the EC, WHO and UNECE (39, 40). In addition, the criteria document by Lindvall has been used. The national targets are (i) the concentration over the growing season (April to September, between 0900 and 1600) should not exceed $50 \mu\text{g} \cdot \text{m}^{-3}$ in order to protect crops and forests, (ii) the 1-h mean value of ozone should not exceed $120 \mu\text{g} \cdot \text{m}^{-3}$ more often than 12 h per year, and $150 \mu\text{g} \cdot \text{m}^{-3}$ should not be exceeded at any time. For work premises the maximum allowed concentration of the gas is related to the time of exposure. The Swedish Board of Occupational Safety and Health requires that the concentration over 8 h must not exceed $200 \mu\text{g} \cdot \text{m}^{-3}$ and that the concentration over any 15-min period during the workday should not exceed $600 \mu\text{g} \cdot \text{m}^{-3}$.

Table 2. Critical concentration levels for ozone with reference to crops (35).

Time (hours)	Concentration ($\mu\text{g} \cdot \text{m}^{-3}$)
0.5	300
1	150
2	110
4	80
8	60
Vegetation season ^a	50

^a The critical concentration for the vegetation or growing season refers to April to September, between 0900 and 1600 daily.

Summary and concluding remarks

Pollution by photochemical oxidants is caused by anthropogenic emissions of nitrogen oxides and volatile organic compounds. Ozone is the most important photochemical oxidant; others are peroxy acetyl nitrate (PAN) and hydrogen peroxide. Photochemical pollution occurs predominantly in summer, during which episodic high levels and elevated background concentrations are encountered. The present Swedish guideline or target value for the protection of human health [$120 \mu\text{g} \cdot \text{m}^{-3}$ (1-h mean) less than 12 h per year] is exceeded about 130 h per year in southern Sweden, but sometimes also in northern Sweden. A complementary guideline (limit value) states that the ozone concentrations should be lower than $150 \mu\text{g} \cdot \text{m}^{-3}$ at all times; this guideline is also exceeded in most parts of Sweden.

The indoor-to-outdoor ratio for the ozone concentration is typically in the range of 0.1 to 0.8, with low values at low ventilation rates and high values at high ventilation rates. Buildings with a low ventilation rate, such as single family homes, can have an indoor-to-outdoor ratio between 0.1 and 0.5, with the lowest values for single family houses and higher values for buildings such as offices or schools (possibly between 0.3 and 0.8). Because of the relatively long periods people spend indoors, total exposure is largely dependent on indoor levels. The variation in personal exposure is largely due to the time spent at home, outdoors, and at work.

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Absorption and metabolic fate of ozone – the molecular basis of ozone-induced toxicity

by Ian A Cotgreave

It is the purpose of this section to define and analyze the currently available data concerning the chemical basis of the reactivity of ozone with biological molecules. The material also illustrates some of the consequences of ozone reactivity for the maintenance of biochemical processes and cell viability and phenotype. Ultimately, the goal is a better appreciation of both the complexity of the molecular mechanism(s) through which ozone executes its pulmonary toxicity and how much remains to be investigated. For this purpose the review has been divided into several parts (figure 1).

The first part deals with the close interplay between ozone, a nonradical molecule, and reactive oxygen metabolites, such as superoxide ($\cdot\text{O}_2^-$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\cdot\text{H}$), as well as other free radicals (figure 1). This description is essential, as many of the consequences of the exposure of biological material to ozone can be wholly or partially due to the conversion of ozone into other reactive species upon aqueous dissolution. The first section also details the limited data available on the absorption of ozone into the lung.

The second part deals with the chemical reactivity of ozone and progeny reactive species. It also shows how such species form the basis for the reaction of ozone with biological molecules, particularly with lipids, proteins, and nucleic acids.

The third part discusses the potential of such alterations to important biological molecules interfering with biochemical processes in cells, and therefore leading to altered cell phenotype, cytotoxicity, and genotoxicity.

It is not the purpose of this review to provide a comprehensive description of all of the literature on the subject. However, selected examples have been used to illustrate each point raised. At the end of each part, the current level of understanding is briefly summarized and specific areas of research are suggested which may, in the future, provide useful information, particularly with regard to possible sensitive biomarkers of exposure to ozone in humans.

The purpose of this structured approach is to put the complex chemical and biochemical events occurring in response to ozone at the molecular and cellular levels into perspective to help the reader evaluate data on the effects of ozone on more complex biological systems, such as the lung. An understanding of the initial biochemical events through which ozone exposure causes

alterations to cellular function is a prerequisite for (i) an understanding of how ozone affects normal lung physiology, (ii) the interpretation of biochemical effects resulting from prolonged ozone exposures, (iii) the identification of risk factors in humans, and (iv) the development of biomarker technology to detect biological responses and early signs of detrimental health effects.

Chemical relationships between ozone and reactive oxygen metabolites

Many early studies have clearly indicated that ozone toxicity can be explained in terms of free radical reactions. Indeed, ozone was considered to have radiomimetic properties and therefore to induce the generation of reactive species and free radicals and cause the induction of chromosome aberrations and facilitate interactions with antioxidant defense systems (1–6). Nevertheless, ozone is not a free radical, as is molecular oxygen. But it does have some of the properties of such a

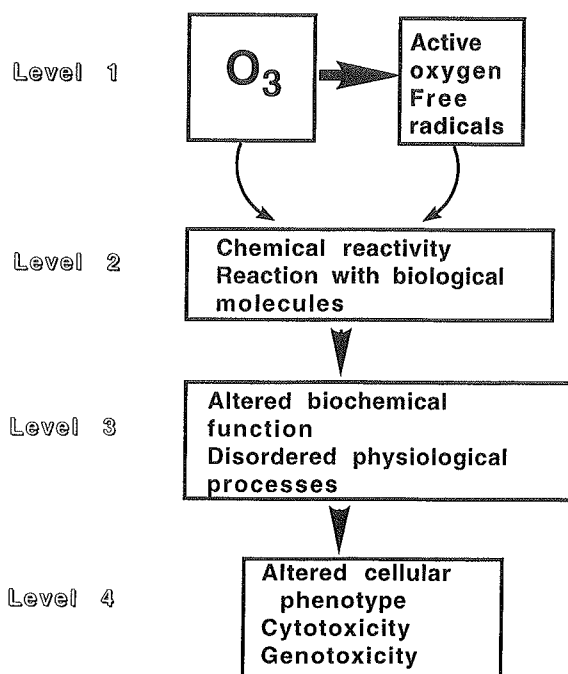


Figure 1. Schematic representation of the molecular basis of ozone toxicity.

radical, and it is clearly reactive, with a redox potential of +2.07 V (7).

The work of Taube (8) and Baxendale & Wilson (9) led Hoigne & Bader (10) to suggest that many of the actions of ozone in solutions of physiologically relevant pH are due to its decomposition to the hydroxyl radical (10). Several processes are thought to be involved (figure 2) in which the decomposition is initiated by hydroxyl ions (11), the presence of hydrogen peroxide (12), or irradiation with ultraviolet light (10, 13).

A consensus of opinion now exists that the reaction of ozone with organic molecules in aqueous solutions of near neutral or alkaline pH is a combined effect of the reactivity of ozone itself and the reactivity of a number of other reactive oxygen metabolites, particularly that of the hydroxyl radical. The hydroxyl radical is much more reactive than the parent ozone molecule, and it is more indiscriminate in its reactions. This relationship provides one of the biggest barriers to understanding the molecular basis of ozone toxicity, but for the purposes of this review, when the reactivity of ozone is considered in biological systems, the involvement of other oxidants is implicit.

Absorption of ozone into the lung

Before considering the biological effects of ozone in the lung, one must first address the question of the extent to which ozone is absorbed from the atmosphere into the lung.

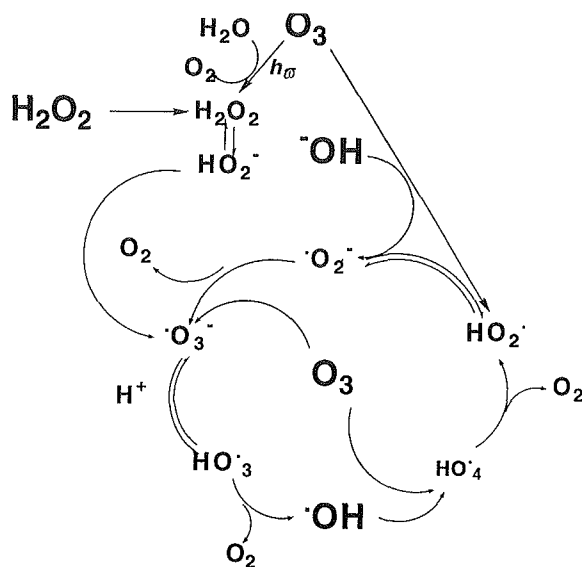


Figure 2. Cyclic decomposition of ozone catalyzed by the hydroxyl ion, hydrogen peroxide, or ultraviolet irradiation.

Elegant experiments by Ultman et al (14), using morphometric analysis of inhaled and exhaled ozone, have shown that the penetration of the gas into the lungs is mainly dependent on the rate of airflow, the tidal volume, and the manner of breathing. Thus during quiet, mainly nasal breathing most ozone is absorbed extrathoracically. On the other hand, during exercise, proportionately more ozone is absorbed by the lower respiratory airways. These authors have also shown that the rate of ozone diffusion around the airways of the lung and eventual chemical or absorption processes are proportionate to the concentration of ozone in the inhaled air (14). These morphometric studies with humans indicate that a large portion of inhaled ozone, as much as 90%, is absorbed by the total respiratory tract, with a large proportion, 30 to 40%, being absorbed by the nasal or oral airways. In addition, these studies indicate that the centriacinar region of the bronchial tree receives the largest dose of ozone when the gas is inhaled. However, these studies do not reveal the metabolic fate of the gas in the lung.

The close interplay between ozone and reactive oxygen metabolites, as well as its reactivity with biological molecules, presents a quandary with respect to the degree of penetration of inhaled ozone into and across the pulmonary epithelium, into the interstitium, and then out into systemic circulation. Limited experimental evidence is available to suggest that these considerations are of primary importance if the mechanisms by which ozone elicits its pulmonary toxicity are to be understood fully. Pryor has postulated that ozone is absorbed into the body via the lungs, but he suggests that ozone itself does not penetrate the epithelial lining fluids in the airways, except in areas of minimal lining thickness or lack of surfactant. Thus it was proposed that ozone-induced cytotoxicity and alterations to lung function result from the reactions of more stable, but nonetheless reactive products of the interaction of ozone with biomolecules, such as lipids. These species, among others, would then propagate the ozone signal into the underlying cellular layers of the lung (figure 3).

In addition, there has been limited use of isotopically-labeled ozone, $^{18}\text{O}_3$, in clinical experiments. Recently it was shown that the isotope was recoverable in lavage cells after inhalation and that the recovery was increased by exercise (17). It is clear that this approach presents definite possibilities to identify the extent of the penetration of ozone into the tissues and to identify the eventual reaction products of its interaction with biological macromolecules, particularly those of lipid origin.

In summary, present knowledge suggests that although most inhaled ozone is absorbed into the respiratory tract during quiet breathing, the molecule is itself not appreciably absorbed into the body via the lungs.

Reactivity of ozone with biological molecules

General reactivity with organic molecules

Ozone reacts readily with unsaturated compounds such as alkenes, alkynes, and aromatic systems (18), as well as with aromatic and aliphatic amines (19). In general, the aromatic systems should be activated towards electrophilic substitution. Such compounds include phenols and aromatic ethers (20). Despite the importance of studying the reactions of ozone with aromatic compounds, particularly from the point of view of ozonolysis processes in water purification, few studies have investigated the by-products of such reactions. However, it appears that, irrespective of the aromatic systems studied, ring-hydroxylations and ring-opening reactions are prevalent (21), in a manner reminiscent of the direct reaction of aromatic compounds with hydroxyl radicals.

Reaction with biological antioxidants

In the context of reactivity it is natural initially to define how ozone interacts with endogenous electron donors which function as antioxidants. One of the best studied targets in this context is glutathione. This thiol-containing tripeptide, along with the associated activities of enzymes such as the glutathione peroxidases, glutathione transferases, and glutathione reductase, represents one of the major biological antioxidant defense mechanisms, which is directed primarily against reactive oxygen metabolites, particularly against oxygen peroxide. The system is also directed against the accumulation of secondary products of the interaction of reactive oxygen metabolites with biological macromolecules, such as the lipid peroxides and the reactive aldehydes produced during lipid peroxidation (22).

Ozonation of simple glutathione-containing solutions causes rapid oxidation of the molecule (23). On the other hand, and, surprisingly, there have been no reports of an observation of acute oxidation of cellular glutathione upon the exposure of isolated cells to ozone. This lack may be due to the rapid reduction of oxidized glutathione by glutathione reductase at the expense of cellular NADPH (nicotinamide adenine dinucleotide phosphate), an event which might only be overwhelmed by exposures to extremely high and irrelevant concentrations of ozone. However, limited work with isolated A-549 epithelial cells from the lung has clearly indicated that cellular glutathione is a front-line antioxidant against ozone, as the depletion of the tripeptide prior to exposure to ozone enhances the acute cytotoxicity of the gas (24). This work also strongly suggested that the protective function of glutathione in these cells is primarily due to its serving as a co-factor for the glutathione transferase-dependent detoxication of lipid ozonides, rather than in

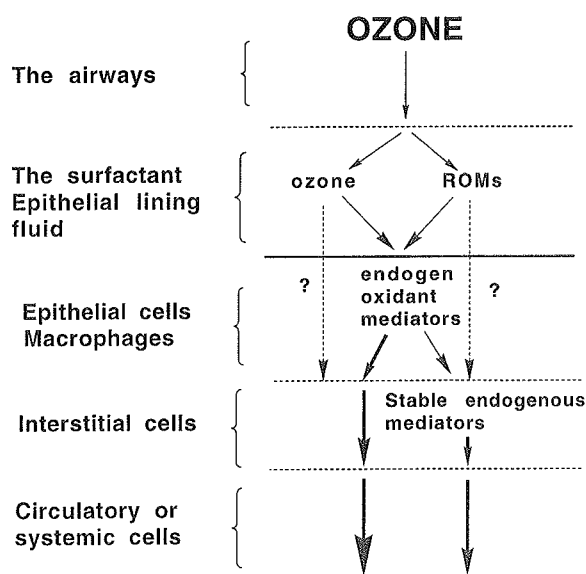


Figure 3. Potential amplificatory cascade of reactive and stable endogenous mediators derived from ozone in the lung. (ROM = reactive oxygen metabolites)

the glutathione peroxidase-dependent detoxication of hydrogen peroxide or lipid peroxides (25).

In contrast to these effects of acute exposure *in vitro*, chronic exposure of animals to ozone has been shown to induce the levels or activities of various components of the pulmonary glutathione-dependent antioxidant defense. Increases in pulmonary glutathione (26–29), glutathione peroxidase (27, 28, 30–32), and glutathione reductase (31) activities have been shown, and it has been suggested that this is an adaptive response aimed at increasing the antioxidant protection of the lung during prolonged exposure. On the basis of such observations, it was envisaged that fluxes in the glutathione system could provide a marker of pulmonary exposure to ozone. This possibility is, however, complicated by certain factors. First, in addition to the obvious dose-response relationships, the extent of these inductions is often time- and species-dependent. For instance, Tepper et al (29) noted that the increase in lung glutathione which occurred in response to ozone was maximal during the first 2 d of exposure and thereafter declined to control levels by day 4. Similarly, Ichinose and his co-workers clearly showed that nonprotein thiol (> 95% glutathione) and glutathione peroxidase activity increases in rat lung in response to ozone over a two-week period, while, in response to similar levels of ozone, the corresponding parameters in guinea pig were shown to decrease (27). This difference suggests interspecies variation for the response. A further complication arises from the fact that the inductions are often quantitatively minor and their significance may decrease or disappear if the data are corrected for the cell proliferation and increased DNA (deoxyribonucleic acid) syn-

theses which occur in the lung in response to ozone (33). Thus the induction of glutathione peroxidase and reductase noted in rat lungs in response to exposure to ozone for 3 d was not significantly different from that of controls when corrected for increases in cell number in the lung (31).

In addition to glutathione, ascorbate is another major water-soluble component of the intracellular antioxidant defense network (22). Pryor (16) has shown that ozone reacts efficiently with ascorbate in simple solutions. Nevertheless, controlled studies on the effects of the acute exposure of cells to ozone *in vitro* on ascorbate levels are absent from the literature. In parallel with glutathione, pulmonary ascorbate levels have been shown, however, to be enhanced by the chronic exposure of animals to ozone (26, 29).

Finally, the major protective antioxidant in biological membranes is vitamin E. Although vitamin E reacts directly with ozone, the reaction rate is inferior to that between ozone and ascorbate (16). Few controlled studies have been performed on the effect of ozone exposure on vitamin E levels in cells *in vitro* or in animals *in vivo*. Elsayed and his co-workers (34) have shown that vitamin E levels increase in the lung of vitamin E-supplemented rats in response to ozone exposure over several days. The importance of vitamin E in the protection of the lung from the effects of ozone is more clearly demonstrated by the observation that the depletion of vitamin E from rats prior to the exposure to ozone clearly exacerbates the toxicity of the gas (33, 34). The importance of vitamin E at the cellular level will become clear as the effects of ozone on lipid metabolism in cells are discussed.

In addition to being present within the intracellular milieu, both glutathione and ascorbate occur at relatively high concentrations in the epithelial lining fluid of the lung. Thus Pryor (25) has suggested that, due to the relatively poor penetrability of ozone into the epithelial layers of the respiratory tract, ozone may only directly react with these biological antioxidant molecules in the extracellular space. Thus any detected effects of ozone on the bulk of pulmonary antioxidant pools may be mainly due to secondary reactive oxygen metabolites generated from ozone or to other reactive metabolites generated from the interaction of ozone with components of the surfactant and epithelial cell lining fluid (16).

In summary, currently available data indicate that the reaction of ozone or derived reactive oxygen metabolites with endogenous low-molecular weight antioxidants is a dominating factor for controlling the disposition of these oxidants in the lung. Their combined activity constitutes an effective antioxidant defense against the reactivity of ozone with biological macromolecules.

Reaction with lipids

For many years it has been clear that one of the major cellular macromolecular targets for ozone is the phospholipids constituting biomembranes. On the other hand, only now is an understanding of the complexity of these reactions in the biological setting being attained. This research has traditionally focused on stable, lipid-derived metabolites that can be assayed in body fluids of the exposed persons.

Classically, these reactions involve an initial, non-radical reaction of ozone with poly-unsaturated fatty acids (PUFA) across carbon-carbon double bonds, the yield being a 1,2,3-trioxalane which rearranges via carbonyl oxide and aldehyde intermediates to an ozonide via a Criegee reaction. This ozonide then decomposes to yield lipid peroxides or hydroperoxides (35, 36). More recently, however, evidence provided by Pryor (16) has suggested that, although ozonides are likely to break down to lipid peroxides in the lipophilic environment of biomembranes, in the aqueous environment of the airways the ozonides are likely to decompose via hydroxyhydroperoxy intermediates to yield further aldehyde products and hydrogen peroxide, which could then partake in metal-catalyzed Fenton reactions to generate hydroxyl radicals (figure 4).

Further evidence for these later reactions in the interaction of ozone with unsaturated fatty acids comes from the demonstration of hydroperoxy intermediates in the ozonation of unilamellar micelles of phospholipids (37). These studies also clearly demonstrate the ultimate accumulation of hydrogen peroxide, aldehydes, and carboxylates in the *in vitro* test system. These reactions have also been recently shown to occur to arachidonic acid in both subcellular systems and in cultures of pulmonary epithelial cells, the yield being aldehydic substances and hydrogen peroxide (38).

Thus, at least from work with *in vitro* systems, it appears that reactive, lipid-derived ozonides, peroxides and aldehydes, as well as hydrogen peroxide, all potentially play a role in ozone-induced toxicity in biological systems. It is also clear that, as new data become available as to the nature of the reaction products of ozone with lipid membranes, better biological markers of exposure will be defined. Indeed, there is definitely such a need as, hitherto, it has been difficult to detect products of the interaction of ozone with lung lipids *in vivo*. Early studies by Goheen et al (39) failed to show the oxidation of linoleate and arachidonic acid in the lungs of rats exposed to ozone for up to four weeks. However, Rabinowitz & Bassett (40) clearly demonstrated the accumulation of stable carboxylate oxidation products of ozonides and aldehydes derived from tissue oleate and arachidonate in the lungs of rats exposed acutely to ozone for 4 h. Similarly nonenal and heptanal were shown to

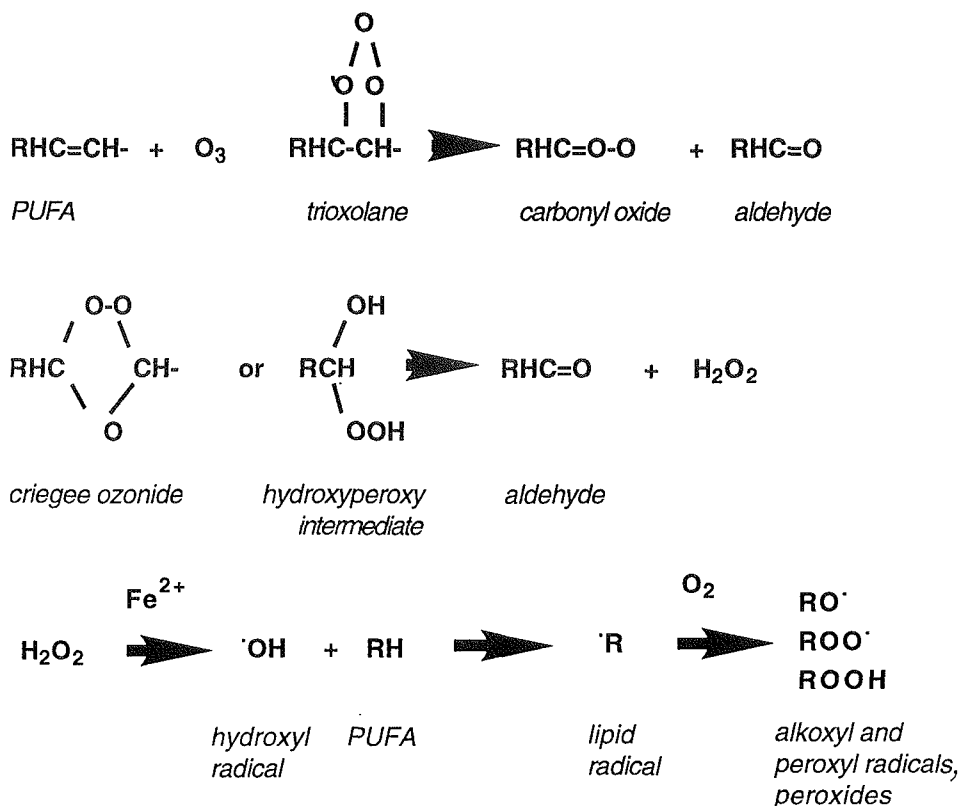


Figure 4. Major products of the interaction of ozone with polyunsaturated fatty acids (PUFA).

accumulate in the bronchoalveolar lavage fluid of animals exposed to ozone for 12 h (41). Other markers of lipid peroxidation, such as the exhalation of pentane and ethane, and the accumulation of malondialdehyde, detected as thiobarbituric acid-reactive substances in body fluids, have failed to provide convincing evidence of rife lipid peroxidation in the lungs in response to the inhalation of ozone (16, 27).

In summary, the results of in vitro experiments indicate that the reaction of ozone with the polyunsaturated fatty acids esterified in the lipids of biological membranes represents one of the major pathways of the reaction of the oxidant with biological macromolecules. Evidence is accumulating to suggest that such reactions, particularly in relation to the induction of lipid peroxidation, play a major role in the acute cytotoxicity of ozone. However, it is still uncertain if such reactions, and the production of lipid ozonides and their breakdown products, play an important role in chronic pathophysiological changes, such as the induction of inflammation, in persons exposed to ozone.

Reaction with proteins

Several amino acids in proteins are targets for direct reactions with ozone, including cysteine, methionine, tryptophane, and tyrosine (figure 5). Oxidation of these amino acids may have a variety of consequences for the

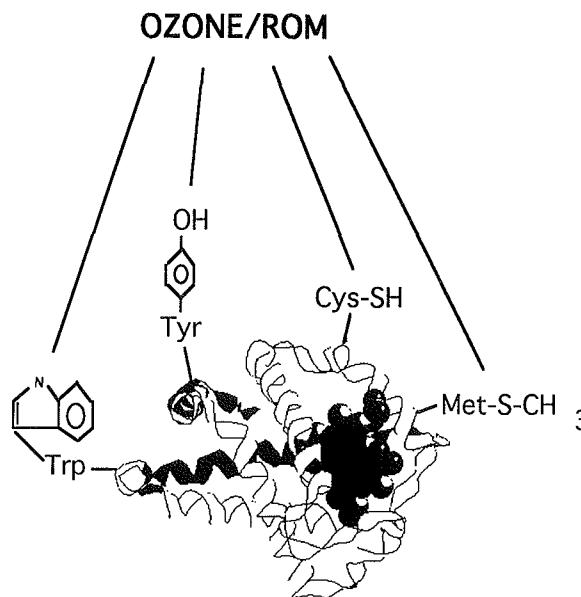


Figure 5. Amino acid targets for the reaction of ozone or reactive oxygen metabolites (ROM) or both with proteins.

function of enzymes, receptors, and other cellular proteins (42).

One of the best studied examples of such oxidation is the effects of ozone on the redox status of cysteinyl

thiols of proteins. Several studies in vivo have shown that the inhalation of ozone causes the oxidation of pulmonary protein thiols via the formation of mixed disulfides with glutathione (43). This reaction is accompanied by the loss of activity of several thiol-dependent enzymes in lung homogenates (44–46). It should be noted that no studies have been performed on the potential of ozone to oxidize protein thiols to higher oxidation states, such as the corresponding sulfeinic, sulfinic, and sulfonic acids. Similarly, although not directly related to thiol oxidation, many oxidant stimuli have been shown to induce the accumulation of protein carbonyls in cells and tissues (47). Such stable oxidation products might also offer promise as biomarkers of exposure, particularly in bronchoalveolar lavage samples.

Although the molecular basis of the interactions of ozone with the amino acids of proteins have, without exception, never been fully clarified, there are many specific examples of such interactions. Thus the acute exposure of animals to ozone over a few hours has been shown to inhibit P_{450} -dependent oxidation of a variety of substrates (48, 49). More recently, many studies with rodents have shown that more chronic exposures to ozone in vivo, over a period of days or weeks, causes the induction of P_{450} and NADPH-cytochrome P_{450} reductase in cytochromes (48, 49). This phenomenon causes a general elevation in the ability of the lung to metabolize xenobiotics, including airborne carcinogens. In addition, some of the mitochondrial enzymes that are coupled to oxidative phosphorylation have been shown to be inhibited in the lung by acute exposure to ozone in vivo (52, 53). The activities of other enzyme systems affected by the exposure to ozone include prostaglandin synthetase (54) and cyclooxygenase (55), which are important in the generation of important prostaglandin and leukotriene mediators involved in the induction and maintenance of inflammation. Finally, ozone has been shown to alter readily the structure and function of rodent pulmonary surfactant protein A in vivo (56), presumably by an oxidative mechanism involving the oxidation of critical methionine and tryptophane residues in the protein through the mediation of ozone or hydrogen peroxide (57).

In summary, although model studies with purified proteins clearly indicate that ozone reacts with several chemically reactive groups in the constitutive amino acids of a wide variety of proteins, it should be noted that there have been only few reports on the effect of exposure to ozone on human pulmonary proteins, particularly during the inhalation of the gas. Thus the only clear example is the fact that ozone effectively oxidizes and inhibits the human α -1-anti-trypsinase inhibitor (58). Clearly, further work is indicated to determine the extent of the chemical interaction of ozone with other human pulmonary proteins.

Reactions with nucleic acids

Many studies have suggested that ozone is genotoxic, possessing both mutagenicity and carcinogenicity in mammalian systems (59). It should be remembered, however, that genotoxicity can also be envisaged in terms of altered regulation of the expression of genetic information (ie, in terms of altered "gene dosage").

By definition, one of the central components in these processes is the ability of ozone to interact with DNA. Early studies with purine and pyrimidine bases, nucleotides and nucleotides, as well as with nucleic acids, all showed that ozone is capable of directly degrading these important materials (60). Since then, many studies have shown that ozone, or ozone-derived hydroxyl radicals, can oxidize and disturb the complex nature of nucleic acids at a variety of levels.

Thus ozonation of the double-stranded plasmid DNA was unequivocally shown to cause single- and double-strand breaks, as well as a loss of structural integrity of the plasmid by linearization, with concomitant loss of transforming action (61, 62). Similarly, in intact cells, single-strand breaks, interstrand cross-links and cross-links between DNA and protein have all been noted in response to ozone exposure in vitro (figure 6) (63). It should be remembered, however, that these experiments are often performed at irrelevantly high concentrations of ozone and that the systems often negate any effect of the normal cellular DNA repair mechanisms. Similarly, little attention has been paid to the potential interactions of ozone with RNA (ribonucleic acid) species (figure 6).

Thus ozone or reactive oxygen metabolites can cause a variety of alterations in the structure of DNA, including, from left to right in figure 6, double-strand breaks, sugar peroxidation, base hydroxylation, single-strand breaks, and protein-base cross-links.

Many questions remain to be answered as to the mechanisms and consequences of the interaction of ozone with cellular nucleic acids. From the point of view of potential biomarkers of exposure, further studies are required to determine the chemical nature of the interaction of ozone with nucleic acids. It would be of particular interest to see if the exposure of DNA to ozone in chemical, cellular, and in vivo systems induces the accumulation of hydroxylated DNA bases and products of sugar oxidation in the system, as has been proposed for DNA damage mediated through the hydroxyl radical (64). Thus the urinary excretion of 8-OH guanosine has been proposed as a marker of whole-body oxidative stress in humans (65).

It is, at present, unclear whether the interaction of ozone with DNA demonstrates some specificity. It remains to be determined, for instance, if the patterns of mutation induced by ozone in cellular DNA are entirely random.

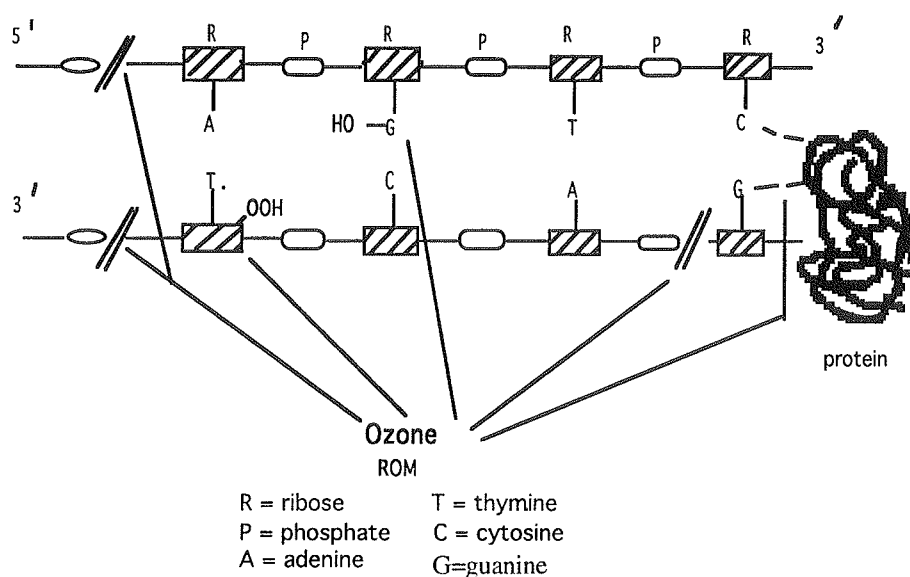


Figure 6. Various molecular targets for the interaction of ozone or reactive oxygen metabolites (ROM) with DNA (deoxyribonucleic acid).

In summary, nucleic acids present attractive sites of reaction with ozone. Current knowledge of such interactions is largely limited to DNA-damaging effects at unrealistically high concentrations in *in vitro* systems. See the section on genotoxicity and carcinogenicity in this issue (66). However, in view of the mutagenic potential of ozone and its debated ability to induce cancer, the ability of ozone to react with cellular nucleic acids, such as DNA, must receive more attention in future studies.

Ozone-induced alterations to macromolecule structure and function and their influence on biochemical processes

In this section selected examples are used to illustrate the serious perturbations in integrated biochemical processes due to the reactivity of ozone with, and the alteration of the structure of, lipids, proteins and nucleic acids. It is hoped that this section will provide a tangible bridge from events at the molecular level to the expression of alterations in the cellular phenotypes, cytotoxicity, and genotoxicity, which have been detected in the lung morphologically, cytologically, and histologically after the inhalation of ozone.

Lipid-dependent processes

Two consequences of the oxidation of phospholipids on the biochemical functionality of membranes can be considered. Experiments using isolated perfused rat lung obtained from animals exposed to ozone over a period of 3 d clearly demonstrated increased synthesis of free fatty acids, phosphatidic acid, phosphatidyl choline, and sphin-

gosine-containing lipids in response to the gas. This phenomenon was interpreted as an increased requirement for membrane synthesis during the proliferative repair response, in particular in the epithelium of the airways (67). Similarly, other changes in the phospholipid composition of pulmonary membranes have been reported, including decreases in the relative proportions of oleic and linoleic acids and increases in arachidonic acid esterified into phospholipids (68). If these compositional changes are coupled to the continuous degradative pressure elicited by the nonradical and radical-dependent attack on unsaturated fatty acids leading to lipid peroxidation, it is clear that the fundamental fluidity and permeability characteristics of cell membranes is affected in the lung.

These changes would constitute important components of the alterations in transepithelial permeability, which is characteristic of the inflammatory state instigated by ozone in the lung (69, 70). Thus changes in membrane permeability and fluidity have been detected in alveolar macrophages obtained from ozone-exposed animals (71, 72). Short-term exposure to ozone *in vivo* has even been shown to affect the integrity of intracellular membranes such as those present in the mitochondria of pulmonary cells (53). In addition, ozone has been shown to increase the permeability of macromolecules in either direction between the interstitium and the airways. Thus ozone exposure increases the albumin and immunoglobulin G (IgG) content of the bronchoalveolar lavage fluid of exposed animals (73). Similarly, ozone exposure increases the uptake of instilled neutral red from the airways into the pulmonary interstitium and circulation (74).

As has already been mentioned, short- and long-term exposure to ozone instigates changes in membrane ara-

chidonate metabolism by both stimulating lipid peroxidation and changing the relative proportion of esterified arachidonic acid in membranes. These alterations to this particular component of biological membranes have important ramifications for many biochemical processes, particularly those involved in inflammation. Thus arachidonic acid metabolites, particularly those generated by 5'-lipoxygenase (leukotrienes and thromboxanes) and cyclooxygenase (prostaglandins), have been implicated in changes in airway permeability, in stimulating the infiltration, or chemotaxis, of granulocytes and lymphocytes into the lung, and in stimulating smooth muscle hyperactivity in airways.

Many studies have shown that lung cells, particularly alveolar macrophages, release elevated amounts of eicosanoids, including PGE₂ (prostaglandin E₂) (75, 76) and PGF_{2α} (prostaglandin F_{2α}) (75), thromboxane B₂, 15-HETE, LTB₄ (leukotriene B₄) and LTD₄ (leukotriene D₄) when exposed to ozone in vitro or in vivo (76). Similar effects have also been demonstrated in vitro in epithelial cells of airways (77). These results have been interpreted both in terms of an increased availability of arachidonic acid through lipid peroxidation or specific, oxidant-stimulated release mechanisms and stimulation of the synthesis of the relevant enzymes themselves. Similarly, eicosanoids, particularly various prostaglandins, have been shown to be elevated in the bronchoalveolar lavage fluid (78, 79) and plasma (80, 81) of ozone-exposed animals. It should be noted, however, that there have been few studies attempting to test the effect of ozone on the release of eicosanoids from either human alveolar macrophages or bronchial epithelial cells and their potential accumulation in bronchoalveolar lavage fluid. See the section on animal studies of ozone toxicity (82).

In summary it is clear that ozone interacts with lipid components of biological systems and generally results in stimulated degradation of membrane integrity. This process has both structural and functional implications for the viability of affected cells. Despite the ample evidence from in vitro experiments that uncontrolled lipid peroxidation is one of the primary effects of the interaction of ozone with biomembranes, evidence for this in vivo is not conclusive. However, more subtle effects (eg, the release of arachidonic acid metabolites), which have been amply described in animal studies, may be more relevant to the mechanism of the toxicity of the gas in humans. Nevertheless, it is not currently positive whether stimulated eicosanoid metabolism plays a role in ozone-induced pulmonary toxicity in humans.

Protein-dependent processes

It is difficult to present one single, representative example of how ozone-induced damage to protein affects a specific biochemical process that may be critical in the expression of the toxicity of the gas. Proteins are in-

involved in most processes in cells and, as has already been shown, it is clear that ozone, and the reactive species generated from it, oxidizes many functional groups of proteins and results in potential changes in function. It is perhaps because of this potential plethora of simultaneous events that little work has been done to identify potentially critical protein ozone "targets," which might then be useful in explaining the molecular mechanism of ozone toxicity.

In addition to causing disturbances in lung surfactant lipid structure, ozone also causes the oxidation of the surfactant protein A. This collagen-like protein constitutes 3% of the surfactant and is thought to have a variety of functions, including control of transepithelial absorption and macromolecule secretion, particularly of phospholipid components of the surfactant. The protein also stimulates various macrophage functions, including phagocytosis and the release of oxidants (83). Ozone alters the physicochemical properties of the molecule and causes an impairment of self-association and association with phospholipid vesicles (56), as well as an impaired ability to interact with alveolar macrophages (57). These effects can thus contribute to an impaired functional integrity of the protective surfactant layer of the lung. It remains to be seen if this phenomenon allows for an increased penetration of ozone and other airborne materials into the pulmonary epithelium.

One of the only other groups of human proteins which have been studied is the tear proteins secreted by lachrymal glands into tear fluid. Schmut et al (84) showed that the exposure of human tears from healthy volunteers to ozone in vitro caused rapid and extensive degradation of the tear proteins. This phenomenon may present ramifications for the maintenance of ocular function and the occurrence of eye infections during exposure to the gas.

The acute exposure of animals to ozone has been shown to affect mitochondrial function in pulmonary tissues. Thus the coupling of mitochondrial oxidative phosphorylation has been found to be reduced dramatically in pulmonary mitochondria after acute ozone exposure in vivo (52, 53). The authors proposed that this phenomenon was due to both stimulated lipid peroxidation in the inner and outer mitochondrial membranes and the oxidation of pyridine nucleotides and mitochondrial thiols. It is well established that many of the critical proteins involved in the generation of adenosine-5'-triphosphate via oxidative phosphorylation are thiol-containing and thus are sensitive to redox changes (85). Although these early studies were never followed up, particularly with respect to the identification of the proteins targeted by ozone, the example clearly illustrates how protein modification induced by ozone can seriously compromise a pivotal biochemical process supplying energy equivalents to many other cellular processes.

One area of protein research that has been totally neglected is the possibility that ozone directly or indirectly alters the redox status of receptors or signal transduction systems in pulmonary cells. A growing number of protein receptors has been shown to contain critical thiol groups whose redox status controls the reception and transduction of extracellular hormonal, growth factor, and other stimuli (86).

In addition to the oxidative modifications of proteins already presented, there is a strong possibility that aldehydic products derived from lipid peroxidation induced by ozone or reactive oxygen metabolites can interact with cellular proteins. For instance, it is well known that certain aldehydes interact directly with irritant receptors in the airways once inhaled. These reactions lead to bronchoconstriction, which may help to facilitate the development of inflammation in the airways.

In summary, the multiplicity of potential sites of reaction between ozone and proteins, coupled to the variation in cellular protein structure and the intricacy of their functional integration into biochemical processes, indicates the potential for ozone-induced alterations to protein structure and function. With the increasing availability of molecular biological and immunologic tools, it should prove possible to detect ozone-induced oxidation of proteins with increasing sensitivity and selectivity and clarify the role of such changes in the pulmonary toxicity of the gas.

DNA-dependent processes

It is clear that ozone causes alterations in the expression of normal genetic programming and ultimately leads to cell transformation. See the section on mutagenicity and carcinogenicity of ozone in this issue (66). By definition, changes in the structure of genomic DNA or the expression of genetic information must underlie these processes to a great extent. Despite the demonstration of mutagenicity for ozone (59), there are no studies reported in the literature defining whether the pattern of ozone-induced mutations is random or whether some selectivity is expressed. In addition, there have been few attempts to determine ozone-induced mutations in genes relevant to the control of cell cycling and differentiation (ie, protooncogenes and tumor suppressor genes). However, a very recent observation indicates that life-time exposure of BC63f1 mice to ozone significantly increases the frequency mutation of the K-ras protooncogene in lung neoplasms (87).

In addition to relying on a mutational basis, DNA-dependent toxicity can also be defined in terms of altered levels of expression for native genes and thus it alters normal patterns of gene "dosage." A few studies have shown the induction of the expression of specific cellular genes in response to exposure to ozone. For instance, the levels of transcription of antioxidant enzymes such as

glutathione peroxidase, catalase and superoxide dismutase (32), various cytochromes P₄₅₀ (51), a variety of pulmonary extracellular matrix proteins, including fibronectin (88) and collagen (89), various macrophage proinflammatory proteins (90, 91), and an, as yet, uncharacterized gene coding for a 45KDa substrate from bronchial epithelial cells (92) are all altered in response to ozone.

It is uncertain whether ozone directly interacts with the respective genes to change the rate and extent of expression or whether the interaction occurs at some other level of the transcriptional or translational regulation. For instance, it is becoming increasingly clear that the activity of many mammalian transcription factors, proteins which bind to specific regions of promoters upstream from the coding region of genes, may be regulated by redox changes to the proteins themselves (93). In addition, it may be that metabolites derived from the interaction of ozone with biomolecules are involved in the execution of these induction events.

In summary, it is becoming clear that many of the phenotypic changes seen within different cell populations in the lung in response to the inhalation of ozone are, in the long-term, controlled by stimulated alterations to gene expression. Irrespective of the mechanisms involved, further work may well identify specific gene inductions that may provide sensitive biomarkers for early exposure to ozone. In this respect, analysis of the transcription rates of various proinflammatory cytokines, such as tumor necrosis factor (TNF)- α , interleukin-1 (IL-1), IL-6 and IL-8 in cells of bronchoalveolar lavage or peripheral blood might provide useful tools. Indeed, recent studies of both human pulmonary epithelial cells and macrophages exposed to ozone *in vitro* have shown induction of the expression of all of the aforementioned inflammatory cytokines from macrophages (94, 95), while others have suggested that epithelial cells may be the primary source of ozone-stimulated IL-6 and IL-8 release in the lung (94).

Summary and concluding remarks

It is clear that the chemical reactivity of ozone and its rapid degradation to reactive oxygen metabolites, such as the hydroxyl radical, at biologically relevant pHs, facilitate a reaction with most classes of biological macromolecules. Similarly, this reactivity, in all probability, restricts the existence of ozone itself to the lumen of the lung after the gas is inhaled. These concepts present serious problems with respect to efforts to define the critical events leading to the pulmonary toxicity of ozone at the molecular level. Many reactions are possible. However, it is clear that reactions with polyunsaturated fatty

acids in biological membranes, the induction of lipid peroxidation, and the release of biologically active metabolites such as arachidonic acid may play a role in both the acute cytolytic toxicity of the gas and the development of chronic toxicity, such as the induction of inflammation. On the other hand, less is known of the potential importance of the interaction of ozone with other cellular macromolecules, particularly with respect to the oxidation of proteins and DNA and the consequences of these events for the long-term maintenance of cellular phenotype by adaptation within the lung.

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Ozone-induced toxicity in experimental animals and isolated cell systems

by Lars E Gustafsson & Ian Cotgreave

The scope of this section is extensively covered by the document Air Quality Criteria for Ozone and Related Photochemical Oxidants published by the Environmental Protection Agency (EPA) in the United States (1). The literature on animal experiments on exposure to ozone is vast, and it was felt not within the scope of this report to make a full review. Instead, emphasis has been put on literature covering principles and on more recent literature, especially that dealing with the mechanisms underlying various effects of ozone, such as proinflammatory effects, effects of low concentrations in vivo, and effects on primates.

Effects on respiratory system morphology

Changes in lung morphology constitute a very early sign of the effects of ozone, but the dose required to elicit such changes varies between species, and also within species. An important issue is how animal data can be related to the effects of ozone on humans. Many studies have been performed on rodents. The modeling of data obtained from studies on rodents suggests that existing anatomical differences within primates would cause the rodents to be more sensitive to damaging effects of the gas in the distal airways and alveoli (2). Therefore, studies on nonhuman primates are of importance. Earlier studies have used rather high concentrations of ozone and have put considerable emphasis on effects on alveolar cells. Recent data indicate, however, that bronchiolar cells might be the most sensitive cell group. An important advancement in striving to reduce variability in results is the systematic microdissection of identical airway segments between different animals and the use of double-blind and automated techniques for morphological evaluations (2).

Affected cells and sites

The most striking effects of ozone occur in the proximal alveolar region and in the most distal parts of the airways, the respiratory bronchioles.

Effects on lung morphology in primates

When exposed to an ozone concentration of $300 \mu\text{g} \cdot \text{m}^{-3}$, 8 h a day for 6 or 90 d, the Macaque monkey exhibits

significant nasal epithelial lesions with ciliated cell necrosis, diminished cilia number, and hyperplasia of Clara cells (3).

When the Macaque monkey was exposed to 300 or $600 \mu\text{g} \cdot \text{m}^{-3}$ for 8 h a day for 6 or 90 d, hyperplasia and hypertrophy of nonciliated cuboidal epithelial cells were observed in terminal and respiratory bronchioles (4). The effect was fully developed already at the lowest dose rate, since there was no further increase with increased ozone concentration or exposure time. Intraluminal accumulation of macrophages was also seen in the respiratory bronchioles. The validity of the results was underlined by the use of systematic microdissection and the identification of matching bronchiolar segments in control and exposed animals.

Effects on morphology in other species

A study employing identical concentration-versus-time exposures to ozone and to the combination of ozone and nitrogen dioxide has been reported for rats (5). The cell proliferation in larger, as well as peripheral, airways was monitored after bromo-deoxy-uridine had been incorporated into airway cells, which were continuously infused subcutaneously by miniosmotic pumps. Alveolar cell labeling was unaltered by 400 and $1600 \mu\text{g} \cdot \text{m}^{-3}$ of ozone, whereas a marked effect was seen in the bronchiolar cells. The large airway cells showed increased labeling only at the highest dose rates. There was a clear synergy with nitrogen dioxide for the effect on airway epithelium. Hypertrophy and hyperplasia were verified by electron microscopy.

In both juvenile and adult rats, epithelial alterations were observed after exposure to an ozone concentration of $500 \mu\text{g} \cdot \text{m}^{-3}$ for 12 h a day for six weeks. The lesions were localized to the proximal parts of the most peripheral parts of the lungs, namely, in the proximal alveolar region (6). A decrease in the surface area of type I epithelial cells of alveoli was noted; the cells also doubled in number and increased in thickness. In addition, the number of type II cells and alveolar macrophages increased. No age-related difference in sensitivity was seen. In addition, adult animals showed an increased number of tissue macrophages, a clear sign of inflammation. Adult animals were also exposed to $240 \mu\text{g} \cdot \text{m}^{-3}$ and showed significant changes in the number and size of alveolar type I cells already at this exposure level. The

findings are consistent with a dose dependently increased turnover rate of alveolar cells and show that the effect occurs already at $240 \mu\text{g} \cdot \text{m}^{-3}$.

Rats (Jcl:Wistar, male, 8 weeks old) exposed to an ozone concentration of $800 \mu\text{g} \cdot \text{m}^{-3}$ continuously for 14 d showed a 214% increase in lung cytochrome P₄₅₀ 2B1, the major pulmonary P₄₅₀ enzyme in rats (7). A hypertrophy of Clara cells, the unciliated cuboidal cells of terminal bronchioles, was seen in histological sections. The Clara stained immunologically positive for P₄₅₀ 2B1. The appearance of a cell type intermediate in morphology between types I and II alveolar cells was also seen in proximal alveolar regions, and these cells also expressed P₄₅₀ 2B1. The data suggest that ozone exposure may activate P₄₅₀-mediated xenobiotic metabolism in pulmonary epithelial cells and that ozone exposure leads to alveolar cell metaplasia.

At higher ozone concentrations (1400 to $6000 \mu\text{g} \cdot \text{m}^{-3}$) several studies have shown effects on alveolar cells, with a decrease in the number of the flat type I cells and hyperplasia of type II (lamellar bodies containing) cells clearly evident. The lamellar bodies were shown to be uneven in density, as well as undergoing hypersecretion into the alveoli, suggesting increased secretion of surfactant.

In the rat, bronchiolar epithelium exhibits changes when exposed to inhaled ozone at $500 \mu\text{g} \cdot \text{m}^{-3}$ (8). The effects included loss of surface area contributed by cilia, a decrease in the luminal surface area contributed by Clara cells, and a decrease in the number of brush cells per square millimeter of bronchiolar basement membrane.

When rats were exposed to $1900 \mu\text{g} \cdot \text{m}^{-3}$ of ozone for 3 d, ciliary damage and necrosis occurred in the trachea, with a concomitant increased density of intermediate cells and an increased thymidine labeling index (9). After 60 d of exposure the only remaining effect was shortened cilia of the ciliated cells. At 42 d postexposure no effect remained.

Another study (10) showed that cell renewal takes place at an increased rate, as indicated by thymidine incorporation, in rats exposed to 700 or $1000 \mu\text{g} \cdot \text{m}^{-3}$ of ozone (30-day-old male Sprague-Dawley rats exposed continuously up to 8 d). The increase in thymidine incorporation occurred during the first 2 d of exposure and returned to base line at day 4. This first "wave" of increase was due to thymidine incorporation in all types of alveolar cells. Animals which received an increase in ozone exposure from 700 to 1000 or $1400 \mu\text{g} \cdot \text{m}^{-3}$ from day 4 showed a second "wave" of increase in thymidine incorporation. The second increase was mainly due to thymidine incorporation into type II alveolar cells. This finding shows that, despite the initial adaptation, a new response can be evoked by a second "wave" of ozone if the concentration is higher.

The effects of long-term ozone exposure on pulmonary morphology has been studied in rats and mice in conjunction with the NTP study on the potential carcinogenesis of ozone (11). Rats were exposed for two years to ozone at 240 , 1000 or $2000 \mu\text{g} \cdot \text{m}^{-3}$ for 6 h a day, 5 d a week for 104 weeks. No effects on alveolar cell morphology were seen in control animals. The animals exposed to $240 \mu\text{g} \cdot \text{m}^{-3}$, both males and females, exhibited minimal, but significant, alveolar cell metaplasia. The changes were further enhanced at higher concentrations of ozone. Mild, but significant infiltration of histiocytes (macrophages) into alveoli and interstitial fibrosis were noted at 1000 and $2000 \mu\text{g} \cdot \text{m}^{-3}$.

Comparison between ozone and oxygen toxicity

Detrimental effects of ozone on alveolar cells occur at $200 \mu\text{g} \cdot \text{m}^{-3}$, which is only slightly above current ambient background levels (12). Another gas which is toxic at concentrations only slightly above background levels is oxygen. It is well known that prolonged exposure to twice the normal concentration of oxygen in inspired air causes pulmonary injury with functional impairment in humans (13). Exposure of primates (baboons) to 60% oxygen (ie, three times the normal concentration) for two weeks with subsequent eight weeks of recovery led to significant morphological changes (14). Changes noted directly after exposure included increases in type II alveolar cells and macrophages and signs of interstitial fibrosis. The fibrosis progressed further during the eight-week recovery period. These alveolar changes are similar to those observed in Macaque monkeys exposed to ozone, as has already been described. If it is assumed that the defense mechanisms against oxygen and ozone are similar, the effects of low concentrations of ozone on morphology in animals are, therefore, likely to have a high degree of relevance for humans, for whom similar studies cannot be performed for ethical reasons.

Summary

Alveolar macrophages proliferate in rats exposed to an ozone concentration of $200 \mu\text{g} \cdot \text{m}^{-3}$ of ozone. Effects on bronchiolar cells also occur at the lowest ozone level tested, $200 \mu\text{g} \cdot \text{m}^{-3}$, and these effects indicate both a growth of and an increased number of epithelial cells. This response was still seen 4 d after the cessation of a 3-d exposure, and it was thus not a transient response. Macrophages proliferate and changes occur in bronchiolar cells, with consequent overgrowth into alveoli, also in primates, and these effects might have implications both as to an understanding of the mechanisms behind direct ozone lesions in the lung and an understanding of indirect effects such as increased sensitization to allergens and hypersensitivity to bronchoconstrictor agents. Thus an increased number of macrophages, which is seen both in the alveoli and in the

interstitial tissue, increases the possibilities for these cells to adsorb allergens and present them to cells of the immune system. This phenomenon facilitates an increase in the development of antibodies.

The morphological changes and biochemical measurements performed provide evidence of increased rates of cell division, and this increase may be important in that it increases the possibilities for a malignant transformation of lung cells. Oxygen, another gaseous oxidant, is toxic to animal and human lung already at twice the normal ambient level. Considering the similarity between the defense against the mechanism of action and ozone and oxygen effects, the toxicity of ozone in animals at levels only slightly above ambient levels might be relevant to the estimation of toxicity in humans. Indeed, morphological changes are seen in monkeys exposed for more than 60 d at ozone levels near the present ambient concentrations. Likewise, in mice subjected to life-long ozone exposure, morphological changes and fibrosis are present in increased frequency in the exposed groups. The fibrosis also remains despite the cessation of ozone exposure. Therefore, the morphological changes must be regarded as a nontransient, long-term effect.

Host defense mechanisms

Host defense mechanisms of the lung are directed either at transporting foreign material out of the lungs, or at inactivating the offending material at the place of intrusion. The elimination from alveoli is mainly accomplished through the action of alveolar macrophages, or through diffusion or penetration of matter into the lung parenchyma, where recruited macrophages and other inflammatory cells engulf it. Above the alveolus the bronchial mucociliary transport mechanisms are of major importance, and the concerted actions of cilia and the secreted mucous lining fluid are necessary for transport. These mechanisms are nonspecific, whereas specific immunological mechanisms can be activated both in the parenchyma and in bronchi with the emergence of inflammatory cells and mediator and cytokine release. The response may involve both the action of preformed antibodies and a long-term activation of antibody formation.

Mucociliary clearance

For normal mucociliary clearance it is necessary that normal mucous production occurs, that ciliary cells function normally, and that mucus membrane function and lung morphology are normal (15, 16). Ozone inhalation can result in increased mucous secretion, but, if other pulmonary functions are disturbed, for example, by smooth muscle contraction or inflammation with

consequent invasion of the mucosum by white blood cells, the net effect may be negative for a person with, for example, an accumulation of mucus in the airways (13). The numbers of ciliated cells in the terminal bronchioli of rats are decreased by the inhalation of ozone at a concentration of $500 \mu\text{g} \cdot \text{m}^{-3}$ for 12 h a day for six weeks (7). The surface area contributed by ciliated cells in the bronchioli is also diminished. These effects are equivalent in adult and newborn rats. Such effects on morphology might impair ciliary transport mechanisms in a long-term perspective, despite having a short-term stimulatory influence.

In hamsters, *in vivo* exposure to the gas for 3 h at $200 \mu\text{g} \cdot \text{m}^{-3}$ does not alter ciliary beating frequency when assessed postexposure *in vitro* (17). However, the inhalation of combinations of ozone and sulfuric acid, or ozone and nitrogen dioxide, have been found to inhibit ciliary beating and mucociliary transport (15). In rabbits, exposure to $1200 \mu\text{g} \cdot \text{m}^{-3}$ for 2 h causes retarded particle clearance (18). In rats, combined exposure to $120 \mu\text{g} \cdot \text{m}^{-3}$ for 13 h a day, in combination with spikes of $500 \mu\text{g} \cdot \text{m}^{-3}$ for 9 h a day, for 5 d a week with a total duration of six weeks causes the pulmonary retention of asbestos fibers to increase (19). Increased and decreased clearance have been found in rabbits, the former in exposures to 200 and $1200 \mu\text{g} \cdot \text{m}^{-3}$ and the latter at $2400 \mu\text{g} \cdot \text{m}^{-3}$ (20). It is possible that the variation in response type is due to the presence or absence of effects on glycoprotein secretion (1). Glycoprotein is a component of mucus, and its increase is a sign of increased mucous secretion. The increased secretion would be expected to carry foreign material out of the airways more rapidly.

Thus the most sensitive effects of ozone on host defense are the stimulation of clearance at $200 \mu\text{g} \cdot \text{m}^{-3}$ in rabbits and the inhibition of clearance at $500 \mu\text{g} \cdot \text{m}^{-3}$ in rats.

Alveolar macrophages

Alveolar macrophages increase in number, decrease in their ability for phagocytosis, decrease bactericidal activity, and diminish superoxide production upon exposure to ozone. The concentration dependency of these effects varies (1). In general effects can occur at $200 \mu\text{g} \cdot \text{m}^{-3}$ and seem well established at $500 \mu\text{g} \cdot \text{m}^{-3}$. A recent *in vivo* study in rats showed that a one-week exposure to $200 \mu\text{g} \cdot \text{m}^{-3}$ increased alveolar macrophage numbers, as well as macrophage enzyme activities in rats (21). Of importance for other effects of the gas might be that the mitotic index of macrophages is increased at $540 \mu\text{g} \cdot \text{m}^{-3}$ (12) and at $1600 \mu\text{g} \cdot \text{m}^{-3}$ (18, 22). The number of lavageable macrophages increases at concentrations from 200 to $2000 \mu\text{g} \cdot \text{m}^{-3}$, but can be unaffected or even decreased at concentrations ranging from $1600 \mu\text{g} \cdot \text{m}^{-3}$ up, perhaps as a sign of more drastic cytotoxicity at the higher con-

centrations. This is of importance for choosing and evaluating exposure concentrations.

Immunologic mechanisms

The effect of short-term ozone exposure on antibody response and on the thymus was investigated in mice (23). Mice were continuously exposed to an ozone concentration of $800 \mu\text{g} \cdot \text{m}^{-3}$ and $1600 \mu\text{g} \cdot \text{m}^{-3}$ for 1, 3, 7 or 14 d. Already from day 1 and throughout the exposure, both concentrations caused an increase in relative lung weight per body weight, and from day 3 and on there was a significant decrease in relative thymus weight. In addition, spleen weights were depressed at all time points by the higher concentration of ozone, whereas at $784 \mu\text{g} \cdot \text{m}^{-3}$ there was a drop only on day 14. The ability to develop an antibody response to sheep red blood cells was also reduced by the higher concentration of ozone, and this effect was seen already after the first day of exposure. Thus an immunodepressant effect of ozone exposure was found in parallel with the induction of an inflammatory response (edema and weight increase) in the lungs. The authors concluded that the impairment was due to a suppressed T-cell response, since the response to other antigens, independent of T-cells, was normal.

The action of ozone on the thymus and on pulmonary lymph nodes has been investigated in mice during acute and subchronic inhalation (24). Female mice (CD-1) were exposed to $1400 \mu\text{g} \cdot \text{m}^{-3}$ for 20 h a day for 1 to 28 d. The thymus showed an atrophic response, whereas the mediastinal lymph nodes showed a hyperplastic response, with the presence of blastic cell forms. The atrophy in the thymus was especially seen in the cortical regions, and it was markedly reversed if the adrenal glands had been removed before ozone exposure. This finding might suggest that steroid hormones, or some other factor derived from the adrenals, are involved in the ozone-induced depressant effect on the thymus. The removal of the thymus reduced the hyperplastic response caused in the mediastinal lymph nodes in response to ozone exposure. The lungs showed signs of oxidant injury, with terminal bronchiolitis and alveolitis evident and with the infiltration of white blood cells, septal thickening, type I cell damage, type II cell proliferation, and macrophage accumulation. It seems likely that mediators from the lungs caused the changes in lymph node and thymus morphology. These results substantiate that exposure to relatively high concentrations of ozone can have an immunomodulatory effect.

In a follow-up study, athymic mice were exposed to an ozone concentration of $1400 \mu\text{g} \cdot \text{m}^{-3}$ for 20 h a day for 7 or 14 d (25). The athymic mice showed more marked lung damage than the control mice did, with more pronounced increases in lung weight and more marked peripheral damage to alveolar ducts and alveoli,

which exhibited a more-pronounced inflammatory reaction. The results support the hypothesis that the mediastinal lymph node reaction seen in normal animals is part of a response aimed at minimizing lung damage.

In female CD-1 mice exposed to an ozone concentration of $200 \mu\text{g} \cdot \text{m}^{-3}$ for 5 h a day, 5 d a week for 103 d, splenic T-lymphocyte function, measured as the response to mitogens and alloantigens, was reduced (26). In addition the *in vitro* bacteriostatic action of peritoneal macrophages was enhanced. However, the mortality of mice infected with group C streptococci was enhanced, despite an increased bactericidal effect on pulmonary alveolar macrophages. Co-exposure with sulfur dioxide or ammonium sulfate aerosol did not increase the effect above that of ozone, but it reversed the effect on splenic lymphocytes.

By use of immunofluorescence against T- and B-lymphocyte markers it was possible to demonstrate an increased T-cell infiltration in lungs from mice exposed to ozone at $1400 \mu\text{g} \cdot \text{m}^{-3}$ for 20 h daily for 4 or 14 d (27). Furthermore, B lymphocytes were decreased in number in the lung parenchyma. The lungs of the mice were significantly increased in weight at both days 4 and 14, whereas spleen and thymus showed decreased weights at day 4, an effect normalized by day 14.

In the NTP study with long-term ozone inhalation (11) no changes in thymus morphology were seen after either two-year or life-long exposure to ozone in either rats or mice. However, in this study a four-week inhalation of $2000 \mu\text{g} \cdot \text{m}^{-3}$ caused a significant decline in thymus weight in female rats. This effect was absent in male rats or in mice of either sex.

Interactions between infectious agents

A potentiating interaction with infectious agents is one of the most sensitive effects of ozone on animals. Effects are seen from $200 \mu\text{g} \cdot \text{m}^{-3}$ in mice, female mice being more sensitive than males. Despite an ozone-induced increased bactericidal effect on alveolar macrophages at $200 \mu\text{g} \cdot \text{m}^{-3}$, exposed female animals infected with group C streptococci showed an increased mortality (23). A detailed account of the interaction of ozone with infectious agents can be found in a document of the EPA in the United States (1).

Effects on some cellular responses in vitro

Arachidonic acid was found to react with ozone *in vitro* to form aldehydic derivatives (28) when the arachidonic acid was exposed to an ozone concentration of 200 or $2000 \mu\text{g} \cdot \text{m}^{-3}$ for 30 min. When such incubates were given to natural killer (NK) cells, the cells exhibited decreased cytotoxicity against K562 target cells. In addition, a human bronchial epithelial cell line was found to release arachidonic acid-derived aldehydic substances when exposed to 200 or $2000 \mu\text{g} \cdot \text{m}^{-3}$. The results

indicate that a specific mechanism of arachidonic acid metabolism involving aldehyde generation, rather than general lipid peroxidation, might function in bronchial epithelial cells and that this phenomenon may exert inhibitory effects on NK cell cytotoxicity and T-cell proliferation.

With the use of a culture technique in which the basolateral side of cells is exposed to medium while the top (apical part) of the cells is kept moist, human airway epithelial cells and alveolar macrophages were exposed to ozone in vitro (29). The primary epithelial cultures were exhibited after 1 h of exposure to an ozone level of $200 \mu\text{g} \cdot \text{m}^{-3}$ and macrophages already at 30 min. The human bronchial epithelial cell-line BEAS excreted increased amounts of interleukins 6 and 8 (IL-6, IL-8) and fibronectin upon ozone exposure, whereas the macrophages did not. The IL-6 excretion from the BEAS cells was directed only from the apical surface at 4 h of exposure, whereas by 24 h a secretion in both directions was at hand. The results suggest that epithelial cells may have a preferential role in the secretion of cytokines upon ozone exposure and that the secretion is stimulated at low ozone concentrations.

Summary

Ozone exerts immunodepressant effects on mice in that the thymus may undergo hypoplastic changes. As another, possibly secondary, effect the formation of antibodies from lines derived from B cells may be deranged. The effects occur from $800 \mu\text{g} \cdot \text{m}^{-3}$ or with 3 d of continuous exposure. After exposure to $200 \mu\text{g} \cdot \text{m}^{-3}$ for 5 h a day for 130 d in vivo, immune-competent cells from mice show abnormal immunologic responses when tested ex vivo. The cytokine-releasing effects of ozone on airway epithelial cells may be an important step in the further activation of the airway response to ozone. If so, it might explain some of the local proliferative effects on the cells and the immigration of inflammatory cells into the airways. (See the later section on inflammation.) The effect occurs at $200 \mu\text{g} \cdot \text{m}^{-3}$ already after 1 h of exposure. It is not possible to convert the in vitro exposure level directly to an inhaled concentration of the gas in vivo. It is noteworthy that the in vitro effects are observed at ozone concentrations which seem highly relevant for those effective in in vivo studies. The in vitro findings are important confirmatory observations of results obtained in vivo and may offer important mechanistic explanations for effects observed in morphological studies.

Sensitization to allergens

An important aspect of ozone toxicity is whether exposure to the gas can lead to enhanced sensitization to

allergens, and especially whether sensitization occurs in more persons in an ozone-exposed group than in a group similarly exposed to an allergen alone.

In an early study on guinea pigs (30) exposure to an ozone concentration of $10\,000 \mu\text{g} \cdot \text{m}^{-3}$ or $20\,000 \mu\text{g} \cdot \text{m}^{-3}$ for 30 min a day caused increased mortality due to anaphylaxis in animals exposed to albumin by inhalation. Hemagglutination tests showed increased antibody formation against the allergen in the animals exposed to $10\,000 \mu\text{g} \cdot \text{m}^{-3}$. The increased sensitization occurred only for inhaled antigen. Thus no increased sensitization was seen upon skin testing with an allergen in animals sensitized to albumin by intraperitoneal injection when a comparison was made between control animals and animals exposed to $2000 \mu\text{g} \cdot \text{m}^{-3}$ by inhalation.

In a primate model (cynomolgus monkey; *Macaca fascicularis*), exposure to $2000 \mu\text{g} \cdot \text{m}^{-3}$ for 6 h a day, 5 d a week for 12 weeks was found to increase allergic sensitization to inhaled platinum salt dust (ammonium hexachloroplatinate, $200 \mu\text{g} \cdot \text{m}^{-3}$), which was given simultaneously with the ozone exposure (31). As a group, the dose of allergen required to evoke a similar airway response decreased to half of that in animals not exposed to ozone. The model was chosen as a simulation of platinum dust exposure in a platinum refinery. Exposed workers develop asthma and rhinitis, and also show positive skin tests and immunoglobulin E (IgE) antibodies to platinum salts, and this phenomenon was therefore investigated in the animals in parallel with lung function tests. Three groups of animals (7 to 8 animals in each group) were studied (ozone alone, platinum salt dust alone, and combined exposure to ozone and platinum salt dust). Exposure to the combination of ozone and platinum salt dust caused a statistically significant increase in positive skin tests, as compared with exposure to platinum salt dust alone, whereas the results of immunoglobulin E (IgE), IgG, or radioallergosorbent tests did not differ between the three groups of animals. In addition, the enhanced sensitization manifested itself as an increase in airway resistance, occurring at a significantly lower concentration of inhaled allergen in the group exposed to ozone and platinum salt dust, as compared with the group exposed only to platinum salt dust. The increased sensitization was not accompanied by any change in base-line airway resistance between the three different groups. That specific sensitization to the allergen occurred was validated by the increase in positive skin tests, and the positive reaction could be transferred by the injection of serum from sensitized animals into the skin of nonsensitized animals, as expected for an immunoglobulin-mediated response. The sensitivity to inhaled methacholine was significantly also increased in the group exposed to the combination of ozone and platinum salt dust. Sensitivity to methacholine was unaltered in

the two other groups. This finding suggests that nonspecific airway sensitivity occurred in the combined exposure group.

A study on relatively low ozone concentrations was performed with mice (32). It showed a sensitizing effect of ozone on the allergenic action of ovalbumin. The mice were exposed continuously for 4 d to either filtered air or to ozone at concentrations of 200, 260, 320 or 480 $\mu\text{g} \cdot \text{m}^{-3}$. On the fourth day the animals received an inhalation of the allergen, ovalbumin, and then remained in ambient air for a week, whereafter the ozone and allergen exposure was repeated to a total of four ozone exposure periods. The ozone-exposed animals exhibited a significantly increased frequency of anaphylactic shock when challenged intravenously with ovalbumin one week after the four exposure periods, as compared with controls exposed to filtered ambient air and allergen. The effect of ozone was significant from exposure levels of 260 $\mu\text{g} \cdot \text{m}^{-3}$ and up.

There are several possible mechanisms for the enhanced sensitization to allergen after ozone exposure. One possibility is that ozone causes decreased elimination of the antigen from the lungs. Evidence for this phenomenon is found in the increased retention of radio-labeled albumin instilled into the trachea of guinea pigs previously exposed to 16 000 $\mu\text{g} \cdot \text{m}^{-3}$ for 30 min (33). In the same study an increased uptake of allergen into blood was also found. A mechanism for the increased retention or uptake of allergen might involve inflammation of the respiratory tract in relation to ozone exposure. This possibility is supported by an experiment with dogs in which acute exposure to 4200 $\mu\text{g} \cdot \text{m}^{-3}$ for 2 h caused an increase in the number of neutrophil granulocytes in the airway mucosa (34). The ozone exposure caused increased airway responsiveness to acetylcholine, and the degree of increased responsiveness correlated with an increase in the mucosal neutrophil number. In an ensuing study it was found that neutrophil depletion in dogs inhibited the effect of ozone on airway hyperresponsiveness (35). Thus a contribution from inflammatory mechanisms seems likely.

Another interesting possibility is that ozone exposure might cause changes in the production and secretion of regulatory proteins, such as cytokines, and these changes might shift the immune response to an antigen towards the development of an allergic pattern. Evidence for such cytokine formation has been found in experiments with cultured human bronchial epithelial cells exposed to ozone. In these cells the formation of the cytokines IL 8 and tumor necrosis factor alpha was increased by ozone exposure (36). Other cytokines may be involved in the increased allergen-sensitizing effect of ozone (37).

Increased cytokine formation can be expected to lead to increased antibody formation. Indeed, evidence has been found for an increased antibody formation in lungs

as a result of ozone exposure. In mice exposed to 1000 or 1600 $\mu\text{g} \cdot \text{m}^{-3}$, ozone exposure continuously for 15 or 24 d caused an increase in pulmonary interstitial cells producing IgA (38), and an increase in the secretion of IgA into the respiratory tract also occurred, as indicated by increments of IgA in lung lavage fluid. Respiratory tract concentrations of IgG and IgM were also increased, but as a result of a passive leakage from plasma, as indicated by a parallel increase in albumin leakage.

Measurement of IgE-containing cells in mice exposed to inhaled ovalbumin or to ovalbumin after ozone inhalation revealed an increase in IgE-positive cells near the mucosa in the ozone-exposed animals (39). In response to only the inhalation of ovalbumin there was a ninefold increase in IgE-containing cells. Exposure to an ozone concentration of either 1000 or 1600 $\mu\text{g} \cdot \text{m}^{-3}$ prior to ovalbumin sensitization increased the number of IgE-positive cells by a factor of 34, and this increase was a significant enhancement of the effect of the allergen. Ozone-exposed animals exhibited positive passive cutaneous anaphylaxis, whereas this sign of antibody formation was not present in the animals exposed to ovalbumin alone.

The increased sensitization to allergen caused by ozone inhalation might be accompanied by the activation of immune regulatory mechanisms modifying the response. Thus mice were subjected to 1600 $\mu\text{g} \cdot \text{m}^{-3}$ of ozone for one, two or four weeks and then subjected to a single albumin inhalation followed by intraperitoneal immunization with albumin a week later. The IgE response to albumin was suppressed in the ozone-exposed mice (40). The suppression was due to a modifying effect of the inhaled albumin on the immune response to the intraperitoneal injection given one week later. Thus it seems clear that ozone can enhance antibody formation when the exposure to allergen is through inhalation and that it is important to use allergen exposure schemes that do not run the risk of obscuring a real effect of allergen sensitization.

In summary, ozone causes increased sensitization to inhaled allergens in animals. The increase in sensitization seems well documented since it has been found in several species and involves increased airway sensitivity, as demonstrated by both the decreased allergen dose necessary and the number of exposed persons that become sensitized. The increased sensitization is also displayed as increased anaphylactic reactions or enhanced passive cutaneous skin tests for allergen.

Effects on lung function

Breathing pattern, airway resistance and lung compliance

A sensitive parameter describing lung function is that of lung compliance. In guinea pigs exposed to an ozone

concentration of 400, 800, or 1600 $\mu\text{g} \cdot \text{m}^{-3}$ for 1 to 2 h, a significant decrease of 22% to 24% in compliance (increase in "lung stiffness") occurred at 800 $\mu\text{g} \cdot \text{m}^{-3}$ and above (41). In guinea pigs exposed to 200 or 1600 $\mu\text{g} \cdot \text{m}^{-3}$ for 1 h, followed by histamine challenge, a significant augmentation of the decrease in compliance caused by histamine occurred already at 200 $\mu\text{g} \cdot \text{m}^{-3}$. The effect was the most marked 2 h after the ozone exposure (42). At 400 to 600 $\mu\text{g} \cdot \text{m}^{-3}$ the airway resistance to gas flow was affected and sensitivity to bronchoconstrictor agents increased. An increase in respiratory frequency occurred with a concomitant decrease in breath volume (tidal volume) at ozone concentrations of 600 $\mu\text{g} \cdot \text{m}^{-3}$ and above. Overt airway constriction was found to occur in guinea pigs and cats, respectively, at 800 and 1000 $\mu\text{g} \cdot \text{m}^{-3}$. The changes seem to parallel the effect of a 1500 $\mu\text{g} \cdot \text{m}^{-3}$ exposure of humans (43).

Airway responsiveness

The hyperresponsiveness to the immunizing effects of allergens as a result of ozone exposure has already been discussed. Another phenomenon of relevance is that ozone can cause an increased responsiveness to inhaled bronchoconstrictor agents. This phenomenon can be called hyperresponsiveness. This effect also involves an increased response to allergen, but it is an effect which is not due to increased immunization and is likely to be a sign of increased effects of released mediators.

Hyperresponsiveness to bronchoconstrictors occurs in several species. In the dog and sheep the hyperresponsiveness caused by a 2-h exposure to an ozone concentration of 1400 and 1000 $\mu\text{g} \cdot \text{m}^{-3}$ is the most marked 1 d after the exposure (44, 45). The effect is likely to be due to lung inflammation. The inflammation sensitizes the lung to allergens and agonists. The hyperresponsiveness decreases but does not disappear during a 7-d period after exposure.

In guinea pigs ozone exposure to concentrations of 2000 or 6000 $\mu\text{g} \cdot \text{m}^{-3}$ for 18 h prior to challenge with inhalation histamine or allergen (ovalbumin) caused similar enhancements of bronchial obstruction (46).

Summary

It is clear that inhaled ozone causes changes in breathing pattern, bronchial obstruction, and hyperresponsiveness to bronchoconstrictive agents in animals. The effects occur at concentrations of the gas that are slightly higher than those necessary to cause changes in morphology, changes in mucociliary transport, and aberrant host defense. Inflammation is likely to be involved in these effects.

Proinflammatory effects of ozone in animal models

The inhalation of ozone has been shown to result in detrimental effects in the lungs of all the animal species tested, ranging from rodents up to nonhuman primates. Among the major observed effects, the induction of pulmonary inflammation and the resultant increase in airway permeability seem to be a recurrent phenomenon, particularly after acute exposures (47). This section deals with the signs and symptoms of ozone-induced inflammation in animals and tries to relate them systematically to exposure factors, such as the concentration of the gas inhaled and the duration and pattern of exposure.

Inflammatory process, friend or foe?

Acute inflammation is a *normal* stress response to local injury or infection within a tissue. The overall process involves the integrated activity of specialized cell types, whose combined activity ensures the destruction of infectious pathogens, the involution of damaged cells within the tissue, and the processes of tissue repair or replacement (figure 1).

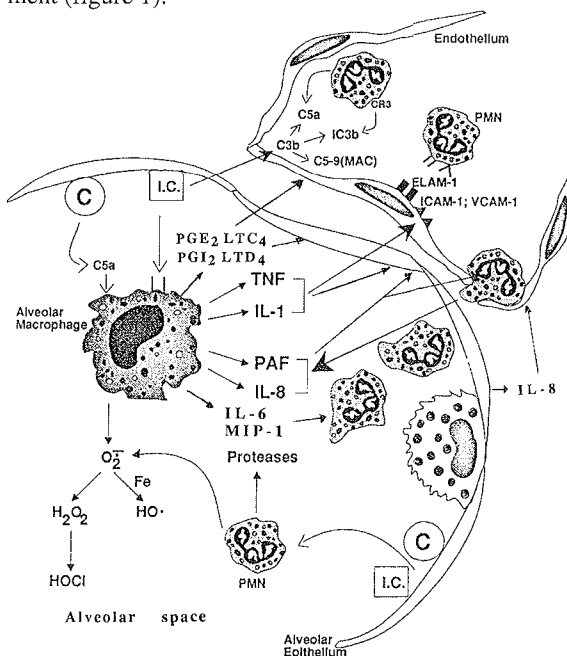


Figure 1. The cells and intracellular mediators involved in alveolar inflammation. (C = complement factor, C3b = complement factor 3b, C5—9 (MAC) = complement factor 5—9 membrane attack complex, C5a = complement factor 5a, CR3 = complement receptor 3 (CD11/CD18), ELAM-1 = endothelial-leukocyte adhesion molecule-1, Fe = iron, H_2O_2 = hydrogen peroxide, HOCl = hypochlorous acid, $\text{HO}\cdot$ = hydroxyl radical, I.C. = immune complex, IC3b = inhibitor fragment of complement factor 3b, ICAM-1 = intercellular adhesion molecule-1, IL-1 = interleukin-1, IL-6 = interleukin-6, IL-8 = interleukin-8, LTC_4 = leukotriene C_4 , LTD_4 = leukotriene D_4 , MIP-1 = macrophage inflammatory protein-1, O_2^- = superoxide radical, PAF = platelet activating factor, PGE_2 = prostaglandin E_2 , PGI_2 = prostaglandin I_2 , PMN = polymorphonuclear leukocyte, TNF = tumor necrosis factor, VCAM-1 = vascular cell adhesion molecule-1)

Macrophages, such as those present in the alveolar space or in the interstitial tissues of the airways, are generally thought to occupy a key role in instigating the inflammatory process. Macrophages respond to the phagocytosis of foreign (particles) or endogenous material (cellular debris), or to antigen presentation, by releasing many potent biological mediators that serve to amplify tissue response (figure 1).

Of these mediators, arachidonate metabolites (eicosanoids), such as the peptide-leukotrienes (LTC_4 and LTD_4), prostaglandins such as PGE_2 , PGI_2 and $\text{PGF}_{2\alpha}$ and peptide cytokines (various interleukins, eg, IL-1, IL-6 and IL-8), tumor necrosis factor alpha ($\text{TNF}\alpha$), the macrophage inflammatory proteins (MIP), and low molecular-weight agents such as nitric oxide (NO), play major roles in propagating the stress signal. Many of these mediators act by increasing the permeability of epithelial layers, such as the airway epithelium. In the lung this phenomenon is characterized by an increased permeability of the epithelium to interstitial proteins, and the increased permeability causes an accumulation of protein in the lung lumen. These changes also allow the permeability of airborne molecules (ie, radiolabeled tracers and particles) from the airways into the interstitium.

Most other epithelial cell types, particularly those within the airways, are also able to release such inflammatory mediators. Another important function of some of these inflammatory mediators is to activate the local vascular endothelium and therefore stimulate the expression of specific adhesion molecules directed against blood leukocytes and increase the endothelial layer permeability to proteins, solutes, and fluid. Finally, some of these mediators act as chemoattractants for specific blood leukocytes, such as mast cells, neutrophils, eosinophils, basophils, and lymphocytes. Thus, in a highly coordinated manner, these mediators stimulate the influx of cells from the circulation into the area of damage or infection, pave the way for their rapid infiltration to the initial area of the affected tissue or organ, and ensure that the cells are correctly activated to function optimally in tissue defense and repair. It should be remembered, however, that extremely vigorous acute inflammations can lead to considerable tissue damage.

In contrast to these acute events, the long-term maintenance of the inflammation response may lead to detrimental effects within tissues. For instance, many of the inflammatory cells recruited to the site of inflammation release agents, such as nitric oxide and reactive oxygen metabolites, proteolytic enzymes and cytotoxic proteins, and these cells can cause extensive tissue damage and loss of defined tissue function. Such damage may also lead to extensive cellular proliferation and lead to increased cellularity in the tissue, such as thickening of the airways, or to an imbalance in the cellular composition of the tissue, such as the replacement of type I

epithelial cells by proliferating type II cells in the alveolus of the airways. In extreme situations, stimulated proliferation of fibroblasts can occur and lead to the development of the fibrotic lesions characteristic of many chronic inflammations.

Ozone and pulmonary inflammation in animals

As the effects of exposure to ozone are dependent both on the concentration of the gas and the pattern and duration of exposure and as actual exposure to ozone within the human population is likely to be of a largely chronic nature, interspersed by acute periods of elevated exposure, for the purpose of this review, we have segregated the description of the proinflammatory effects of ozone into those after acute exposure (continuous exposure up to 24 h) and those after more chronic exposures of >1 d, either continuous or repeated intervals of exposure. Due to the multiplicity of studies in these areas, we have limited our description to selected examples which best illustrate the major findings in each case.

Acute inflammatory responses to ozone

The objective of the many studies on the acute proinflammatory effects of ozone have been to define characteristic symptoms of pulmonary response and relate these symptoms to the factors governing exposure, such as the minimum concentration required to induce a response in animals and the effect of the duration and pattern of exposure.

Early work by Alpert et al (47) demonstrated dose-dependent (200 to $5 \text{ mg} \cdot \text{m}^{-3}$) transient increases in lavage albumin and IgG concentrations in rats exposed acutely to ozone; these increases were then confirmed for other species (48, 49). These pioneering studies clearly show a threshold concentration of ozone in the range of 200 to $400 \text{ mg} \cdot \text{m}^{-3}$ for the induction of these types of changes in airway permeability, which are characteristic of inflammation. Since then, a plethora of information has become available describing the mechanism of the inflammatory response in more detail and defining suitable markers of animal species differences in response to the gas and the complex relationship between the concentration of inhaled ozone and the time of exposure. All of these parameters are important to the eventual assessment of the risk to human health.

One of the biggest problems in determining the lowest threshold for an acute inflammatory response to ozone is presented by the choice of markers. Thus, in contrast to the preceding permeability observations, it seems that the release of inflammatory mediators such as PGE_2 and $\text{PGF}_{2\alpha}$ into lavage fluid after acute exposures (2 h) of

rabbits only occurs at ozone concentrations of >1 ppm, whereas $200 \text{ mg} \cdot \text{m}^{-3}$ was totally without activity (50, 51). In another study, Fouke et al (52) exposed dogs and baboons to ozone levels of $1 \text{ mg} \cdot \text{m}^{-3}$ for 2 h and also failed to detect eicosanoid release into the lavage fluid.

In an extensive permeability study, in which several animal species were acutely exposed to ozone between 400 and $4 \text{ mg} \cdot \text{m}^{-3}$ for 4 h, species differences in response were clearly evident. Thus guinea pigs were shown to respond to $400 \text{ mg} \cdot \text{m}^{-3}$ with an increase in lavage protein 20 h after treatment. On the other hand, mice, rats, and hamsters were relatively insensitive, responding first to an ozone level of $2 \text{ mg} \cdot \text{m}^{-3}$, while rabbits were relatively resistant, requiring $4 \text{ mg} \cdot \text{m}^{-3}$ to initiate a response (53).

In addition to detecting permeability changes and the release of eicosanoids in response to relatively short-term acute exposure to ozone, several studies on rats exposed to ozone between 3 and $240 \text{ mg} \cdot \text{m}^{-3}$ for 6 h have shown transient increases in lavageable macrophages and polymorphonuclear leukocytes (PMN) (54, 22).

Interestingly, although the threshold for an increase in PMN in the total lavage was $1.6 \text{ mg} \cdot \text{m}^{-3}$ in these studies, when the airways were differentially lavaged, $240 \text{ mg} \cdot \text{m}^{-3}$ was shown to increase significantly the number of PMN lavaged from the nasal portion of the airways (22). It should be stressed that lavageable PMN are only one component of the inflammatory response to consider and that changes in the interstitial populations of macrophages may also be relevant. This point is considered later in detail.

Three studies have been published that probe the interrelationship of concentration (C) and time (T) in controlling the response of animals to acute, continuous exposure to ozone. Rombout and his co-workers exposed rats for 1 to 8 h to 760 or $4.08 \text{ mg} \cdot \text{m}^{-3}$ during daytime conditions and for 4 to 12 h to 260 to $760 \text{ mg} \cdot \text{m}^{-3}$ under nighttime conditions. The minimal C x T product required to increase the lavageable protein in the lung lumen was $260 \text{ mg} \cdot \text{m}^{-3}$ for 4 h. Animals exposed under nighttime conditions were more sensitive to ozone. Multivariate analysis revealed that the influence of time increased with increasing concentration and that the influence of time was still important at the lowest concentration of the gas tested ($260 \text{ mg} \cdot \text{m}^{-3}$) (55). Similar results were obtained for guinea pigs, but there was some disparity in the relationship between concentration and time at low C x T products (56). The diurnal sensitivity of animals to the acute effects of ozone has been confirmed in a study by Van Bree et al (57), who demonstrated greater increases in lavage protein and PMN in rats exposed acutely to 12 h of $800 \text{ mg} \cdot \text{m}^{-3}$ at night versus a similar exposure under daytime conditions.

Many studies have been performed using single ozone exposures to $>1.6 \text{ mg} \cdot \text{m}^{-3}$. Using radiolabeled tracers, either injected into circulation or introduced into airways, Bhalla and his co-workers have exposed rats to $1.6 \text{ mg} \cdot \text{m}^{-3}$ for 2 h and demonstrated a transient permeabilization of the lung in both directions (58–60). The same authors showed that exercise enhanced the response of the animals to ozone under these conditions (61) and that the effects of ozone were attenuated in leukopenic rats, or rats pretreated with indomethacin, a cyclooxygenase inhibitor, and antiinflammatory agent (62). In addition, the PMN isolated from control rats treated with ozone under these conditions (58–60) showed morphological changes indicative of activation and increased cellular adhesiveness (63).

In more-detailed temporal studies of the influx of PMN into the lung and the relationship of this influx to increased epithelial permeability, Young & Bhalla (64) have shown that increases in tracheal permeability in response to $1.6 \text{ mg} \cdot \text{m}^{-3}$ for 3 h clearly preceded the influx of PMN into the lumen (64). In addition, it was shown that the influx of PMN into the lumen was clearly preceded by a transient increase in interstitial PMN, which began immediately after the inhalation was terminated, and that this later event correlated best of all with increases in airway permeability (65).

Higher concentrations of ozone ($>2 \text{ mg} \cdot \text{m}^{-3}$) have been generally shown to induce acute proinflammatory responses rapidly in the lung. In a unique study (66), in which dogs were exposed to $2 \text{ mg} \cdot \text{m}^{-3}$ for only 5 min, in the form of a bolus in the intubated lung, even this short exposure was sufficient to elevate the interstitial PMN and lavageable PMN transiently at 1 to 3 h and 24 h postexposure, respectively. In addition, unlike the lack of eicosanoid response to lower levels of ozone (52, 53), a 1- to 2-h exposure to $2 \text{ mg} \cdot \text{m}^{-3}$ has been clearly shown to elevate the levels of eicosanoids, such as PGE_1 , PGE_2 , LTB_4 and thromboxane A_2 , in lavage fluid from a variety of species (67, 68). Interestingly, Gunnison et al (67) clearly demonstrated age-related differences in the released eicosanoids, younger animals clearly favoring the release of prostaglandin metabolites, while the release of leukotrienes, the increase in epithelial permeability, and the influx of PMN into the lung lumen were not altered by age. It should be mentioned that some of the stimulated release of eicosanoids may not have any functional bearing on the development of the inflammatory reaction. Recent experiments by Stevens et al (69) have demonstrated that the administration of MK 0591, a phospholipase-activator protein antagonist, did not diminish the response of dogs exposed acutely to ozone. Thus these authors postulate that leukotrienes do not play a role in the response of dogs to ozone.

After 6 h of ozone exposure of rats to $2 \text{ mg} \cdot \text{m}^{-3}$, Gunnison et al (70) noted frank cytotoxicity in the

laved PMN population of young but not mature animals. In a recently published study Pino et al showed that the exposure of rats to $2 \text{ mg} \cdot \text{m}^{-3}$ for between 4 and 24 h resulted in time-dependent increases in PMN in the lavage and in biopsies of the centriacinar region of the lung, as well as in increased airway permeability. They noticed, however, that the number of lavageable macrophages decreased after exposure, while the interstitial macrophage numbers remained elevated (71). Using a neutrophil-deficient rat model, these authors also showed that the induction of epithelial cell permeability was independent of the presence of neutrophils in the lung (72).

Only a limited number of acute exposure studies have been performed with concentrations of ozone above $2 \text{ mg} \cdot \text{m}^{-3}$ (73, 74). Despite showing signs of inflammation in most cases, a clear decrease in the number of lavageable macrophages was noted in rats exposed to $3.6 \text{ mg} \cdot \text{m}^{-3}$ for 2 to 4 h (73). In a recent study utilizing some interesting inbred mice strains, ozone exposure to concentrations of up to $4 \text{ mg} \cdot \text{m}^{-3}$ for 3 h was shown to attenuate the transepithelial electric potential of the tracheal airways of C57-BL/6J mice prone to inflammation after ozone exposure. In contrast, the ozone-resistant C3H/HeJ strain showed no such acute alteration in transepithelial potential (75). In a similar series of experiments, Tankersley et al (76) also studied the proinflammatory effects of ozone in the C57BL/6J mice, which were again shown to respond to both high-level exposure ($4 \text{ mg} \cdot \text{m}^{-3}$, 3 h) and relatively low-level exposure ($600 \text{ mg} \cdot \text{m}^{-3}$, 7 h), whereas the resistant C3H/HeJ strain showed little response. Comparative genetic studies performed on these mice strains indicate the involvement of two critical, and as yet unidentified, genes in the susceptibility to ozone-induced lung inflammation (76). In another series of experiments with susceptible and resistant mice strains, Kleeberger et al (77) showed that WBB6F1-W/Wv mice with a mast cell deficiency were resistant to the influx of PMN into the lung lumen and the increase in lavage protein caused by $3.5 \text{ mg} \cdot \text{m}^{-3}$ for 3 h. On the other hand, the C57-BL/6J mice and mature WBB6F1-W/Wv, which had received a bone marrow transplant, showed a considerable inflammatory response under these conditions. These results indicate a potentially critical role for interstitial mast cells in mediating the acute inflammatory response to ozone, at least in mice, at these relatively high concentrations.

In a recent study on the differential cellular response to the inhalation of very high ozone levels ($4 \text{ mg} \cdot \text{m}^{-3}$) for 4 h, Schultheis & Bassett (78) have shown that lavaged cells obtained from guinea pigs over a period of 2 to 14 d postexposure had transient changes in the numbers of mast cells, macrophages, and eosinophils in the lung after exposure. Using conventional lavage and lavage following the collagen digestion of pulmonary connective tissue, these studies also confirmed (64, 65) that

the progression of the pulmonary inflammation was best reflected in the interstitial cell response, rather than in the responses detected by conventional lavage of the lung lumen (78).

Only very few studies have been performed on the effect of the acute co-exposure of animals to ozone and other agents. In a recent study, Bhalla (79) co-exposed rats to ozone levels of 1.2 or $1.6 \text{ mg} \cdot \text{m}^{-3}$ and an enzyme system generating hydrogen peroxide in the trachea for 2 h. The study clearly showed an additive effect between the two agents in the induction of tracheal permeability.

In summary, it appears that a single acute exposure to ozone between 200 and $4 \text{ mg} \cdot \text{m}^{-3}$ results in several changes in animal pulmonary physiology which are characteristic of the induction of acute inflammation. One primary characteristic of the acute response is that the effects are often delayed after exposure to about $1 \text{ mg} \cdot \text{m}^{-3}$ or below, whereas concentrations above $1.6 \text{ mg} \cdot \text{m}^{-3}$ can result in immediate proinflammatory changes after the termination of exposure. It also appears that the longer the exposure time, the more exaggerated the response, both in terms of the recruitment of proinflammatory mechanisms and the threshold of the response. Nevertheless, the molecular mechanism underlying acute ozone-induced inflammation remains questionable. Several studies have also revealed interspecies variations in the sensitivity of animals to ozone, and these variations complicate extrapolation to human exposure. The vast majority of the studies indicate, however, that single exposures to ozone for up to 24 h result in transient inflammatory changes in the lung, but that the changes eventually appear to subside. It must be stressed, however, that appropriate studies have not been performed which critically probe for long-term effects of acute exposure to the gas. In addition, careful comparison of the studies reveal intraspecies variation in the response of the lungs to ozone inhalation. Further work is required to determine the molecular basis of this variation in order to clarify eventual extrapolations from animal studies to situations of human exposure.

Chronic inflammatory responses to ozone

In contrast to the plethora of studies on the acute, proinflammatory effects of ozone in the lungs of animals, there have only been a few controlled studies on the effects of repeated interval or continuous exposures to the gas over extended periods. Such experimental exposure patterns more realistically represent the conditions under which humans are exposed to ozone in the environment.

Early work by Castleman et al with primates (80) and by Moore & Schwartz with rodents (81) clearly demonstrate proinflammatory effects of chronically administered ozone in a concentration range similar to that in-

ducing acute effects (ie, 100 to 400 $\text{mg} \cdot \text{m}^{-3}$). In addition to demonstrating effects on PMN infiltration into the lung, these studies closely detailed long-term ultrastructural changes in tissue composition (ie, thickening of the epithelial-interstitial layers) which are generally not studied after acute exposure. However, these and more recent studies clearly show that repeated exposures of animals to ozone often result in an attenuation of the response with time and indicate the induction of an adaptive response (82).

Several subchronic studies have been performed which highlight adaptive responses to ozone inhalation in animals. In a study in which rabbits were exposed to ozone concentrations between 200 and 2.4 $\text{mg} \cdot \text{m}^{-3}$ for 2 h a day over a two-week period, Driscoll et al (83) demonstrated an increase in alveolar macrophages 7 d after a single exposure to as little as 200 $\text{mg} \cdot \text{m}^{-3}$. However, even upon repeated exposures for up to two weeks, significant elevations in lavageable macrophages and PMN were only evident after more than 6 d of repeated exposure. However, the infiltration response occurred more rapidly. In another study in which rats were exposed to 400 or 1.6 $\text{mg} \cdot \text{m}^{-3}$ for 7 h a day over 4 d, no significant increase in total lavage cells was noted over control values, but PMN were increased at concentrations above 1.2 $\text{mg} \cdot \text{m}^{-3}$ after 1 d of exposure; the levels then decreased to control values thereafter (84). Tepper et al (85) demonstrated an adaptive change (attenuation of the response with time) in pulmonary function after exposure to 1 $\text{mg} \cdot \text{m}^{-3}$ in rats exposed for 2.25 h a day for 5 d, but not in inflammatory cell infiltration into the lung lumen. Indeed, this study revealed significantly more PMN in the lung and a persistence of elevated protein levels in the lavage after repeated ozone administration over this period. More recently, van der Val and his colleagues (86) demonstrated that preexposing rats to daily intervals of near ambient levels of ozone for 7 d, significantly diminished the subsequent inflammatory cell response to an acute challenge with high concentrations of the gas.

Several long-term studies have been published utilizing interval exposures. Last and his co-workers (87) exposed rats to between 60 and 2 $\text{mg} \cdot \text{m}^{-3}$, 6 h a day, 5 d a week, for 20 months. In rats exposed to concentrations above 1 $\text{mg} \cdot \text{m}^{-3}$, increases in collagen deposition were noted in the centriacinar region of the lung; this change is characteristic of the induction of mild, persistent fibrosis. In a recently published study on primates, exposure to 300 $\text{mg} \cdot \text{m}^{-3}$, 8 h a day for up to 90 d was clearly shown to cause morphological changes in the lung. The changes included increases in epithelial thickness and the number of cuboidal epithelial cells and increased cellular proliferation in the interstitium (3). These results are disturbing in terms of the risks to human health as the levels of ozone employed were

close to ambient values, and the clearly hyperplastic response can have serious implications for situations of co-exposure with other airborne pollutants, particularly carcinogens.

In addition to the concentration of ozone and duration of exposure, the pattern of exposure clearly affects the response of animals to the gas. Several studies have been performed using continuous exposure for extended periods. Bassett and his co-workers (88) have exposed rats to 1.4 $\text{mg} \cdot \text{m}^{-3}$ for 3 days continuously. Increases in alveolar macrophages, PMN, and albumin were noted as early as 12 h into the exposure. In contrast, while PMN numbers rapidly subsided after exposure, the elevated protein and macrophage number persisted in the lavage fluid over several days. Similar effects were obtained by Canning et al (89) after the continuous exposure of mice to 1 $\text{mg} \cdot \text{m}^{-3}$ for up to two weeks. Thus PGE_1 and protein peaked in lavage fluid by the third day of the exposure, declining thereafter. These authors noted declining numbers of cells with time in the lavage following 1–3 d of exposure.

With the induction of lung fibrosis in mind as a consequence of chronic exposure to ozone, it is interesting to note that the continuous exposure of rats to 1 ppm of the gas for up to two weeks caused considerable induction of the expression of both fibronectin and type 1 collagen mRNA. An induction of the growth-related protooncogene *c-myc* was also noted during the treatment (90). However, it should be emphasized that these effects were seen with relatively high concentrations of ozone.

Extending the exposure of rats to between 8 and 200 $\text{mg} \cdot \text{m}^{-3}$ continuously for between 1 and 12 weeks has been shown to induce persistent infiltration of cells into the lung lumen. However, in view of the C x T products used, relatively little proinflammatory action was noted after extended periods of inhalation. Thus the lowest effect limit was 400 $\text{mg} \cdot \text{m}^{-3}$, which caused a significantly higher accumulation of macrophages, but not PML, only after 11 weeks of exposure (12). In a very interesting study by Chang and his co-workers (91) rats were continuously exposed to ozone over a long period using a cyclic pattern matched to environmental variations of the gas. Thus animals received ambient concentrations of the gas (120 $\text{mg} \cdot \text{m}^{-3}$) for 13 h, followed by a short peak of 500 $\text{mg} \cdot \text{m}^{-3}$, returning to ambient levels over 9 h. These authors clearly demonstrated an inflammatory response in the lungs by one week of exposure, interstitial macrophages being increased in the proximal airways and the terminal bronchioles and interstitial edema being present. Once again, adaptive responses were noted in these experiments, as the levels of interstitial macrophages returned to control values after three weeks of exposure to the cycle. However, importantly, some evidence of interstitial fibrosis in the proximal airways was noted after the exposure was stopped.

Finally, as the human population is simultaneously exposed to many airborne pollutants, it is of interest to determine any additive or synergistic effects of other agents on ozone-induced inflammation. One agent which has received considerable attention is nitrogen dioxide, and this possibility has been the subject of many reviews (83). Apart from this oxidant gas, the literature is beginning to reveal interactions between ozone and other agents. For instance, the concomitant chronic inhalation of carbon-black particles with high concentrations of ozone ($3 \text{ mg} \cdot \text{m}^{-3}$) clearly potentiated the inflammatory response of mouse lung (92). Such interactions, although a possibility, may not, however, be plainly evident, and revealing them requires a careful study design. In a very recent study by Cassee & Feron (93), in which rats were intermittently exposed for 3 d to formaldehyde (3.6 ppm), a known nasal or respiratory tract irritant, ozone ($800 \text{ mg} \cdot \text{m}^{-3}$) and combinations thereof, little or no synergism was demonstrated despite each agent causing its own spectrum of inflammatory and cell proliferative responses. Further interaction studies involving chronic exposure of animals to ozone and other airborne agents are needed if we are to define the risks to human health from the inhalation of ozone better, particularly in the urban environmental setting.

Summary

Perusal of the available studies clearly shows that the initial inflammatory response of animals to the inhalation of continuous or repeated periodic bouts of ozone

resembles the response of the animal to acute exposures. However, with increasing lengths of exposure, it is clear that adaptive responses in lung function and biochemistry occur which instigate tolerance to the gas. The relevance of such adaptive responses in animals to the situation of human exposure to ozone is clear. However, the demonstration of such a response in humans may be difficult as the threshold for response in the animal models tested appears to be between 240 and $300 \text{ mg} \cdot \text{m}^{-3}$, which is readily achieved in the urban environment at present. In addition, the pattern of exposure ($C \times T$ product), more than the absolute exposure concentration, seems to dominate the response. Thus continuous exposure to ambient levels of ozone, interspaced by short peak exposures to the gas, seems to elicit as strong a proinflammatory response as does continuous exposure to the peak level used. The detrimental inflammatory and cell proliferative responses of animal lungs to chronic exposures to these near-ambient concentrations of ozone are disturbing in terms of the risks to human health.

Summary and concluding remarks

Exposure of animals to ozone causes effects on lung morphology and function, as well as inflammation and decreased resistance to infectious agents. Significant effects are seen at $200 \text{ } \mu\text{g} \cdot \text{m}^{-3}$ and above, and dose-dependency occurs from this concentration and above, mak-

Table 1. Summary of the low-level effects of ozone and effects of ozone on primates (selected for low effective concentrations, 0.1 ppm approximated to $200 \text{ } \mu\text{g} \cdot \text{m}^{-3}$).

Reference	Effect	Ozone exposure ($\mu\text{g} \cdot \text{m}^{-3}$)	Species
Morphology			
Mochitate et al 1992 (21)	Alveolar macrophages, increase in number and enzyme activity	200	Rat
Barry et al 1985 (6)	Increase in alveolar type I cell turnover	240	Rat
Harkema et al 1993 (3)	Bronchiolar cell proliferation	300	Monkey
Harkema et al 1987 (4)	Nasal epithelial lesions	300	Monkey
Raijini et al 1993 (5)	Bronchiolar cell proliferation	300	Rat
Rithideh et al 1989 (12)	Increase in alveolar macrophage mitotic index	540	Rat
Mucociliary clearance			
Driscoll et al 1986 (20)	Increase	200	Rabbit
Pinkerton et al 1989 (19)	Decrease	500	Rat
Infection defense			
Aranyi et al 1983 (26)	Decreased resistance	200	Mouse
Sensitivity to allergen or bronchoconstrictors			
Osebold et al 1988 (32)	Increased sensitization to allergen; inhaled ovalbumin, i.v. anaphylaxis	260	Mouse
Biagini et al 1986 (31)	Inhaled platinum salt dust, inhaled and skin tests	2000	Monkey
Gordon & Amdur 1980 (42)	Increased sensitivity to bronchoconstrictive effect; increased sensitivity to histamine	200	Guinea pig

ing the observed effects highly reliable. A summary of some important effects at low levels or in primates is given in table 1. Apart from effects on lungs, the effects of ozone can be seen in thymus and lymphoid tissues after acute exposure. Very disturbing, from a health perspective, are recent, well-controlled studies on monkeys showing that, at $300 \mu\text{g} \cdot \text{m}^{-3}$, an increased proliferation of both epithelial and inflammatory cells occurs in the airways. Thus effects on animals are apparent at concentrations which are at, or near, background levels of ozone. In mice subjected to life-long ozone exposure, morphological changes and fibrosis are present in increased frequencies. The fibrosis also remains despite the cessation of ozone exposure. Therefore, the morphological changes must be regarded as nontransient, long-term effects.

The lowest effective concentration of ozone evoking morphological changes and decreased defense against infectious agents in rodents and changes in mucociliary clearance in rabbits is $200 \mu\text{g} \cdot \text{m}^{-3}$. The morphological effects are seen both after acute exposure and after 90-d (monkeys) or life-long (mice, rats) exposure. In primates morphological changes are seen with exposure to $300 \mu\text{g} \cdot \text{m}^{-3}$, involving changes in alveolar morphology in the lung and alterations in nasal epithelium morphology. Regardless of differences in sensitivity between species, it is clear that ozone can cause effects on animals from $200 \mu\text{g} \cdot \text{m}^{-3}$ up and that ozone causes increased sensitization to allergens from $260 \mu\text{g} \cdot \text{m}^{-3}$ on.

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Genotoxicity and carcinogenicity of ozone

by Katarina Victorin

A review of the genotoxicity of ozone has been published earlier (1) and can be used for a more-detailed description of individual studies.

Genotoxic effects in vitro

Reactions with DNA and RNA

Ozone has been shown to react with DNA (deoxyribonucleic acid) in vitro, preferentially with thymine and guanine residues, and cause degradation of the DNA structure and DNA strand breaks (2—11). In aqueous solution, ozone decomposes to give hydrogen peroxide, superoxide and hydroxy radicals. See the section on the absorption and metabolic fate of ozone (12). According to van der Zee et al (13) DNA damage proceeds both directly, via ozone itself, and indirectly, via hydroxy radicals when solutions of nucleotides or DNA are treated with ozone. DNA adducts can also be formed (14) (eg, hydroxymethyluracil, thymine glycol, 8-hydroxyguanine, and various unidentified adducts).

Ozone has also been clearly shown to cause damage to viral DNA and RNA (ribonucleic acid) (15—19).

Genotoxic effects on microorganisms, plants and insects

All the studies on genotoxic effects on microorganisms, plants, and insects are summarized in table 1.

The bubbling of ozone through a suspension of *Escherichia coli* or *Bacillus subtilis* bacteria caused both forward and reverse mutations in their respective genomes, as well as DNA strand breaks (20—25). Similar treatment of the yeast *Saccharomyces cerevisiae* caused a variety of genetic events (26). It should be noted that, in these experiments, the observed effects may not only be due to ozone, but also to degradation products of ozone in water, such as hydroxy radicals and hydrogen peroxide.

One negative study concerning DNA strand breaks was performed with aerosolized *E coli* bacteria in air containing ozone. However, the ozone concentration was low ($0.08 \text{ mg} \cdot \text{m}^{-3}$) in this study, which was actually a control experiment as part of a larger study (27).

The Ames *Salmonella* assay has been applied in three studies. Ozone in air was passed over agar plates inoculated with *Salmonella* TA 100, without lids, in an exposure chamber for 20 h in one study and for 6 h in another. No mutagenic effects could be found in a single experi-

ment with an ozone level of $1.0 \text{ mg} \cdot \text{m}^{-3}$ (28), or with several concentrations in the range of 0.2 to $4.0 \text{ mg} \cdot \text{m}^{-3}$, either with or without metabolic activation (S9) of the test system (29). Concentrations of the gas above $4 \text{ mg} \cdot \text{m}^{-3}$ were toxic to the bacteria in the latter study. In the third study, several *Salmonella* strains were exposed to ozone in air (30). Strain TA 102, but not strains TA 100, TA 104 or TA 98, experienced a slight mutagenic response after 35 min of exposure to ozone concentrations in the range of 0.04 to 0.08 and 0.4 to $1.0 \text{ mg} \cdot \text{m}^{-3}$, both with and without S9 addition.

The genotoxic effect of ozone has also been studied in plants. Chromosome aberrations were found in *Vicia faba* (31, 32), and in the *Tradescantia* test system ozone was reported as positive in this respect in one study (33) but negative in two others (34, 35). One of the latter studies also reported negative results in *Nicotiana tabacum*.

Erdman & Hernandez (36) exposed male *Drosophila virilis* flies to an ozone concentration of $60 \text{ mg} \cdot \text{m}^{-3}$ for 3 h. Longer exposure times were not tolerated by the flies. Ozone induced dominant lethal mutations in the offspring, as calculated by the proportion of eggs that failed to develop into pupae. Dominant lethals were also seen in another study, along with a life-span shortening effect on male offspring from exposed females and a decrease in the hatchability of the eggs (37).

Cell cultures

All the studies performed with cell cultures are summarized in table 2. Different modes of exposure have been used in the studies, see Victorin (1) or the original references.

Cytogenetic effects. Fetner (38) was the first to show the chromosome-damaging effects (chromatid deletions) of ozone in cell cultures using the human KB cell line. Gross chromosome effects, such as rounded or lysed cells, condensed nuclei, and clumped metaphase chromosomes, were caused by high concentrations of ozone (1400 to $14\,000 \text{ mg} \cdot \text{m}^{-3}$) in another study using embryonic chick fibroblasts (39). Gooch et al (40) studied the effect of bubbling ozone through suspension cultures of human peripheral leukocytes. When the cells were treated in the S-phase, the frequency of chromatid deletions increased.

Guerrero et al (41) exposed 24-hour old cultures of human fetal lung cells (WI-38) to ozone levels of 0.5 ,

1.0, 1.5, or 2.0 mg · m⁻³ for 1 h, the result being a concentration-related increase in sister chromatid exchanges (SCE). An increase in chromatid deletions also occurred at 2 mg · m⁻³, but no increase in chromosome-type aberrations were noted.

Exposure to ozone at 0.07 mg · m⁻³ for 1 h did not appear to induce SCE in V79 Chinese hamster lung fibroblasts attached to cellulose membrane filters (42), whereas 0.1 mg · m⁻³ caused a substantial loss in the fraction of cells recovered from the filters.

Shiraishi & Bandow (43) exposed V79 cells for 2 h to concentrations of ozone ranging from 0.26 to 2.0 mg · m⁻³, which induced SCE and produced a concentration-related inhibition of cell growth. In a review paper on research at the Fudan University in China (44), it was reported that ozone induced SCE in human lymphocytes

at concentrations ranging from 0.3 to 1.5 mg · m⁻³ (treatment time not mentioned).

Effects on DNA. In a study by Rasmussen (45), V79 cells were exposed to ozone for 1 h. DNA replication, as measured by ³H-thymidine incorporation, was depressed in a concentration-dependent manner over the range 2 to 20 mg · m⁻³. Concentrations higher than 4 mg · m⁻³ were cytotoxic. The results were interpreted to indicate that ozone interacts with DNA in a manner which inhibits replication.

The induction of DNA single strand breaks was the end point chosen in three studies. In the first (46), L929 murine fibroblast cell cultures were exposed for 10 s every 30 s to a stream of ozone in oxygen at a very high concentration (25 µmol O₃ per ml = 1 200 000 mg · m⁻³).

Table 1. Genotoxic effects on microorganisms, plants and insects. (+ = positive, - = negative, *E. coli* = *Escherichia coli*, DNA = deoxyribonucleic acid)

Reference	Test organism	End point	Ozone exposure	Result
Hamelin & Chung, 1974 (20)	<i>E. coli</i> , K ₁₂	Reverse mutations	Bubbling of 0.1–2.0 mg · m ⁻³ through cell suspension; 30–60 min	+
Hamelin & Chung, 1975 (21)	<i>E. coli</i> , K ₁₂	Forward mutations	Bubbling of 0.2 mg · m ⁻³ through cell suspension; 60 min	+
L'Hérault & Chung, 1984 (23)	<i>E. coli</i> , different strains	Forward mutations, effect on the rec-lex error-prone repair system	Bubbling of 100 mg · m ⁻³ through cell suspension; 1–20 min	+
Nover & Botzenhart, 1985 (24)	<i>E. coli</i> , K ₁₂	Forward mutations	Bubbling of 1.0 mg · m ⁻³ through cell suspension; 4.5 h	+
Hamelin & Chung, 1989 (22)	<i>E. coli</i>	DNA strand breaks	Bubbling of 100 mg · m ⁻³ through cell suspension; 15 min	+
De Mik & De Groot, 1978 (27)	<i>E. coli</i> , MRE 162	DNA strand breaks	0.08 mg · m ⁻³ ; 30–45 min in aerosolized bacteria	-
Song & Chung, 1983 (25)	<i>Bacillus subtilis</i>	DNA repair	Bubbling of 10–100 mg · m ⁻³ through cell suspension; 30 min	+
Shepson et al, 1985 (28)	<i>Salmonella</i> TA 100	Reverse mutations	1.0 mg · m ⁻³ ; 20 h	-
Victorin & Ståhlberg, 1988 (29)	<i>Salmonella</i> TA 100	Reverse mutations	0.2–4.0 mg · m ⁻³ ; 6 h	-
Dillon et al, 1992 (30)	<i>Salmonella</i> TA 102	Reverse mutations	0.04–1.0 mg · m ⁻³ ; 35 min	+
Dillon et al, 1992 (30)	<i>Salmonella</i> TA 98, TA 100, TA 104, TA 1535	Reverse mutations	0.04–1.0 mg · m ⁻³ ; 35 min	-
Dubeau & Chung, 1982 (26)	<i>Saccharomyces cerevisiae</i>	Forward and reverse point mutations, gene conversion mitotic crossing-over	Bubbling of 100 mg · m ⁻³ through cell suspension; 60 min	+
Fetner, 1958 (31)	<i>Vicia faba</i>	Chromosome-type aberrations in root meristem cells	8000 mg · m ⁻³ ; 15, 30 or 60 min	+
Fetner, 1958 (31)	<i>Vicia faba</i>	Chromatid-type aberrations	8000 mg · m ⁻³ ; 15, 30 or 60 min	-
Janakiraman & Harrey, 1976 (32)	<i>Vicia faba</i>	Chromosome aberrations in buds	4 mg · m ⁻³ ; 4 or 8 h	+
Ma et al, 1982 (34)	<i>Tradescantia</i>	Micronuclei in pollen	10 mg · m ⁻³ ; 5.5 h	-
Schairer et al, 1979 (33)	<i>Tradescantia</i>	Somatic mutations in stamen hairs	10 mg · m ⁻³ ; 6 h	+
Gichner et al, 1991 (35)	<i>Tradescantia</i>	Somatic mutations in stamen hairs	0.3–0.6 mg · m ⁻³ ; 13–15 d	-
Gichner et al, 1991 (35)	<i>Nicotiana tabacum</i>	Somatic mutations in leaves	0.2–0.6 mg · m ⁻³ ; 18 d	-
Chigusa & Nakada, 1972 (37)	<i>Drosophila</i>	Dominant lethals	54 mg · m ⁻³ ; 1–2 h, repeated exposures	+
Erdman & Hernandez, 1982 (36)	<i>Drosophila</i>	Dominant lethals	60 mg · m ⁻³ ; 3 h	+

Table 2. Studies using cell cultures. (+ = positive, – = negative, SCE = sister chromatid exchanges, DNA = deoxyribonucleic acid)

Reference	Cell line	End point	Ozone exposure	Result
Fetner, 1958 (31)	KB human cell line	Chromatid-type aberrations	16 mg · m ⁻³ ; 5 or 10 min	+
Sachsenmaier et al, 1965 (39)	Embryonic chick fibroblasts	Gross chromosome effects	1 400–14 000 mg · m ⁻³ ; 30 min	+
Gooch et al, 1976 (40)	Human lymphocytes	Chromatid-type aberrations	Bubbling of ozone through cell suspension	+
Gooch et al, 1976 (40)	Human lymphocytes	Chromosome-type aberrations	Bubbling of ozone through cell suspension	–
Guerrero et al, 1979 (41)	W1-38 human cell line	SCE	0.5–2 mg · m ⁻³ ; 1 h	+
Guerrero et al, 1979 (41)	W1-38 human cell line	Chromatid-type aberrations	0.5–2 mg · m ⁻³ ; 1 h	+ (2 mg · m ⁻³)
Guerrero et al, 1979 (41)	W1-38 human cell line	Chromosome-type aberrations	0.5–2 mg · m ⁻³ ; 1 h	–
Rasmussen & Crocker, 1982 (42)	V79 Chinese hamster cells	SCE	0.07 mg · m ⁻³ ; 1 h	–
Rasmussen & Crocker, 1982 (42)	V79 Chinese hamster cells	Inhibition of DNA replication	0.07 mg · m ⁻³ ; 1 h	–
Rasmussen, 1986 (45)	V79 Chinese hamster cells	Inhibition of DNA replication	2–20 mg · m ⁻³ ; 1 h	+
Shiraishi & Bandow, 1985 (43)	V79 Chinese hamster cells	SCE	0.26–2.0 mg · m ⁻³ ; 2 h	+
Hsueh & Xiang, 1984 (44)	Human lymphocytes	SCE	0.30–1.5 mg · m ⁻³	+
van der Zee et al, 1987 (46)	L929 mouse fibroblasts	DNA single strand breaks and interstrand cross-links	1 200 000 mg · m ⁻³ ; 2 h	+
Kozumbo & Agarwal, 1990 (48)	CCD-18Lv human lung fibroblasts and A549 human lung type II epithelial cells	DNA single strand breaks	Ozonated buffer (buffer exposed to 2 mg · m ⁻³ ; 2 h)	–
Borek et al, 1988 (47)	Human epidermal cell-line RHEK	DNA single strand breaks	10 mg · m ⁻³ ; 10 min	–
Borek et al, 1986 (49)	Primary hamster embryo cells and C3H/10T-1/2 mouse fibroblasts	Neoplastic transformation	10 mg · m ⁻³ ; 5 min	+
Thomassen et al, 1991 (52)	Primary rat tracheal epithelial cells	Neoplastic transformation	2 or 20 mg · m ⁻³ ; 40 min	—
Thomassen et al, 1991 (52)	Primary rat tracheal epithelial cells	Neoplastic transformation	1.4 mg · m ⁻³ ; 40 min bi-weekly for 9 exposures	+

Using a DNA unwinding technique, ozone exposure for 2 h was shown to generate single strand breaks. However, with the alkaline elution technique, no increased rate of DNA elution was noted, which might have been the result of the generation of DNA-interstrand cross-links. Ozone-induced cross-links were also formed between DNA and protein. No mention was made in the paper of cytotoxic effects on the cells. However, at the ozone concentrations used, the cells must have suffered considerable cytotoxicity.

Borek et al (47) exposed the RHEK human epidermal cell line to an ozone concentration of 10 mg · m⁻³ for 10 min. DNA single strand breaks were assayed with the alkaline elution technique and alkaline sucrose gradient centrifugation. No significant induction of DNA strand breaks was detected. A high background may have contributed to the negative outcome of this study.

Control experiments with ozonated buffer were performed in a study on the formation of genotoxic reaction products by the ozonation of arylamines (48). Human CCD-18 lung fibroblasts and transformed type II epithelial A549 lung cells were used as the test organisms. Buffer that had been preexposed to an ozone concentration of 2 mg · m⁻³ did not induce DNA single strand breaks in these cell types.

Cell transformation in vitro. In a study by Borek et al (49) it was shown that ozone induced neoplastic transformation in primary hamster embryo cells and in (C3H/10T1/2) mouse fibroblast cultures, both exposed to 10 mg · m⁻³ for 5 min. Ozone acted synergistically (more than in an additive manner) with ionizing radiation, but only when delivered after the radiation (49, 50). In a similar study (51), ozone and ultraviolet radiation induced cell transformation in an additive fashion.

Preneoplastic transformation was also shown to be induced by ozone in a study by Thomassen et al (52), but only after multiple exposures to the gas. Primary rat tracheal epithelial cells were exposed to single exposures of 1 ppm or 10 ppm for 40 min or to multiple exposure of 0.7 ppm twice weekly for five weeks.

Summary. The bubbling of ozone through a suspension of bacteria or yeast cells induces forward and reverse mutations, as well as DNA strand breaks. Mutations in bacteria after airborne exposure have been more difficult to determine, although there is one positive study with *Salmonella* TA102. Chromosome aberrations have been induced in the plant *Vicia faba*, but only in one out of three studies with *Tradescantia* or *Nicotiana tabacum*. Ozone has also induced dominant lethals in *Drosophila*.

Ozone has been shown to induce chromatid-type aberrations, SCE, and neoplastic transformation in cell cultures, but data concerning single strand breaks in DNA are inconsistent.

Overall, the data discussed indicate that ozone is genotoxic to microorganisms and cell cultures, a finding in accordance with the damaging effect of ozone on naked DNA and viral DNA. Genotoxic effects in cell cultures (eg, SCE) have occurred at 0.3 to 2 mg · m⁻³.

Genotoxic effects in vivo

Animal experiments

The cited studies with animal experiments are summarized in table 3.

The first of several inhalation experiments with laboratory animals was performed by Zelac et al (53). These authors used female Chinese hamsters; four animals each were exposed to ozone at 0.48 and 0.60 mg · m⁻³ for 5 h, and four other animals served as controls. The frequency of chromosome-type aberrations was significantly higher in peripheral lymphocytes from the exposed groups, as compared with the control group, immediately after exposure and 15 d after exposure. (The increase 6 d after exposure was not significant.)

In an attempt to duplicate the positive findings of Zelac et al, Tice et al (54) exposed Chinese hamsters in groups of three to seven animals, both males and females, to 0.80 mg · m⁻³ for 5 h. An increase in chromatid-type aberrations in peripheral lymphocytes (deletions and gaps) was noted one and two weeks after the treatment, but not immediately after it. No chromosome-type aberrations were seen. Bone-marrow preparations from a few animals showed no induced aberrations. The frequencies of SCE in peripheral lymphocytes from the Chinese hamsters were not increased, nor were the SCE levels in bone-marrow cells from groups of three to four C57B16 female mice, exposed to 4 mg · m⁻³ for 6 h and examined 0, 1, 2, and 3 weeks after the exposure.

Gooch et al (40) exposed mice and hamsters to ozone. No mention was made of the number of animals used in these experiments, but male mice, strain C₃H, were exposed to 0.30 mg · m⁻³ or 0.42 mg · m⁻³ for 5 h and to 2.0 mg · m⁻³ for 2 h. No significant induction of chromosome or chromatid damage in peripheral lymphocytes resulted from the ozone exposure. Mouse spermatocyte preparations were made eight weeks after the exposure and analyzed for the presence of reciprocal translocations. No such effect was seen. Nor was there any induction of chromatid or chromosome aberrations in bone

Table 3. Genotoxic effects in laboratory animals. (+ = positive, - = negative, SCE = sister chromatid exchanges)

Reference	Species	End point	Ozone exposure	Result
Zelac et al, 1971 (53)	Chinese hamster, females	Chromosome-type aberrations in lymphocytes	0.48 and 0.6 mg · m ⁻³ ; 5 h	+
Gooch et al, 1976 (40)	Mouse, strain C ₃ H, males	Chromosome and chromatid-type aberrations in lymphocytes	0.30 and 0.42 mg · m ⁻³ for 5 h; 2.0 mg · m ⁻³ for 2 h	-
Gooch et al, 1976 (40)	Mouse, strain C ₃ H, males	Reciprocal translocations in spermatocytes	0.30 and 0.42 mg · m ⁻³ for 5 h; 2.0 mg · m ⁻³ for 2 h	-
Gooch et al, 1976 (40)	Chinese hamster	Chromosome and chromatid-type aberrations in bone marrow cells	0.46 mg · m ⁻³ for 5 h; 10 mg · m ⁻³ for 6 h	-
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromosome-type aberrations in lymphocytes	0.86 mg · m ⁻³ ; 5 h	-
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromatid-type aberrations in lymphocytes	0.86 mg · m ⁻³ ; 5 h	+
Tice et al, 1978 (54)	Chinese hamster, males + females	Chromosome and chromatid-type aberrations in bone marrow cells	0.86 mg · m ⁻³ ; 5 h	-
Tice et al, 1978 (54)	Chinese hamster, males + females	SCE in lymphocytes	0.86 mg · m ⁻³ ; 5 h	-
Tice et al, 1978 (54)	Mouse strain C ₅₇ B ₁₆ , females	SCE in bone marrow cells	4.0 mg · m ⁻³ ; 6 h	-
Zhurkov et al, 1979 (55)	Rat, males	Chromosome and chromatid-type aberrations in bone marrow cells	0.15 mg · m ⁻³ ; 8 h daily for 7 days or 5.6 mg · m ⁻³ continuously for 5 days	-
Rithidech et al, 1990 (56)	Rat, strain F334/N, females	Chromosome-type aberrations in pulmonary macrophages	0.43 and 1.3 mg · m ⁻³ ; 6 h	-
Rithidech et al, 1990 (56)	Rat, strain F334/N, females	Chromatid-type aberrations in pulmonary macrophages	0.43 and 1.3 mg · m ⁻³ ; 6 h	+
Thomassen et al, 1991 (52)	Rat, strain F334/N, males	Preneoplastic transformation in tracheal epithelial cells	0.28, 1.2 and 2.4 mg · m ⁻³ ; 6 h/day, 5 days/week, for 1, 2 or 4 weeks	-
Sills et al, 1995 (71)	Mouse, strain B6C3F ₁ , females	Mutations in <i>K-ras</i> oncogene in lung tumors	1.0 and 2.0 mg · m ⁻³ ; 6 h/day, 5 days/week, 2 years and life-time	+

marrow cells from Chinese hamsters exposed to either $0.46 \text{ mg} \cdot \text{m}^{-3}$ for 5 h or $10 \text{ mg} \cdot \text{m}^{-3}$ for 6 h.

Another negative study on chromosome aberrations (including chromatid breaks) in bone marrow cells was reported by Zhurkov et al (55). These authors exposed male white rats, five in each concentration group, to either $0.15 \text{ mg} \cdot \text{m}^{-3}$ 8 h daily for 7 d or to $5.6 \text{ mg} \cdot \text{m}^{-3}$ for 5 d. Under the latter conditions bone marrow mitotic activity was substantially depressed.

Pulmonary alveolar macrophages were used by Rithidech et al (56) to study genotoxic effects of ozone in lung cells. Female F344/N rats, five in each concentration group, were exposed to ozone at 0.20, 0.43, or $1.3 \text{ mg} \cdot \text{m}^{-3}$ for 6 h. Exposure to 0.43 and $1.3 \text{ mg} \cdot \text{m}^{-3}$ significantly increased the proportion of cells with chromatid deletions and gaps. The highest level of ozone used also induced a significant increase in the mitotic index of the macrophages. No chromosome-type aberrations were seen, however.

Thomassen et al (52) studied the potency of ozone in inducing preneoplastic transformation in rat tracheal epithelial cells after the inhalation of 0.24, 1.2, or $2.4 \text{ mg} \cdot \text{m}^{-3}$, 6 h a day, 5 d a week for one, two, or four weeks. After the exposure of male F344/N rats, the tracheal epithelium cells were isolated, plated, and scored for preneoplastic variants after five weeks of incubation. Ozone failed to cause statistically significant increases in transformation frequency, as compared with that of control rats breathing air only.

Human exposure

The cited studies involving human exposure are summarized in table 4.

In a study of the effect of ozone on pulmonary function in humans, chromosome aberrations in peripheral lymphocytes were also analyzed (57). Two persons were exposed to $1.0 \text{ mg} \cdot \text{m}^{-3}$ for 6 h and four persons to $1.0 \text{ mg} \cdot \text{m}^{-3}$ for 10 h. The subjects served as their own controls, blood samples being drawn both pre- and post-exposure. In addition, the group of four persons was also tested two weeks and six weeks after exposure. No chromosome-type aberrations were seen in any of the subjects. However, for most of them an increase in

achromatic lesions (gaps) and chromatid deletions was found after the exposure. The number of chromatid deletions seemed to be highest two weeks after the exposure. The statistical significance of the results was not evaluated, however.

Guerrero et al (41) analyzed the SCE frequency in peripheral lymphocytes from 31 male and female college students one day before and immediately after exposure to $1.0 \text{ mg} \cdot \text{m}^{-3}$ of ozone for 2 h. There was no significant difference in the mean SCE frequency per chromosome for all the subjects before, compared with after, exposure. However, in this study, the individual SCE levels were not reported, nor was there any mention of the smoking habits of the subjects.

McKenzie et al (58) have also performed cytogenetic studies with ozone-exposed subjects. In their initial study 26 healthy, nonsmoking male college students were exposed to $0.8 \text{ mg} \cdot \text{m}^{-3}$ for 4 h. Blood samples were taken immediately before and after the exposure, as well as three days, and two and four weeks after the exposure. The pooled results from all the subjects showed no statistically significant increase in the number of cells with chromosome or chromatid-type aberrations (including gaps) after exposure to the gas.

In a later study reported by McKenzie (59), neither ozone exposure to $1.2 \text{ mg} \cdot \text{m}^{-3}$ for 2 h nor $0.8 \text{ mg} \cdot \text{m}^{-3}$ for 4 h, both administered repeatedly for 4 d, gave statistically significant increases in structural aberrations or SCE levels immediately after, three days after, or two or four weeks after exposure (≥ 10 subjects). No experimental details were given in this paper.

Sarto & Viola (60) noticed a statistically significant increased frequency of chromatid gaps, but no other chromosome aberrations, in lymphocytes from 10 workers exposed to nearly $0.6 \text{ mg} \cdot \text{m}^{-3}$ for one to three years, when the workers were compared with 10 unexposed controls. No mention was made of the smoking habits of the subjects or possible confounding air pollutants in the workplace, which produced plastic bags.

Summary

The results derived from the in vivo cytogenetic studies with laboratory animals are contradictory. Concerning

Table 4. In vivo cytogenetic effects in humans (lymphocytes). (+ = positive, - = negative, SCE = sister chromatid exchanges)

Reference	End point	Number of individuals	Ozone exposure	Result
Merz et al, 1975 (57)	Chromosome-type aberrations	6	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h or 10 h	-
Merz et al, 1975 (57)	Chromatid-type aberrations	6	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h or 10 h	+
Guerrero et al, 1979 (41)	SCE	31	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 2 h	-
McKenzie, 1977 (58); McKenzie, 1982 (59)	Chromosome- and chromatid-type aberrations or SCE	10-30	$0.8 \text{ mg} \cdot \text{m}^{-3}$; 4 h, once or repeatedly for 4 days; $1.2 \text{ mg} \cdot \text{m}^{-3}$; 2 h	-
Sarto & Viola, 1980 (60)	Chromatid gaps	10	$0.6 \text{ mg} \cdot \text{m}^{-3}$; 1-3 years	+
Sarto & Viola, 1980 (60)	Other chromosome- and chromatid-type aberrations	..	$0.6 \text{ mg} \cdot \text{m}^{-3}$; 1-3 years	-

chromosome aberrations in lymphocytes of Chinese hamsters, one study noted chromosome-type aberrations, but another study noted only chromatid-type aberrations. SCE were not induced by the same treatment. In mice, no aberrations of either type were observed. No cytogenetic effects have been seen in bone-marrow cells or spermatocytes. Only one study with lung cells has been published, in which chromatid-type aberrations in alveolar macrophages were induced by ozone exposure of $0.43 \text{ mg} \cdot \text{m}^{-3}$ for 6 h.

The experimental and epidemiologic studies with human subjects do not allow a conclusion regarding the cytogenetic effects of ozone in lymphocytes. In only one study did the subjects serve as their own controls, but relatively few persons participated in this study. In two of the other three studies smoking or other potential confounding factors were not taken into consideration.

Tumor induction

The cited studies on tumor induction, except those with coadministration of known carcinogens, are summarized in table 5.

Stokinger (61) reported that ozone induced lung tumors (adenomas) in a susceptible strain of mice (strain

not mentioned), exposed to $2 \text{ mg} \cdot \text{m}^{-3}$ daily for 15 months. However, no experimental details were presented in this article.

Werthamer et al (62) exposed Swiss-Webster mice (sensitive to the development of lung adenomas) to ozone ($9.0 \text{ mg} \cdot \text{m}^{-3}$) during 2 h every third day for a period of up to 75 d. A statistically significant increase in cellular alterations in the lung between ozone-exposed mice and control animals was reported, although the control data were not shown. The cellular alterations included the induction of hyperplasia, metaplasia, and adenomas. There were also indications of the induction of inflammatory reactions.

Hassett et al (63) exposed female mice (strain A/J, sensitive to the development of lung adenomas) to an ozone concentration of either $0.62 \text{ mg} \cdot \text{m}^{-3}$ intermittently 103 h a week every other week for six months or $1.0 \text{ mg} \cdot \text{m}^{-3}$ 102 h a week during the first week of each month for six months. In these studies, animals were killed five months and three months, respectively, after the final ozone exposure. In both studies the presence of tumor nodules (adenomas) on the pleural surface of the lung was reported to be significantly higher in the exposed animals, as compared with those in control animals. However, in a later paper from the same research group (64), only the increase in the second study was referred to as being statistically significant. The tumor

Table 5. Induction of lung tumors in experimental animals. (+ = positive, - = negative, (+) = originally reported as positive, but later (64) as not statistically significant)

Reference	Species	End point	Ozone exposure	Result ^a					
				Males			Females		
				A	C	A + C	A	C	A + C
Stokinger, 1965 (61)	Mice (species not mentioned)	Adenomas	$2 \text{ mg} \cdot \text{m}^{-3}$; 15 months	+					
Werthamer et al, 1970 (62)	Mice, Swiss Webster	Adenomas	$9 \text{ mg} \cdot \text{m}^{-3}$; 2 h every third day, 75 d	+					
Hassett et al, 1985 (63)	Mice, A/J, females	Adenomas	$0.62 \text{ mg} \cdot \text{m}^{-3}$; 103 h a week, every other week, 6 months	(+)					
Hassett et al, 1985 (63)	Mice, A/J, females	Adenomas	$1.0 \text{ mg} \cdot \text{m}^{-3}$; 102 h a week, first week of each month, 6 months	+					
Last et al, 1987 (65)	Mice, A/J, males	Adenomas	0.8 and $1.6 \text{ mg} \cdot \text{m}^{-3}$; 8 h a day, 18 weeks	+	($1.6 \text{ mg} \cdot \text{m}^{-3}$)				
Last et al, 1987 (65)	Mice, Swiss Webster, males	Adenomas	0.8 and $1.6 \text{ mg} \cdot \text{m}^{-3}$; 8 h a day, 18 weeks	-					
Ichinose & Sagai 1992 (69)	Rats, Wistar, males	Adenomas and carcinomas	$0.1 \text{ mg} \cdot \text{m}^{-3}$; 10 h a day, 13 months	-					
Witschi et al, 1993 (68)	Hamsters, Syrian Golden, males	Adenomas and carcinomas	$1.6 \text{ mg} \cdot \text{m}^{-3}$; 23 h a day, 7 d/week, 6 months	-					
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	0.24 , 1.0 and $2.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h/d, 5 d a week, 105 weeks (2 years)	-	-	-	-	+ ^a	+ ^a
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	1.0 and $2.0 \text{ mg} \cdot \text{m}^{-3}$; 6 h/d, 5 d a week, 130 weeks (life-time)	-	+ ^b	-	+ ^a	-	-
NTP, 1993 (70)	Mice, B6C3F, males and females	Adenomas (A), carcinomas (C) or both (A+C)	Both studies combined (2 year and life-time)	-	+ ^a	+ ^a	+ ^a	-	+ ^a
NTP, 1993 (70)	Rats, F344/n, males and females	Adenomas (A), carcinomas (C) or both (A+C)	As for mice except 104 and 124 weeks	-	-	-	-	-	-

^a $2.0 \text{ mg} \cdot \text{m}^{-3}$

^b 1.0 , $2.0 \text{ mg} \cdot \text{m}^{-3}$.

inducing activities of urethane, as a single intraperitoneal injection one week before exposure to ozone was begun, was not significantly enhanced by ozone in the first experiment. However, when the injection of the carcinogen urethane followed immediately after each ozone exposure in the second experiment, a higher than expected tumor incidence was produced.

In a similar study, Last et al (65) exposed both A/J and Swiss-Webster male mice to either 0.8 or 1.6 mg · m⁻³ for 8 h a night, 7 nights a week for 18 weeks. Upon examination, 1.6 mg · m⁻³, but not 0.8 mg · m⁻³, induced a significant increase in lung adenomas in the A/J mice, but not in the more resistant Swiss-Webster strain. In one part of the experiment, the animals were pretreated by a single intraperitoneal injection of urethane one day before the ozone exposure started. In these animals, ozone actually caused a decrease in the number of tumors per lung. This finding can be explained in terms of a cytotoxic action of ozone upon initiated cells destined to grow into tumors (66, 67). In contrast, the cocarcinogenic action of ozone observed by Hassett et al (63) may be explained by the cell proliferative activity of ozone, which might have expanded the cell population at risk to undergo transformation. Thus the sequence of exposure can determine whether the effects of ozone on lung tumor development are beneficial or detrimental (66).

In further examining the modulation of lung tumors by ozone, Witschi et al (68) treated male Syrian Golden hamsters with subcutaneous injections of N-nitrosodiethylamine twice a week for six months, during which the animals were continuously exposed to 1.6 mg · m⁻³ (23 h per day, 7 days per week). It was found that the animals kept in ozone developed half as many peripheral lung tumors as did the animals kept in air. However, the difference between these groups was not statistically significant. Tumors of the trachea, bronchi, nasal cavity, and liver developed with the same incidence irrespective of whether the animals were exposed to ozone or not. No tumors were found in a group of animals (N = 16) injected with sodium chloride and exposed to ozone.

In another experiment (69) male Wistar rats were given a single intraperitoneal injection of N-bis(2-hydroxypropyl)nitrosamine and were then exposed to an average ozone concentration of 0.01 mg · m⁻³ (10 h per day) or 0.05 mg · m⁻³ (10 h per day), supplemented with a nitrogen dioxide concentration of 0.75 mg · m⁻³ (24 h per day), for a total of 13 months. The animals were then kept in clean air for another 11 months before being killed. There were no lung tumors in the control groups treated with ozone only or the nitrosamine only (N = 36). However, in the groups treated with the nitrosamine + ozone or nitrosamine + ozone + nitrogen dioxide, three and five pulmonary tumors (adenomas and adenocarcinoma) were detected, respectively. The latter enhancement was statistically significant.

The results of a thorough cancer study from the National Toxicology Program (NTP) in the United States (70) with life-long inhalation exposure to ozone were recently presented. Male and female F344/N rats were exposed in groups of 50 animals to an ozone concentration of 0.24, 1.0, or 2.0 mg · m⁻³ of ozone, 6 h a day, 5 days a week for 104 weeks and to 1.0 and 2.0 mg · m⁻³ of the gas for 124 weeks. Male and female B6C3F₁ mice were similarly exposed for 105 weeks (2 years) and 130 weeks (life-time). There was no increased tumor incidence in the rat study. However, ozone induced alveolar or bronchiolar tumors (adenomas and carcinomas) in mice. For the male mice, the increase was statistically significant ($P \leq 0.05$) for carcinomas at 1.0 and 2.0 mg · m⁻³ in the life-time study, and for carcinomas and adenomas + carcinomas at 2.0 mg · m⁻³ when the results from the two-year and the life-time studies were combined. In the two-year study there was a significant positive trend in the incidence of adenomas + carcinomas, although no single concentration group was significantly increased. For female mice the increase was statistically significant ($P \leq 0.05$) for carcinomas and adenomas + carcinomas at 2.0 mg · m⁻³ in the two-year study, for adenomas at 2.0 mg · m⁻³ in the life-time study, and for adenomas and adenomas + carcinomas at 2.0 mg · m⁻³ when the studies were combined.

In the conclusions from the NTP study it is stated that "there was some evidence of carcinogenic activity" of ozone in the female mice, but "equivocal evidence of carcinogenic activity" in male mice. The latter statement seems to be mainly based on the fact that the combined tumor incidence in the two-year study was within the historical range (Boorman, 1994, personal communication). However, the combined analysis of both the two-year and the life-time studies would also argue for a carcinogenic effect in the male mice study.

The adenomas and carcinomas induced in the female mice in the NTP study were investigated for mutations in the *K-ras* proto-oncogene and the *p53* tumor suppressor gene (71). *K-ras* mutations were detected in 73% of the ozone-induced tumors, as compared with 33% of the lung tumors from control animals; this finding suggests that ozone causes direct or indirect DNA damage within the *K-ras* oncogene. No enhanced expression of the *p53* protein was detected with immunohistochemical methods.

The NTP study (70) also included a separate experiment in which male rats were injected with a nitrosamine (NNK) three times per week for the first 20 weeks and exposed to an ozone concentration of 1.0 mg · m⁻³ for 6 h a day, 5 days a week for 105 weeks. Ozone did not significantly enhance the incidence of lung tumors in these animals.

The effect of ozone on cancer cell metastasis in the lung of mice has been studied in two papers. Kobayashi

et al (72) exposed male C3H/He mice to ozone concentrations of 0.2, 0.4, 0.8, and 1.6 mg · m⁻³ continuously for 1, 3, 7 and 14 d. A suspension of fibrosarcoma NR-FS cells was then infused into the animals via the tail vein. The animals were then sacrificed after 14 d. A significantly increased rate of pulmonary metastasis was observed in the ozone-exposed mice. The maximal enhancement occurred in mice exposed to 1.6 mg · m⁻³ for 1 d. The prolonged exposure (14 d) gradually suppressed this enhancement. In a similar study (73), no significantly enhanced cancer cell colonization of lungs was observed when male C57 BL/6 mice were exposed to 0.3 or 0.6 mg · m⁻³ for 7 h a day, 5 days a week, for a total period of 12 weeks and then infused with melanoma cells (B16F10R1).

Other effects seen in animal experiments may be of relevance when the tumor-inducing activity of ozone is discussed. Cell proliferation (increased DNA replication) and the induction of metaplasia or hyperplasia have been induced in parts of the nasal epithelium in rats and monkeys (74–76). Ozone also induces cell proliferation, assessed as an increase in the DNA-labeling index and mitotic index of type II epithelial alveolar lung cells, alveolar macrophages, and lymphocytes of the lung (77–81). Epithelial hyperplasia in the lung have been observed in the aforementioned long-term studies with ozone (62, 63, 65, 66). In the life-time NTP study (70) the ozone exposure was associated with the induction of hyperplasia and squamous metaplasia in the nose and alveolar metaplasia (extension of bronchial epithelium into the centriacinar alveolar ducts) in the lungs of both rats and mice. There was also interstitial fibrosis in the lung of rats and infiltration of inflammatory cells in mice. However, there was no mention of the induction of hyperplasia in the lung of either rats or mice.

In summary, there is only one cancer study with full life-long exposure to ozone by inhalation. In this study, lung adenomas or carcinomas were induced in B6C3F₁ mice at an ozone concentration of 2.0 mg · m⁻³. This result agrees with the earlier findings of the induction of lung adenomas in short-term bioassays with strain A/J mice. In contrast, ozone has failed to induce tumors in rats or hamsters.

No conclusion can be drawn concerning a possible cocarcinogenic or promoting effect of ozone. An increase as well as a decrease or no effect on the carcinogenicity of known carcinogens has been demonstrated.

Summary and concluding remarks

Ozone has been shown to be genotoxic in vitro. Mutations and DNA strand breaks occurred in bacteria and yeast cells, mainly in experiments in which ozone was

bubbled through suspension cultures of cells, in which case hydroxy radicals and hydrogen peroxide are also formed. The bacteriotoxicity of ozone complicates the demonstration of mutagenicity in *Salmonella*, but a positive response has been observed with ozone in air with strain TA 102 in one study. In cell cultures, chromatid-type chromosome aberrations, sister SCE, and neoplastic transformation have been demonstrated. SCE occurred at 0.3 to 2 mg · m⁻³.

The results from in vivo cytogenetic studies with laboratory animals after inhalation exposure are contradictory. Chromosome aberrations in lymphocytes, but not SCE, have been found in Chinese hamsters, but not in mice. No cytogenetic effects were reported for bone marrow cells or spermatocytes. As ozone is a very reactive compound, which is probably not taken up unreacted from the lung, the positive finding of chromatid-type aberrations in lung macrophages in rats (at 0.43 mg · m⁻³ for 6 h) is important from the risk assessment point of view.

The lung is the target organ for the carcinogenic effects of ozone. Lung adenomas have been induced in the sensitive A/J strain of mice. A recent life-time inhalation study demonstrated an induction of alveolar or bronchiolar adenomas and carcinomas in B6C3F₁ mice, but not in F344/N rats, at an ozone concentration of 2 mg · m⁻³ (70). An increased frequency of mutations in the *K-ras* protooncogene of tumors in ozone-treated female mice suggests a genotoxic origin of the ozone-induced tumors. These findings lead to the conclusion that a possible carcinogenic risk of ozone to humans should not be overlooked.

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Controlled human studies of ozone exposure

by Gunnar Bylin

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_{1,0}. When the airways are obstructed the FEV_{1,0} is decreased. The FEV_{3,0}, 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_{1,0} 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,

FEF₂₅₋₇₅, 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_{1.0} and FVC. However, these indices are less reproducible and show greater normal variation than do FEV_{1.0} 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_{1.0} 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_{1.0} 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_{1.0} 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_{1.0}, FEF₂₅₋₇₅ and SG_{aw}. 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_{1.0} and FEF₂₅₋₇₅ 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_{aw} 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.^a (BSA = body surface area, CE = continuous exercise, FEF = forced expiratory flow, VC = vital capacity, FEF₂₅₋₇₅ = forced expiratory flow between 25% and 75% of VC, FEV_{1.0} = forced expiratory volume in 1 s, FEV_{3.0} = forced expiratory volume in 3 s, FVC = forced VC, IC = inspiratory capacity, IE = intermittent exercise, MVV = maximum voluntary ventilation, NO₂ = nitrogen dioxide, O₂ = oxygen, O₃ = ozone, SG_{aw} = specific airway conductance, SO₂ = sulfur dioxide, SR_{aw} = specific airway resistance, TLC = total lung capacity, V = ventilation, V₂₅ = ventilation at 25% VC, V_{50%} = ventilation at 50% VC, V_E = minute ventilation, V_T = total volume, VO₂ max = maximal oxygen uptake)

Reference	Ozone concentration (μg · 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)	0 500 1000 1500	2 h	.	8 men & 5 women	Healthy adults	FVC decreased with 1000 and 1500 μg · m ⁻³ O ₃ exposure compared with filtered air
Silverman et al, 1976 (2)	0 740 1000 1500	2 h	.	20 men & 8 women	Young healthy adults	Decrease in FEV _{1.0} , V ₂₅ and V ₅₀ with 1500 μg · m ⁻³ O ₃ exposure compared with filtered air
<i>Healthy exercising adult subjects</i>						
Linn et al, 1986 (4)	0 160 200 240 280 320	2 h	IE (4×15 min at V _E =68 l · min ⁻¹)	24 men	Young, healthy adults	No significant changes in pulmonary function measurements
Horstman et al, 1990 (15)	160 200 240	6.6 h	IE (6×50 min at V _E =39 l · min ⁻¹)	22 men	Healthy nonsmokers	FVC and FEV _{1.0} decreased throughout the exposure; FEV _{1.0} decrease at end of exposure was 7.0%, 7.0%, and 12.3%, respectively; FEV _{1.0} change >15% occurred in 3, 5, and 9 subjects at 160, 200, and 240 μg · m ⁻³ , respectively
McDonnell et al, 1991 (17)	160 200	6.6 h	IE (6×50 min at V _E =40 l · min ⁻¹)	38 men	Healthy nonsmokers (mean age=25 years)	FEV _{1.0} decreased 8.4% at 160 μg · m ⁻³ and 11.4% at 200 μg · m ⁻³
Kulle et al, 1985 (13)	0 200 400 500	2 h	IE (4×14 min on treadmill at mean V _E =70 l · min ⁻¹)	20 men	Young, healthy nonsmokers	FVC, FEV _{1.0} , FEF ₂₅₋₇₅ , SG _{aw} , IC, and TLC all decreased with increasing O ₃ concentration, and increasing time of exposure; threshold for response was above 200 μg · m ⁻³ but below 300 μg · m ⁻³
Schelegle & Adams, 1986 (5)	0 240 360 480	1 h	Competitive simulation exposures at mean V _E =87 l · min ⁻¹	10 men	10 highly trained competitive cyclists	Decrease in FVC and FEV _{1.0} for O ₃ exposure of 360 and 480 μg · m ⁻³ compared with filtered-air exposure; decrease in exercise time for subjects unable to complete the competitive simulation at 360 and 480 μg · m ⁻³ , respectively
McDonnell et al, 1983 (14)	0 240 360 480 600 800	2.5 h	IE (4×15 min of treadmill exercise at V _E =65 l · min ⁻¹)	20 men 22 men 20 men 21 men 20 men 29 men	Young, healthy adults	Decrease in FVC, FEV _{1.0} , and FEF ₂₅₋₇₅ at an O ₃ level of 240 μg · m ⁻³ , decrease in V _T and increase in breathing frequency and SR _{aw} at 480 μg · m ⁻³
McDonnell et al, 1985 (22)	240 360 480 600 800	2×2.5 h	IE (4×15 min of treadmill exercise at V _E =35 l · min ⁻¹ · m ⁻² BSA)	8 men 8 men 5 men 5 men 6 men	Young, healthy adults	Pulmonary function variables SR _{aw} and V _E were not significantly different in repeat exposures, indicating that the response to 360 μg · m ⁻³ or higher is reproducible
McDonnell et al, 1993 (23)	240 360 480 600 800	2×2.5 h	IE (4×15 min of treadmill exercise at V _E =35 l · min ⁻¹ · m ⁻² BSA)	290 men	Young, healthy adults	O ₃ concentration and age predicted FEV _{1.0} decrements (ie, older subjects being less responsive to O ₃)
Seal et al, 1993 (35)	0 240 360 480 600 800	2.5 h	IE (4×15 min of treadmill exercise at V _E =25 l · min ⁻¹ · m ⁻² BSA)	15—17 each of white men, black men, white women & black women	Young, healthy whites and blacks	Decreases in FEV ₁ for all levels of O ₃ increase in SR _{aw} with 360 μg · m ⁻³
Gong et al, 1986 (6)	0 240 400	1 h	CE (mean V _E =89 l · min ⁻¹)	15 men 2 men	Highly trained competitive cyclists	Decrease in V _E max, VO ₂ max, V _T max, work load, ride time, FVC, and FEV _{1.0} with an O ₃ exposure of 400 μg · m ⁻³ , but not significant with 240 μg · 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 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 O_3 concentration change with a lag time of $\approx 2 \text{ h}$; 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) 30 (13 men & 17 women)	Healthy nonsmokers (age 22—41 years), 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 O_3 exposure
Avol et al, 1984 (10)	0 320 480 640	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)	0 400	4 h	IE (4×50 min cycle ergometry or treadmill running at $V_E = 40 \text{ l} \cdot \text{min}^{-1}$)	11 men 3 women (filtered-air exposure); 9 men 3 women (O_3 exposure)	Adult, healthy nonsmokers	Decrease in FVC, $\text{FEV}_{1.0}$, V_T , and SR_{aw} and increase in breathing frequency
Adams & Schelegle, 1983 (7)	0 400 700	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 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 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)	0 420	1 h	CE (75% $\dot{V}\text{O}_2$ max)	6 men & 1 women	Well-trained cyclists	Decrease in FVC, $\text{FEV}_{1.0}$, FEF_{25-75} , and MVV with an O_3 level of $420 \mu\text{g} \cdot \text{m}^{-3}$
Folinsbee & Horvath, 1986 (9)	0 500	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 O_3 level of $500 \mu\text{g} \cdot \text{m}^{-3}$
Hazbun et al, 1993 (91)	0 500	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)	0 600	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 SR_{aw} 1 h post- O_3 exposure
Gerrity et al, 1993 (101)	0 800	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 O_3 uptake efficiency in the lower respiratory tract fell by 9% during O_3 exposure

^a 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 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 l $\cdot$ 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_{1.0} 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_{1.0} and the probability of a lower respiratory symptom. The authors estimated that a 10% decrease in FEV_{1.0} 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_{1.0}, 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_{1.0} 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 β_2 -stimulating agent possessing bronchodilatory properties. No effect of salbutamol was seen on either FVC or FEV_{1.0}, 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_{1.0}, or FEF₂₅₋₇₅. The authors concluded that the contribution of β -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 FEF₂₅₋₇₅, 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_{aw}, and also decreases in FVC and FEV_{1.0} 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.^a (BSA = body surface area, CE = continuous exercise, FEF₂₅₋₇₅ = forced expiratory flow between 25% and 75%, FEV_{1.0} = forced expiratory volume in 1 s, FEV_{3.0} = forced expiratory volume in 3 s, FVC = forced vital capacity, IC = inspiratory capacity, IE = intermittent exercise, NO₂ = nitrogen dioxide, O₂ = oxygen, O₃ = ozone, PD = provocative dose, SO₂ = sulfur dioxide, SR_{aw} = specific airway resistance, V = ventilation, V_E = minute ventilation, Vmax₅₀ = maximum expiratory flow at 50% of the vital capacity)

Reference	Ozone concentration (µg · m ⁻³)	Exposure duration	Activity	Number and gender of subjects	Subjects characteristics	Observed effect(s)
<i>Subjects with allergic rhinitis</i>						
McDonnell et al, 1987 (50)	0 360	2 h	IE (4×15 min)	26 men	History of allergic rhinitis	Increased respiratory symptoms, SR _{aw} , and reactivity to histamine and decreased FVC, FVC _{1.0} and FEF ₂₅₋₇₅
Bascom et al, 1990 (51)	0 1000	4 h	Rest	6 men 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 ₃ and filtered air exposure
<i>Adult subjects with asthma</i>						
Weymer et al, 1994 (56)	0 200 500	2 h	Light IE (2×15 min on treadmill, V _E = 10–20 l · min ⁻¹)	7 men	Stable mild asthmatics with FEV _{1.0} >70% and increased methacholine responsiveness	No significant differences in FEV _{1.0} or postexposure exercise challenge
Koenig et al, 1990 (81)	0 240	0.75 h	IE (V _E = 30 l · min ⁻¹ followed by 15 min of exercise while inhaling SO ₂ (300 µg · m ⁻³))	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 ₂ or O ₃ alone did not cause significant changes in pulmonary function; O ₃ followed by SO ₂ resulted in a significant decrease in FEV _{1.0} (8%) and Vmax ₅₀ (15%) and a significant increase in total respiratory resistance (19%)
McBride et al, 1994 (54)	0 210 480	1.5 h	IE (V _E = 25 l · min ⁻¹)	4 men, 4 women (nonasthmatics); 5 men & 5 women (asthmatics)	Physician-diagnosed asthma confirmed with methacholine 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 ₃ exposure in asthmatics only
Linn et al, 1994 (20)	0 240	6.5 h/day	IE [6×50 min (2 days of exposure) and V _E = 31 l · min ⁻¹ (asthmatic) and V _E = 34 l · min ⁻¹ (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 ₂₀ ; mild to severe asthmatics	FEV _{1.0} decreased 8.6% in asthmatics and 1.7% in nonasthmatics, with difference between the two groups not being significant
Molfino et al, 1991 (82)	0 240	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)	0 400	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)	0 500	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)	0 800	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 ₃ in asthmatics; increase in airway responsiveness to methacholine in asthmatics with O ₃ and filtered air; asthmatic subjects had significantly greater decreases in FEV _{1.0} and FEF ₂₅₋₇₅ with O ₃ exposure than did nonasthmatic subjects
<i>Adolescent subjects with asthma</i>						
Koenig et al, 1985 (57)	0 240	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)	0 240	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 O_3 exposure; no significant changes with combined O_3 - NO_2 exposure
Koenig et al, 1987 (58)	0 240 360	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 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)	0 240	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 O_2 saturation
Linn et al, 1983 (61)	0 360 500	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 O_2 saturation
Solic et al, 1982 (63)	0 400	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 O_2 saturation
Kehrl et al, 1985 (62)	0 600	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 O_2 saturation
Kulle et al, 1984 (64)	0 820	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 FEV_3 with an O_3 exposure of $820 \mu\text{g} \cdot \text{m}^{-3}$
<i>Subjects with heart disease</i>						
Superko et al, 1984 (65)	0 400 600	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

^a 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 SR_{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 FEF_{25-75} 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 $\text{FEV}_{1.0}$ in 30 subjects with asthma, starting from the third hour of exposure (20). The ozone-induced decrease in $\text{FEV}_{1.0}$ among the asthmatic subjects, whether expressed in liters or in percentage of preexposure $\text{FEV}_{1.0}$, did not significantly differ from the $\text{FEV}_{1.0}$ 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 $\text{FEV}_{1.0}$ 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_2) (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 $\text{FEV}_{3.0}$ 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 $\text{FEV}_{1.0}$ 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_{1.0}) after repeated daily exposure to ozone.^a (CE = continuous exercise, IE = intermittent exercise V_E = minute ventilation, O₃ = ozone)

References	Ozone concentration (µg · m ⁻³)	Exposure duration and activity ^b	Number of subjects	Percent change in FEV _{1.0} 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 ^c	-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 ^d	-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	—

^a This table is based on table 7-7 in reference 1.^b Exposure duration and intensity of IE or CE were variable; V_E (number in parentheses) given in liters per minute or as a multiple of resting ventilation.^c Subjects especially sensitive on prior exposure to 820 µg · m⁻³ O₃ as evidenced by a decrease in FEV_{1.0} of more than 20%. These nine subjects were a subset of the total group of 21 persons used in this study.^d 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_{1.0}, PEF, or SR_{aw}, 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⁻³, 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⁻³ for 1 to 3 h with intermittent exercise also caused increased

Table 4. Changes in airway responsiveness following ozone exposures.^a (BSA = body surface area, FEV_{1.0} = forced expiratory volume in 1 s, FVC = forced vital capacity, IE = intermittent exercise, O₃ = ozone, PC_{100SRaw} = provocative concentration_{100SRaw}, R_{aw} = airway resistance, SO₂ = sulfur dioxide, SR_{aw} = specific airway resistance, V_E = minute ventilation, Vmax₅₀ = maximum expiratory flow at 50% of the vital capacity)

Reference	Ozone concentration (µg · m ⁻³)	Exposure duration	Activity	Number and gender of subjects	Subject characteristics	Observed effect(s)
<i>Normal subjects</i>						
Horstman et al, 1990 (16)	0 160 200 240	6.6 h	IE at ≈ 39 l · min ⁻¹	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 _{aw} after exposure to O ₃ at 160, 200 and 240 µg · m ⁻³ , respectively
Gong et al, 1986 (6)	0 240 400	1 h	1 h at V _E = 89 l · min ⁻¹ followed by 3—4 min at ≈ 150 l · min ⁻¹	15 men & 2 women	Elite cyclists	Greater than 20% increase in histamine responsiveness in one subject at an O ₃ level of 240 µg · m ⁻³ and in 9 subjects at 400 µg · m ⁻³
Folinsbee et al, 1988 (18)	0 240	6.6 h	IE at ≈ 25 l · min ⁻¹ · m ⁻² BSA	10 men	Nonsmokers 18—33 years old	Approximate doubling of mean methacholine responsiveness after exposure; on an individual basis, no relationship between O ₃ -induced changes in airway responsiveness and FEV _{1.0} 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 _{aw} to a 10-breath histamine aerosol challenge after exposure to O ₃ at 800 µg · m ⁻³ , but no change at 400 µg · m ⁻³ ; 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)	0 800	3 h/d for 5 d in a row		13 men 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 1200	2 h	Rest	5 men & 3 women	Nonsmokers 22—30 years old	300% increase in histamine-induced change in R _{aw} 5 min after O ₃ 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 1200	2 h	IE at 2× resting	11 men & 5 women	9 atopic & 7 nonatopic, nonsmokers	Ten-breath methacholine or histamine challenge increased SR _{aw} ≥150% in 16 nonasthmatics after O ₃ . The increased responsiveness resolved after 24 h
<i>Asthmatic subjects</i>						
Koenig et al, 1990 (81)	240 O ₃ -300 SO ₂ 240 O ₃ -240 O ₃ air-300 SO ₂	45 min in first atmosphere and 5 min in second	IE	8 men & 5 women	Asthmatic, 12—18 years old	Greater declines in FEV _{1.0} and Vmax ₅₀ and greater increase in respiratory resistance after O ₃ -SO ₂ than after O ₃ -O ₃ or air-SO ₂
Molfino et al, 1991 (82)	Air-antigen 240 O ₃ -antigen	1 h	Rest	4 men & 3 women	Asthmatic, 21—64 years old	Increased bronchoconstrictor response to inhaled ragweed or grass after O ₃ exposure compared with air
Kreit et al, 1989 (52)	0 800	2 h with IE at V _E = 53—55 l · min ⁻¹		8 men & 10 women	9 asthmatics (5 women & 4 men) and 9 healthy persons (5 women & 4 men), 18—34 years old	Decreased PC _{100SRaw} from 33 mg · ml ⁻¹ to 8.5 mg · ml ⁻¹ in healthy subjects after O ₃ ; PC _{100SRaw} fell from 0.52 mg · ml ⁻¹ to 0.19 mg · ml ⁻¹ in asthmatic subjects after exposure to O ₃ and from 0.48 mg · ml ⁻¹ to 0.27 mg · ml ⁻¹ after exposure to air

^a 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⁻³ 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⁻³ 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 SR_{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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Epidemiologic studies on ozone

by Fredrik Nyberg & Göran Pershagen

Epidemiologic studies on the detrimental health effects of ozone have a long history. In early studies, exposure was often expressed in terms of the ambient concentration of total photochemical oxidants, while more recent studies use direct measurement of ozone concentrations. Most of the early studies were performed in locations where photochemical pollution was a major problem associated with high oxidant levels, and ozone constituted a chief component of the pollution mixture. With the later improved oxidant measuring methods, in particular, the oxidant levels in parts per billion and the true ozone levels are thus usually very close (1).

Ozone concentrations are sometimes expressed as parts per billion or parts per million, but in recent studies more frequently as micrograms per cubic meter. The conversion factor of parts per billion to micrograms per cubic meter varies according to air pressure (weather, altitude) and temperature (1, 2). A conversion factor of 2 is usually adequate and is used in most studies, as it has also been in this report. However, for Mexico City, where several studies originated, a factor of 1.5 was used because of the extremely high altitude (2).

The epidemiologic evidence relates to ozone exposures in ambient air, where ozone is only one component in a complex mixture of potentially noxious substances, with which it can correlate. It can also correlate with meteorological variables such as temperature. It is thus difficult to define etiologic relationships. In this review, mainly studies which provide data on actual ozone concentrations have been included, and these form the basis for the risk assessment.

Epidemiologic studies of acute health effects of ozone fall into several different groups. Individual-level time-series studies use repeated measurements on the same person over a period of time, in effect using the person as his or her own control, to examine short-term effects. The usual clinical end points evaluated are lung function and symptoms, especially respiratory symptoms. Aggregate-level time-series studies evaluate exposure and short-term temporal variations in outcomes such as mortality, hospital admissions, or outpatient visits in a population as a whole, and the measure of the effect is the number of outcomes in the population in successive time periods. In certain studies simple group comparisons are used, such as the proportion of persons who experience symptoms on days with high ozone levels versus days with low levels. Chronic effects are best elucidated in longitudinal studies, for example, with a cohort design. Cross-sectional studies are also used to evaluate chronic

effects, but it is difficult to separate the effects of short-term and long-term exposure in this type of study.

In epidemiologic research, the relative risk (RR) (ie, the ratio of disease incidence among the exposed to that of the unexposed population) is often the parameter of interest. Nowadays, regression techniques are commonly used to estimate the relative risk and simultaneously control for other exposure factors of relevance. The odds ratio (OR) is equivalent to the relative risk in studies of the case-referent type, given that referents are adequately sampled. In other cases, the odds ratio is also normally a good approximation of the parameter of interest (relative risk, rate ratio, prevalence ratio), particularly if the outcome studied is rare in the population. Rather than P-values, confidence intervals (CI) are usually computed for point estimates. A value of $P < 0.05$ (two-sided test) corresponds to a 95% CI that does not include 1 for an odds ratio or relative risk.

The epidemiologic evidence concerning ozone exposure covers a wide range of detrimental health effects, from acute symptoms, annoyance reactions, and lung function changes to chronic pulmonary disease and effects on hospital admissions and mortality. This review focuses on studies relevant to the risk assessment of low-level exposure to the gas in the general environment. Effects of short-term exposure constitute the main part of the review. First, symptom reports in relation to ozone levels are considered. Pulmonary function disturbances are treated separately for adults and children. Hospital admissions and physician visits, as a reflection of disease or symptom exacerbation, are then discussed. Subsequently, effects on daily mortality are considered, followed by a section on functional or morphological effects. Finally, a section on the effects of long-term exposure covers such outcomes as chronic pulmonary disease and asthma and lung cancer, as well as the long-term effects on mortality the levels of and acute and chronic morbidity.

Symptoms and symptom exacerbation

Temporal variations in symptoms such as eye irritation, cough, phlegm, and chest discomfort are examined in this section, as well as symptom exacerbation among asthmatics and patients with other respiratory conditions. Many studies measure both symptoms and lung function, but only the results concerning symptoms are presented in this section.

Symptoms in healthy subjects

Studies on Los Angeles student nurses. Hammer et al followed 102 student nurses from Los Angeles and Santa Barbara during 931 d, with an average of 61 reporting each day (3, 4). The daily 1-h maximum oxidant levels ranged up to 500 ppb (average 90 ppb). The relative risks for the prevalence of symptoms on high oxidant days (> 400 ppb) ranged from 1.4 for headache to 6.1 for eye discomfort. Temperature, carbon monoxide, or nitrogen dioxide did not explain the associations. The lowest effect threshold levels, as evaluated with an early "hockey stick" linear threshold regression method, were 50 ppb for headache and 155 ppb for eye discomfort.

A modern logistic regression analysis incorporating specific time-series effects replicated a significant association to oxidants for prevalence of eye discomfort and cough, but not for headache and chest discomfort (5). Only for cough was there a clear threshold for an increased prevalence near 200 ppb, close to the value of 260 ppb reported by Hammer et al (4). Since the prevalence of a condition in fact depends on both its incidence and its duration, further reanalysis used the *incidence* of new symptom episodes, defined as the presence of a symptom when the previous day was symptom-free (6). The mean level of total oxidants was 102 (SD 74) ppb and an increase of 1 SD was associated with an increased risk of chest discomfort (OR 1.17, $P < 0.001$) and eye irritation symptoms (OR 1.20, $P < 0.001$). There appeared to be a threshold for the risk of new chest discomfort at 200 ppb. For eye discomfort, the threshold was 150 ppb, below which the risk increased less per unit of exposure. No association with any air pollutant was reported for new cough episodes. However, oxidants were significantly associated with the *duration* of episodes of cough, phlegm, and sore throat (7), with strong signs of a dose-response relationship for cough and phlegm continuing into concentrations well below 120 ppb. Some heterogeneity in response was seen, including a smaller effect on the duration of cough among asthmatics.

The Tucson studies. The Tucson Epidemiological Study of Airways Obstructive Disease was a large investigation of respiratory health and environmental exposures, including indoor exposures. A United States census sample of 1655 households (3805 persons) was evaluated with questionnaires, measurements of peak expiratory flow rate (PEFR), blood samples, and skin prick tests in 1972 to 1973 (8). Robertson & Lebowitz (9) reported results from the weekly diaries regarding 14 symptoms, representing acute respiratory illness, rhinitis, and other conditions, during the period 1974 to 1977. The seasonal mean ozone levels varied from $20 \mu\text{g} \cdot \text{m}^{-3}$ in the winter to $58 \mu\text{g} \cdot \text{m}^{-3}$ in the summer. No obvious correlations to symptoms were found.

In 1979 to 1981 a symptom-stratified geographic cluster subsample of 24 young (5 to 25 years) and 204 adult (45 asthmatic, 68 with obstructive airway disease, 62 allergic and 29 asymptomatic) persons was studied with daily diaries and PEFR measurements during two to four 3-month periods. At various monitoring stations the highest ozone 1-h maximum level during 1980 ranged from 180 to $239 \mu\text{g} \cdot \text{m}^{-3}$, and the annual means from 41 to $65 \mu\text{g} \cdot \text{m}^{-3}$ (10). In an initial analysis, no associations were reported between symptoms and ozone (11). A detailed assessment using spectral time-series analysis of the association of symptoms with a large number of exposure variables (12) found significant associations between ozone in early spring and wheeze and productive cough in asthmatics. In the allergic disease group ozone was related to productive cough during the summer. Path analysis methods with an extended pollen data set (13) revealed no clear association with ozone in any group.

A further study of indoor-outdoor exposures and respiratory effects collected basic data by screening questionnaires from municipal employees of Pima County, Tucson, as well as their families (2322 persons) (14). Later, a subsample of about 500 households (over 1000 persons), primarily families with children, was studied with more-detailed health questionnaires, skin prick tests, blood analyses, and pulmonary function tests. The exposure evaluation included household inventory questionnaires, personal or household samplers for nitrogen dioxide, total suspended particulates (TSP), formaldehyde, and ambient air monitoring data. Despite ozone levels continuously below $240 \mu\text{g} \cdot \text{m}^{-3}$ (the mean 8-h maximum level was $88 \mu\text{g} \cdot \text{m}^{-3}$), an increase in allergic or irritant symptoms was suggested in relation to the interaction between outdoor PM_{10} (particles $< 10 \mu\text{m}$ in diameter) and the 8-h maximum level of ozone (15). Krzyzanowski et al (16) specifically addressed the ozone effect in an evaluation of additional confounders, for a period when the ozone 1-h maximum levels never exceeded $184 \mu\text{g} \cdot \text{m}^{-3}$. The symptom prevalence rates were 25.8% and 32.7% at ozone 1-h maximum levels below and above $112 \mu\text{g} \cdot \text{m}^{-3}$, respectively ($P = 0.011$). Again, interactions between ozone and PM_{10} , as well as ozone and temperature, were indicated (ie, increased effect of ozone at high PM_{10} or on hot days).

Other American studies. For 144 elementary schoolchildren in Pennsylvania (17), who recorded symptoms daily during an eight-month period, only low temperature was significantly related to lower and upper respiratory symptoms, as well as wheezing, in multivariate analyses. The 1-h maximum levels ranged from 0 to $129 \mu\text{g} \cdot \text{m}^{-3}$ for ozone and from -22 to $+20^\circ\text{C}$ for temperature.

Krupnick et al (18) studied 290 families (572 adults and 756 children) during September to March in greater

Los Angeles. The daily 1-h maximum ozone levels were 40 to 860 (mean 198) $\mu\text{g} \cdot \text{m}^{-3}$. Statistically significant and robust effects of ambient ozone were seen on daily reported respiratory symptoms among healthy nonsmoking adults, but not among smokers or children. There were indications of a larger effect among those with chronic respiratory disease. A more-detailed analysis with 321 nonsmoking adults revealed an odds ratio of 1.22 (95% CI 1.11–1.34) for lower respiratory symptoms for a 200 $\mu\text{g} \cdot \text{m}^{-3}$ change in the 1-h maximum ozone level (19). With maximum temperature in the model, the odds ratio decreased to 1.11 (95% CI 0.99–1.25); for upper respiratory symptoms it increased slightly to 1.08 (95% CI 1.00–1.17). The odds ratios for a 200 $\mu\text{g} \cdot \text{m}^{-3}$ change in the 7-h mean ozone level were slightly higher.

In Mexico, the lung function of 148 children aged seven to nine years (83 boys and 65 girls) was tested every second week for six months (2). The ozone 1-h maximum levels were 60 to 510 $\mu\text{g} \cdot \text{m}^{-3}$ with 74% of the days above 180 $\mu\text{g} \cdot \text{m}^{-3}$. The children's reports of cough or phlegm on the day of spirometry were analyzed with the mean ozone levels 1, 2, 4, 24, 48 and 168 h before the testing. The mean 48-h ozone levels showed an association, and an increase from the lowest to the highest (+80 $\mu\text{g} \cdot \text{m}^{-3}$) gave an odds ratio of 1.70 (95% CI 0.98–2.93) for cough or phlegm.

For 83 children in Uniontown, Pennsylvania (20), who recorded PEFR values in summer and reported new symptoms, an increment of 60 $\mu\text{g} \cdot \text{m}^{-3}$ (interquartile range) in the daytime 12-h ozone levels gave an odds ratio of 1.36 (95% CI 0.86–2.13) for the incidence of evening cough. Weighted by the proportion of hours spent outdoors during the previous 12 h, the odds ratio was 2.20 (95% CI 1.02–4.75). Cough was also associated with the level of sulfur dioxide and various particle measures. The ozone 12-h mean levels ranged up to 175 (mean 74.4) $\mu\text{g} \cdot \text{m}^{-3}$.

For children from the Harvard Six Cities Study (21), a comprehensive study of air pollution and health effects in six cities in the United States, single pollutant models for cough gave significant odds ratios for PM_{10} and for ozone (OR 1.27, 95% CI 1.05–1.54 for a 60 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the previous day 24-h ozone level). The odds ratio for ozone decreased only slightly (OR 1.20, 95% CI 0.96–1.49) in an analysis with PM_{10} , and the effect appeared to be independent of the PM_{10} and sulfur dioxide levels. The ozone 24-h levels had a median value of 74 $\mu\text{g} \cdot \text{m}^{-3}$ and a maximum value of 174 $\mu\text{g} \cdot \text{m}^{-3}$.

European studies. The incidence and duration of symptoms reported daily in diaries from May to October for 625 children up to five years of age in Zurich and Basel, Switzerland, were related to the levels of total suspended particles and nitrogen dioxide, but not the daily or six-

week average ozone levels (22). Unfortunately, the ozone levels were not given, but it was reported that 120 $\mu\text{g} \cdot \text{m}^{-3}$ was typically exceeded about 80 to 150 h during April to September in Swiss cities.

At low pollution levels (24-h maximum ozone level 49 $\mu\text{g} \cdot \text{m}^{-3}$), 212 primary schoolchildren in The Netherlands performed base-line lung function measurements and reported symptoms (23). During a later summer smog episode (mean 8-h ozone level >120 $\mu\text{g} \cdot \text{m}^{-3}$ on two consecutive days), they were re-examined. There was a small, statistically significant increase in the prevalence of shortness of breath and eye irritation, but not of other symptoms.

For 300 schoolchildren aged 7 to 11 years in two small nonindustrial towns in The Netherlands, followed during March to July (24), there was no relation between ozone levels on the same or previous day and reports of acute respiratory symptoms, eye irritation, headache, or nausea. The 1-h maximum levels of ozone ranged from 7 to 237 $\mu\text{g} \cdot \text{m}^{-3}$ and frequently exceeded 160 $\mu\text{g} \cdot \text{m}^{-3}$.

No significant ozone-related differences in symptom reports by 41 senior citizens in Munich were seen between one to three low-ozone days [0–80, (mean 61) $\mu\text{g} \cdot \text{m}^{-3}$] and one to three high-ozone days [100–200 (mean 140) $\mu\text{g} \cdot \text{m}^{-3}$] (25).

Summer camp studies. Spektor et al (26) investigated symptoms and lung function in 46 healthy children aged 8 to 14 years in a New Jersey summer camp. The daily ozone 1-h maximum levels were from 80 to 300 $\mu\text{g} \cdot \text{m}^{-3}$. There were no reported symptoms on most days and no consistent associations for symptoms with ozone or acid aerosols. Likewise, in an earlier such summer camp study with ozone levels up to 258 $\mu\text{g} \cdot \text{m}^{-3}$ (27), no ozone-related respiratory morbidity was found.

Berry et al (28) studied the symptoms of 10 camp counsellors and 14 children in a summer camp in suburban-central New Jersey. The afternoon 1-h average ozone levels ranged from 50 to 400 $\mu\text{g} \cdot \text{m}^{-3}$. The children had increased symptoms, especially cough and runny or stuffy nose, as well as eye irritation, at ozone levels above 240 $\mu\text{g} \cdot \text{m}^{-3}$. For the adolescent and adult camp counsellors, no clear associations were found.

Exercise studies. For a group of amateur cyclists studied in The Netherlands during the summer (29), exercise duration was 10 to 145 min (mean 75), with minute ventilation varying from 20 to 153 (mean 80) l, during which the mean ozone levels averaged 87 (range 26 to 195) $\mu\text{g} \cdot \text{m}^{-3}$. Only 3.6% of the observations exceeded 160 $\mu\text{g} \cdot \text{m}^{-3}$. The difference between the pre- and post-exercise acute symptoms was positively related to the ozone levels for shortness of breath (OR 2.45, 95% CI 1.05–5.74 for a 100 $\mu\text{g} \cdot \text{m}^{-3}$ increase in ozone), chest tightness (OR 2.27, 95% CI 0.87–5.93) and wheeze

(OR 5.21, 95% CI 0.99–27.39), but not for cough or eye irritation.

Symptom exacerbation in subjects with respiratory disease

Daily life studies on groups of asthmatics and respiratory disease patients. Three early studies measuring total oxidant levels were performed in Los Angeles and Chicago in the 1960s, but the oxidant levels were not reported (30–32). The first study found a clear correlation with the asthma attacks of 137 asthmatics; the second study of 166 men found indications of an association with symptoms overall among patients with chronic respiratory disease but not among healthy subjects, whereas the third study found no association with symptoms overall for 115 patients with chronic obstructive diseases of the lung.

In the Tucson study (12), some association to ozone was found for the symptoms of asthmatics and allergic subjects. The study by Krupnick et al (18) reported a larger effect among those with chronic respiratory disease. Similarly, Zagraniski et al (33) studied 84 patients of an asthma and allergy clinic and 192 telephone company employees with daily symptom diaries for 10 weeks. The daily 1-h maximum ozone levels varied from 8 to 470 (mean 160) $\mu\text{g} \cdot \text{m}^{-3}$, and the 8-h maximum levels were 4 to 184 (mean 96) $\mu\text{g} \cdot \text{m}^{-3}$. No clear correlations were seen for the 1-h maximum values, but, in a multiple regression analysis, the 8-h and 24-h ozone levels were associated with eye irritation and cough, particularly for smokers and those with predisposing illnesses.

Among 45 asthmatics between 7 and 72 years of age and studied in Los Angeles for one year (34), an "asthma index" composed of daytime wheezing, nighttime wheezing, and medication use showed no strong association with any of the gaseous air pollutants studied. The weekly average of the daily peak ozone levels varied between 30 and 580 $\mu\text{g} \cdot \text{m}^{-3}$, with 84 episodes of an ozone peak above 1000 $\mu\text{g} \cdot \text{m}^{-3}$ or a 400 $\mu\text{g} \cdot \text{m}^{-3}$ 1-h maximum. Another investigation (35) used daily diaries to study 20 asthmatic children 8 to 15 years of age for two years in Chicago. High levels of ozone and carbon monoxide correlated with the number, duration, and severity of the attacks. Unfortunately, data on the ozone levels were only available for 10 months (but not reported), and data were analyzed by a simple correlation analysis, leaving open the possibility of confounding by other variables. A third small study of 24 asthmatics during a three-month period in Denver (36) found only fine nitrates to be significantly associated with increased symptoms and bronchodilator usage. The monthly mean ozone levels were low, ranging from 12 to 56 $\mu\text{g} \cdot \text{m}^{-3}$.

Whittemore & Korn (37) reported on a diary study carried out over 34 weeks of asthma attacks among

443 asthmatics, both children and adults, in areas of greater Los Angeles. The median oxidant levels for different areas were 30 to 150 ppb. In a multiple linear regression analysis, the relative risk of an attack was 1.2 for a 100 ppb increase in the daily oxidant level ($P < 0.01$), when both temperature and the total suspended particle level were controlled for.

Over a period of four weeks Higgins et al (38) studied 75 adults in the United Kingdom with asthma or chronic obstructive pulmonary disease, of whom 36 were "reactors" to a methacholine challenge (38). The ozone 24-h mean levels ranged from 7 to 55 $\mu\text{g} \cdot \text{m}^{-3}$. Symptoms of dyspnea and eye irritation were related to the ozone level for all the subjects. The effects in the subgroup of reactors were generally stronger and significant for dyspnea and wheezing. The odds ratio for bronchodilator use in relation to a 10 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the 24-h mean ozone level was 1.44 and significant for reactors and others.

Summer camp studies. At a special camp for asthmatic children in 1991, the ozone, sulfate, and acid aerosols level were all highly correlated with symptomatic beta-agonist treatment (as a measure of asthma exacerbations) for 50 children aged from 7 to 13 years, but only the sulfate and acid aerosol levels were statistically significant (39). The ozone 1-h maximum reached 308 $\mu\text{g} \cdot \text{m}^{-3}$. A subsequent reanalysis showed a significant effect of ozone on asthma exacerbations in this study, but not in a similar study in 1992 when the ozone 1-h maximum reached only 126 $\mu\text{g} \cdot \text{m}^{-3}$ (40).

Summary

Several studies report no association between symptoms (chiefly respiratory) and ozone. The daily ozone 1-h maximum levels in these studies ranged up to 300 $\mu\text{g} \cdot \text{m}^{-3}$ but were mostly lower. Other studies showing an association had 1-h maximum ozone levels ranging up to 400 $\mu\text{g} \cdot \text{m}^{-3}$ or higher. However, one study with levels below 240 $\mu\text{g} \cdot \text{m}^{-3}$ found an increase in allergic or irritant symptoms in association with the ozone level, chiefly related to interactions between ozone and PM_{10} or temperature, for adults and children combined; in another an ozone effect on cough became significant after weighting by the proportion of hours spent outdoors, at 12-h mean ozone levels up to 175 $\mu\text{g} \cdot \text{m}^{-3}$. Heavy exercise may increase the ozone effect at low concentrations, as shown by one study with effects on adult bikers below 160 $\mu\text{g} \cdot \text{m}^{-3}$ during exercise. Attempts to determine threshold levels for various symptom effects in adults have been made chiefly in the multiple analyses of the data set from the Los Angeles nurses' daily diaries. Most thresholds were found at oxidant concentrations above 200 ppb, but some effects on the duration of symptoms seemed to continue into lower concentrations, possibly as low as 40 to 80 ppb for total oxidant level. The most

common symptom associated with high ozone levels was cough, others were phlegm, sore throat, lower and upper respiratory symptoms, and also such symptoms as wheezing, chest discomfort, shortness of breath and eye irritation, for which the data suggested a possible relationship to higher dose rates (ie, high ozone level or high activity or both).

It is not clear if children or adults are more sensitive. One study showed an association for children, but not for adults, at ozone levels above $240 \mu\text{g} \cdot \text{m}^{-3}$. Limited observations on adults may have precluded a significant finding. However, another investigation with ozone levels up to $860 \mu\text{g} \cdot \text{m}^{-3}$ indicated effects primarily on nonsmoking adults. Investigating children only, one study found effects on children's symptoms at 1-h maximum ozone levels up to $680 \mu\text{g} \cdot \text{m}^{-3}$, whereas several studies have shown no effect at levels ranging only up to $300 \mu\text{g} \cdot \text{m}^{-3}$ or lower.

Few studies have been performed in presumably sensitive subgroups. Increased asthma symptoms have been observed at 1-h maximum ozone levels ranging up to $300 \mu\text{g} \cdot \text{m}^{-3}$ or higher. Two asthma camp studies, in different years, indicated effects in asthmatic children when the ozone 1-h maximum levels ranged up to $320 \mu\text{g} \cdot \text{m}^{-3}$, but not when the levels only ranged up to $126 \mu\text{g} \cdot \text{m}^{-3}$. The evidence is not sufficient to conclude that subjects with asthma show respiratory symptoms following ozone exposure at lower levels than "normal" subjects.

Lung function in adults

The design of and results from studies on ozone and lung function in adults are summarized in table 1 (on pages 78–79), which also includes the reported ozone levels and measurement time frames. In general, linear regression methods were used, providing robust estimates. Some studies have been previously reviewed, and only the results regarding lung function will be detailed in this section. For an explanation of the lung function measures, see the section on controlled human studies in this issue (41).

Daily life studies

The Tucson epidemiologic study of airways obstructive disease has been described in detail. In a subsample of 204 adults (45 asthmatics, 68 with airway obstructive disease, 62 with allergy and 29 with no symptoms), the PEFR for asthmatics was significantly related to the interaction between ozone and temperature (11). In adults with airway obstructive disease, the PEFR showed a highly significant relation to ozone in a multiple regression, but not to the total suspended particle level. A spectral time-series analysis (12) indicated no association between PEFR and ozone for those without respira-

tory symptoms. For the asthmatics there seemed to be an association with ozone in late spring, though carbon monoxide was the pollutant most clearly related to PEFR. A reanalysis using path analysis and an extended pollen data set again revealed the association between PEFR and ozone for subjects with airway obstructive disease, but not for asthmatics or subjects with allergies (13).

In the later Tucson study, the ozone levels never exceeded $240 \mu\text{g} \cdot \text{m}^{-3}$; the average 8-h maximum level was $88 \mu\text{g} \cdot \text{m}^{-3}$. In an initial analysis focused on particulate matter, the outdoor level of both particulate matter and ozone was associated with a decrease in PEFR (15). Krzyzanowski et al (16) specifically addressed the ozone effect and found the evening PEFR to be decreased by ozone exposure, especially for asthmatics who spent much time outdoors on days with higher ozone levels. Interactions with PM_{10} and temperature were confirmed. The 1-h maximum ozone levels ranged up to $184 \mu\text{g} \cdot \text{m}^{-3}$ in these analyses.

Rieder et al (42) performed monthly afternoon spirometry on 40 Swiss hospital employees in 1989. No significant associations to ozone levels were found. However, the 1-h maximum ozone levels were rather low ($4\text{--}196 \mu\text{g} \cdot \text{m}^{-3}$, apart from one day at $272 \mu\text{g} \cdot \text{m}^{-3}$).

In The Netherlands, 712 subjects between 35 and 65 years of age performed spirometry after two sham and two real air pollution alerts (43). Gender-specific regressions on the ozone levels 1 h before the tests, which ranged up to $228 \mu\text{g} \cdot \text{m}^{-3}$, showed a relation to small decrements in inspiratory vital capacity, a measure similar to FVC, and $\text{FEV}_{1.0}$.

In the United Kingdom the lowest daily PEFR was related to the same day 24-h mean ozone levels for 75 adults who had asthma or chronic obstructive pulmonary disease [of whom 36 were "reactors" to a methacholine challenge (38)] and who recorded PEFR values every 2 h daily for four weeks. The results for 1-d or 2-d lag times were similar. The 24-h ozone means ranged from 7 to $55 \mu\text{g} \cdot \text{m}^{-3}$.

No significant ozone-related differences between low and high ozone days were seen for the lung function parameters of 41 senior citizens in the Munich study described previously (25).

Summer camp studies

Berry et al (28) tested 10 summer camp counsellors in 1988. No deleterious effect of ozone was seen in the afternoon spirometric results. In fact, most of the effects were positive, though not significantly so. Similar results were obtained for the morning spirometry values of 10 counsellors at another camp.

Exercise studies

Spektor et al (44) measured the lung function of 30 nonsmokers before and after exercise. The regression

coefficients were negative for all the spirometric indices, and the effects were still significant after limitation to only days with ozone levels below $160 \mu\text{g} \cdot \text{m}^{-3}$.

In a Swiss study (45), 24 office workers exercised on low ozone days and high ozone days (30-min maximum of $145\text{--}205 \mu\text{g} \cdot \text{m}^{-3}$). The high ozone levels produced a significant decrease in FVC and $\text{FEV}_{1.0}$, but not in PEFR. These effects were more pronounced for the nonsmokers. Airway resistance was not affected, but subsequent methacholine provocation produced a significantly increased resistance during the high ozone days; this finding indicates increased bronchial responsiveness.

Near Mexico City, 124 cadets between 16 and 20 years of age from a military academy performed exercise, half the group indoors and the other half outdoors (46). The ozone concentrations during exercise ranged from 6 to $275 \mu\text{g} \cdot \text{m}^{-3}$. The only significant difference was that the mean FVC increased 1.3% after the exercise for the outdoor group and 4.4% for the indoor group. The regressions for postexercise pulmonary function on ozone showed no significant relationships for the outdoor group. The lack of response, according to the authors, may have been due to the vigorous training level or the adaptation of the subjects.

Korrick et al (47) studied 103 adult hikers in Mount Washington in the United States in 1990. The 1-h maximum ozone levels were below $160 \mu\text{g} \cdot \text{m}^{-3}$ during the hikes. All the spirometric indices showed decrements at increasing ozone levels, and the increase seemed to be more pronounced for the younger hikers and after longer hikes. A similar study in 1991, when the 1-h maximum ozone levels ranged up to $188 \mu\text{g} \cdot \text{m}^{-3}$, showed that changes in FVC and $\text{FEV}_{1.0}$ over the hiking period were again associated with the ozone level, and the change was more pronounced with increasing hike duration and history of wheeze (48).

Similarly, the 1991 Dutch study of amateur cyclists found lung function to be significantly related to the ozone level even below $100 \mu\text{g} \cdot \text{m}^{-3}$, and only when restricted to observations below $80 \mu\text{g} \cdot \text{m}^{-3}$ did all the coefficients become insignificant and, for most of the lung function variables, close to zero (29).

Summary

Most of the reviewed studies showed negative effects of ozone, usually measured as the daily 1-h maximum level or average level during a short period of activity, on various lung function parameters (table 1), with regression coefficients in the range of -0.7 to $-1.4 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ for FVC, in the range of -0.12 to $-1.33 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ for $\text{FEV}_{1.0}$, in the range of -0.41 to $-3.0 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ for FEF_{25-75} , and in the range of -0.31 to $-4.8 \text{ ml} \cdot \text{s}^{-1} \text{ per } \mu\text{g} \cdot \text{m}^{-3}$ for PEFR. Effects were observed in several studies on ozone levels below

$160 \mu\text{g} \cdot \text{m}^{-3}$ during physical activity and in one study down to $100 \mu\text{g} \cdot \text{m}^{-3}$. No clear threshold has been established. In most studies the subjects have been physically active. In one study failing to show an association and in studies with low coefficients, the subjects only performed normal daily activities. However, in one study on military cadets, negative effects of ozone were not seen despite high activity and ozone levels up to $275 \mu\text{g} \cdot \text{m}^{-3}$. The age group studied has varied and, in most cases, one summary coefficient has been estimated for the whole group.

For comparison, for an average healthy, 30-year-old man 180 cm tall and a woman 168 cm tall experiencing a $100 \mu\text{g} \cdot \text{m}^{-3}$ increase in ozone, estimating the effect using the maximum coefficients obtained would mean a 2.7% and 3.7% decrease in FVC, a 3.0% and 3.5% decrease in $\text{FEV}_{1.0}$, a 6.1% and 7.5% decrease in FEF_{25-75} and a 4.8% and 6.6% decrease in PEFR, respectively, from the base-line values. Given that many of the coefficients are derived from studies at a low activity level, this estimation appears to be a reasonable estimate for a situation with some exertion. Furthermore, the base-line pulmonary function values of healthy people decline with age to somewhere below half the levels of 30-year-olds. Thus, with the same coefficients, elderly people may risk decrements of 10% to 15%, or more, from their base-line values. People with asthma and other pulmonary diseases also have reduced base-line values and may risk large relative decrements. Even if the same relative decrements should apply, it is clear that the persons with lower base-line values are more vulnerable in terms of the clinical effects of the gas.

Lung function in children

The design of and results from studies on ozone and lung function in children are summarized in table 2 on pages 80–83. However, a few studies which present results in a different form are not included in the table. Many of the investigations have been described previously, and only the results regarding lung function are discussed in this section. For an explanation of the lung function measures, see the section on controlled human studies in this issue (41).

Daily life studies

Five studies have investigated PEFR only, which is relatively unintrusive to measure and easily recorded by children in their normal daily life. A 1966 to 1967 study (49) investigated the influence of oxidants on PEFR in 78 schoolchildren 8 to 9 years of age from two cities in the Los Angeles area. PEFR tests performed at 1300 in the afternoon twice monthly were not associated with the

Table 1. Epidemiologic studies of lung function in adults. (COPD = chronic obstructive pulmonary disease, FVC = forced vital capacity, expiratory flow rate at 25%—75% of vital capacity, NS = not significant, NG = significance not given)

Reference	Ozone levels in analysis ($\mu\text{g} \cdot \text{m}^{-3}$)	Nature of exposure	Outcome measurement	Exposed population	Averaging time for the ozone exposure metric
Lebowitz et al 1985 (11) [methods in (10)]	180—239	Normal daily activity	Evening (1500—1900) PEFR	23 asthmatic adults	1-h maximum
Spektor et al 1988 (44)	<258	Vigorous outdoor recreation for 15—55 min a day	Change in spirometric parameters, post-pre exercise	68 adults with obstructive airways disease 30 healthy adults, 21—44 years of age	Mean ozone level during exercise
	<160 <258		Spirometry result post exercise	29 healthy adults 30 healthy adults	
Keller et al 1990 (45)	<205	15-min exercise outside	Change in spirometric parameters, post-pre exercise	24 office workers, 31—58 years of age	30-min maximum during day
Rieder et al 1991 (42)	4—272	Normal daily activity	Monthly afternoon spirometric parameters	13 nonsmokers (of the 24) All 24	30-min maximum in preceding 24 h
				40 hospital employees, 26—62 years old	8 h before test 24 h before test
Aardema & Schouten 1991 abstract (43)	32—228	Normal daily activity	Spirometric parameters	371 men 35—68 years of age 314 women 35—68 years of age	1 h before test
Berry et al 1991 (28)	~50—400	Continuous, with daytime recreation	Spirometric parameters in late afternoon	10 camp leaders, 14—35 years of age	1-h before test
Krzyzanowski et al 1992 (16)	30—184	Daily living	Evening PEFR	520 adults, 15—64 years of age	8-h before test Same-day 1-h maximum Same-day 8-h maximum, moving average
		More time outdoors		Asthmatic sub-group	Same-day 1-h maximum Same-day 8-h maximum, moving average
Korrick et al 1992 abstract (47)	<160	Hiking on Mt Washington (mean 8 h)	Change in spirometry, post-pre hike	103 healthy adults, 18—53 years old	1-h maximum level during hike
Korrick et al 1993 abstract (48)	<188	Hiking on Mt Washington (mean 8 h)	Change in spirometry, post-pre hike	227 healthy adults, 18—65 years old	1-h maximum, level during hike
Brunekeerf et al 1994 (29)	<240 <120 <100	Outdoor cycling exercise (mean 75 min)	Change in spirometry, post-pre exercise	23 amateur cyclists 18—37 years of age	Ozone levels during exercise
Higgins et al 1995 (38)	8-h maximum <71	Daily activity	Lowest daily PEFR of measurements every 2 h	75 adults 18—82 years with asthma or COPD 36 "reactors" to metacholine challenge	Same day 24-h mean (7—55)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.002$.

midday (1300—1400) oxidant level, which ranged up to 340 ppb, on the same day. Interestingly, higher mean PEFR values and greater variances were noted for the city with the higher oxidant concentrations. Vedal et al (17) studied 144 elementary schoolchildren during nor-

mal daily activity between 1980 and 1981. Neither sulfur dioxide nor particle pollution nor the ozone 1-h maximum levels up to $129 \mu\text{g} \cdot \text{m}^{-3}$ on the previous day were associated with a substantially decreased morning PEFR. For children in Tucson (11, 50), the PEFR adjusted for

IVC = inspiratory vital capacity, $FEV_{1.0}$ = forced expiratory volume at 1 s, PEFR = peak expiratory flow rate, R_{aw} = airway resistance, FEF_{25-75} = forced

Regression coefficients for function on ozone (or significant/nonsignificant difference)				Remarks	
FVC $ml \cdot \mu g^{-1} \cdot m^{-3}$	$FEV_{1.0}$ $ml \cdot \mu g^{-1} \cdot m^{-3}$	PEFR $ml \cdot s^{-1} \cdot \mu g^{-1} \cdot m^{-3}$	FEF_{25-75} $ml \cdot s^{-1} \cdot \mu g^{-1} \cdot m^{-3}$	R_{aw}	
..	..	*	..	..	Tucson, Arizona (United States), 1979—1981, PEFR associated with the interaction of ozone and temperature
..	..	***	..	..	
-1.04**	-0.68**	-4.6**	-3.0**	..	Tuxedo, New York (United States), June—August 1985
-0.71*	-0.65*	-3.3*	-2.76*	..	
-0.95**	-0.6*	-4.8**	-1.95**	..	
***	***	NS	..	NS	Switzerland, 1989, comparison of low-ozone day (30-min maximum 68—102 $\mu g \cdot m^{-3}$) and high-ozone day (30-min maximum 145—205 $\mu g \cdot m^{-3}$)
***	***	NS	..	NS	
NS	NS	..	..	**	Same comparison, but after metacholine provocation
NS	NS	..	NS	..	Switzerland, March to December 1989, no correlation with ozone (Wilcoxon test negative); FEF_{50} used instead of FEF_{25-75}
NS	NS	..	NS	..	
NS	NS	..	NS	..	
-0.24(NS) IVC)	-0.29(NS)	..	..	..	The Netherlands, 1988—1989 35—52 years: -0.19(NS) and -0.2(NS); 52—68 years: -0.32(NS) and -0.05(NS)
-0.18(NS) IVC)	-0.12(NS)	..	..	..	35—52 years: -0.27(NS) and -0.85***; 52—68 years: -0.01(NS) and +0.43(NS)
-0.42*	+0.23(NS)	+0.49(NS)	..	..	YMCA summer camp 1988 in New Jersey (United States)
-0.14(NS)	-0.11(NS)	+0.30(NS)	..	..	
..	..	-0.31(NS)	..	..	Tucson, Arizona, May 1986 — Nov 1988, family sample
..	..	-0.32(NS)	..	..	From two different random effects models used (I and II)
..	..	-0.5(NS)	..	..	
..	..	-0.56*	..	..	
0.7(NG)	-0.75 P=0.07	..	-1.15(NG)	..	Mt Washington, New Hampshire (United States), summer hiking, 1990, longer hikes and younger age gave a greater decrease in FVC and $FEV_{1.0}$, controlled for confounders
0.7(NS)	-0.85*	..	Not associated	..	Mt Washington, New Hampshire (United States), summer hiking, 1991 longer hikes, and history of wheeze, gave a greater decrease in FVC and $FEV_{1.0}$
1.13**	-0.80*	-3.93**	-0.41(NS)	..	Holland, summer 1991, Amateur cycling
1.39*	-1.33(NS)	-4.73(NS)	-2.39(NS)	..	
II four coefficients similar to above, larger standard error					
..	..	..	-2.33*	..	United Kingdom, August—September 1993
..	..	..	-4.67*	..	

the level of total suspended particles, temperature, and stove use in a multiple regression had a significant negative relationship to ozone. In the later Tucson study (16), the association between PEFR and ozone was investigated for 287 children, and negative regression coefficients

of borderline significance were obtained. The values were slightly larger and significant for asthmatics, however. Finally, Neas et al (20) studied 83 children who recorded morning and evening PEFR values in summer in Uniontown, Pennsylvania, when the mean 12-h ozone

Table 2. Epidemiologic studies of lung function in children. [FVC = forced vital capacity, FEV_{1.0} = forced expiratory volume at 1 s, FEV_{0.75} = forced 25%—75% of vital capacity, SO₂ = sulfur dioxide, NO₂ = nitrogen dioxide, ppb = parts per billion, cens = censored (analysis limited to only observations

Reference	Ozone levels in analysis (µg · m ⁻³)	Nature of exposure	Outcome measurement	Exposed population	Averaging time for ozone exposure metric
Lippman et al 1983 (54)	56—244	Continuous with daytime recreation	Spirometric parameters 1300—1600, PEFR morning or lunchtime	58 camp children, 8—13 years old 24 boys 34 girls	Same day 1-h maximum
Lebowitz et al 1985 (11) [methods in (10)]	<239	Normal daily activity	Evening PEFR (1300—1900)	24 young people, 5—25 years of age	Same day 1-h maximum
Lioy et al 1985 (55)	40—280	Continuous with daytime recreation	Early afternoon (1200—1500) spirometric parameters	39 healthy camp children, 7—13 years 17 boys 33 girls 31 children	Same day 1-h maximum
Vedal et al 1987 (17)	0—129	Normal activity	Morning PEFR on school days	144 elementary schoolchildren (mean age 10 years)	Previous day 1-h maximum
Spengler et al 1985 and Özkaynak et al 1985 (in Kinney et al 1988 (56))	28—150	Summer camp activities	Spirometric parameters twice daily	52 children, 8—16 years old, half asthmatics 27 boys 25 girls	1-h maximum
Spektor et al 1988 (27)	<240	Continuous, with active daytime recreation	Spirometric parameters in late afternoon	91 camp children, 8—15 years old 53 boys 38 girls both genders All 91 children	1-h preceding test
	<120 cens <240				4-h preceding test
Kinney et al 1989 (51)	14—156	Continuous, with normal school and after-school activity	Spirometric parameters in late morning (1000—1300)	154 healthy school-children, 10—12 years old	1-h maximum in 24 h before test 2 h preceding test
Brunekeef et al 1991 (52) (on Kinney et al 1989)				146 (FVC) and 141 (FEV _{0.75}) children	1-h maximum in 24 h before test
Higgins et al 1990 (58)	40—490	Continuous, with daytime recreation	Spirometric parameters in morning, midday and late afternoon	43 healthy camp children, 7—13 years old	Test hour (1 h 2 hours before test almost same result)
	<240 cens				6 h preceding test
Avol et al 1990 (59, 60)	120—320	Camp activity, moderate	0800 and 1700 FEV _{1.0}	293 healthy camp children, 8—17 years old	Preceding 1 h
			Change in FEV _{1.0} evening—morning		Preceding 8 h Preceding 24 h 1 h preceding evening test Intervening 8 h
Spektor et al 1991 (26)	<300	Continuous active daytime recreation	Spirometric parameters in late afternoon	35 healthy camp children, 8—14 years old	1 h preceding test
	<240			33 children	0900—1800, same day average
	<300		Spirometric parameters in late morning	35 (34) children	Same day 1-h maximum
	<300		Change in spirometric parameters (evening—morning)	35 children	Previous day 1-h maximum Hour preceding the evening test Time between tests

expiratory volume at 0.75 s, PEFR = peak expiratory flow rate, $V_{\max_{75}}$ = maximal flow rate at 75% of FVC, FEF_{25-75} = forced expiratory flow rate at below given level), NS = not significant]

Regression coefficients for function on ozone				Remarks
FVC (ml · μg^{-1} · m^{-3})	$FEV_{1.0}$ (ml · μg^{-1} · m^{-3})	PEFR (ml · s^{-1} · μg^{-1} · m^{-3})	FEF_{25-75} (ml · s^{-1} · μg^{-1} · m^{-3})	
-0.53**	-0.39**	Correlation not significant	..	Summer camp in Indiana, Pennsylvania (United States), 1980
-0.38(NS) -0.64**	-0.21(NS) -0.52**	..	..	
..	..	*	.	Tucson, Arizona (United States), 1979—1981
-0.06(NS)	-0.14(NS)	-1.50**	-0.28(NS)	Summer camp in Mendham, New Jersey (United States), 1982 (ozone levels up to $380 \mu\text{g} \cdot \text{m}^{-3}$, analysis restricted to <280)
+0.03(NS) -0.15(NS) -0.09(NS)	-0.06(NS) -0.21(NS) -0.03(NS)	-0.87(NS) -1.98** -0.96*	-0.19(NS) -0.34(NS) -0.03(NS)	Analysis of period after smog episode to separate persistent effects of episode from short-term effects on lung function
..	..	No substantial effect	..	Chestnut Ridge, Pennsylvania (United States), 1980—1981, low pollutant levels
-0.07(NS)	..	..	..	Summer camp, Ontario, Canada, July 1983, model also included sulfuric acid and fine particulates Ozone did not appear in stepwise regressions of $FEV_{1.0}$ and PEFR
+0.17(NS) -0.33(NS)	..	..	..	
-0.52*	-0.71*	-3.39*	-1.24*	Summer camp, New Jersey (United States), 1984
-0.67* -0.44* -0.88* -0.53*	-0.53* -0.97* -0.60* -0.76*	-3.56* -3.15* -1.96* -3.49*	-1.04* -1.53* -0.74(NS) -1.28*	
	$FEV_{0.75}$	$V_{\max_{75}}$		
-0.46*	-0.50**	-1.2**	-0.95*	February-April 1981 in Kingston and Harriman, Tennessee (United States)
-0.35(NS)	..	..	..	
-0.65**	-0.58**	..	..	Reanalysis with restricted data set after removal of outliers
0.34**	-0.38**	-0.96**	..	Summer camp, California (United States), June-July 1987; Controlled for temperature, relative humidity, particles
0.25(NS)	-0.28(NS)	-0.98(NS)	..	Analysis using only days with ozone 1-h levels during 6 hours before test all $<240 \mu\text{g} \cdot \text{m}^{-3}$
	Positive (NS)	..	..	Summer camp, southeast of Los Angeles (United States) 1988
	Positive (NS)	..	..	
	Positive (NS)	..	..	
	Approximately 0	..	..	
	Negative*	..	..	
0.90**	-0.82**	-2.19**	-1.11**	Summer camp, New Jersey (United States), 1988
1.05*	-0.85*	-1.21*	-1.25**	
1.29**	-1.25**	-1.36**	-1.35**	
1.24**	-0.25**	-0.66**	-0.40**	Very similar results using only previous-day 1-h maximum $< 240 \mu\text{g} \cdot \text{m}^{-3}$
1.68**	-0.56**	-3.36**	-0.72*	
1.38**	-0.34**	-1.48**	-0.57**	

(continued)

Table 2. Continued

Reference	Ozone levels in the analysis ($\mu\text{g} \cdot \text{m}^{-3}$)	Nature of exposure	Outcome measurement	Exposed population	Averaging time for ozone exposure metric
Berry et al 1991 (28)	50—400	Continuous with daytime recreation	Spirometric parameters in late afternoon	14 healthy camp children, 9—14 years old	1 h preceding test 8 h preceding test
Krzyzanowski et al 1992 (16)	30—180	Daily living Evening PEFR	Noon PEFR	287 children, 6—15 years old Approximately 40 asthmatic children	Same day 1-h maximum Same day 1-h maximum Same day 8-h maximum (moving average)
Castillejos et al 1992 (2)	60—500	School activities	Spirometric parameters 0800—1400, school days every 2 weeks	143 schoolchildren, 7—9 years old 80 boys 63 girls 143 schoolchildren, 7—9 years old	1 h preceding test 48 h preceding test 1 h preceding test 1 h preceding test 1-h maximum in 24 h preceding test 1 h preceding test 48 h preceding test
Thurston et al 1992 abstract (39), 1993 abstract (40)	<320	Daily recreational activities	Daily PEFR	50 asthmatic children, 7—13 years old	1-h maximum
Thurston et al 1993 abstract (40)	<126	Daily recreational activities	Daily PEFR	55 asthmatic children	1-h maximum
Hoek et al 1993 (62)	<240	Sports exercise	PEFR after training	65 children, 7—12 years old 64 children 34 children	Same day 1-h maximum Previous day 1-h maximum
Hoek et al 1993 (53)	7—237	School activities	Spirometric parameters 0800—1200 (–1500), school days every 2—3 weeks	533 schoolchildren, 7—11 years old	1-h maximum on day preceding test
Braun-Fahrländer et al 1994 (63)	40—157 (ozone <245 1-h maximum during whole study period)	10-min cycle ergometer exercise	Change in spirometry post and preexercise, monthly tests	128 schoolchildren, 9—11 years of age 69 boys 59 girls All children	30-min maximum during testing
Neas et al 1995 (20)	20—175 (12-h average)	Normal daily activity	Morning and evening PEFR	83 nonasthmatic schoolchildren, 9—11 years old	1-h maximum

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

levels ranged up to 175 (mean 74.4) $\mu\text{g} \cdot \text{m}^{-3}$. Negative effects on the PEFR were obtained for ozone, sulfur dioxide, particle measures, and particle strong acidity. The ozone-specific coefficients were significant.

Four daily life studies involved full spirometric measurements for the children. In the carefully designed Harvard six cities study, 154 children in normal daily activity performed weekly spirometry during a two-month period (51). Significant negative regression coefficients were obtained for the lung function parameters for the 1-h maximum ozone level (which ranged up to 156 $\mu\text{g} \cdot \text{m}^{-3}$), whereas no association with particulate

levels was seen. A reanalysis without outliers did not alter the results appreciably (52).

The lung function measurements of 143 schoolchildren in Mexico City were examined for associations with ozone levels averaged over 1, 2, 4, 24, 48 and 168 h before the testing (2). In univariate analyses, FVC was significantly related to 1-h ozone levels, and FEV_{1.0} and FEF_{25–75} to 24-h, 48-h and 168-h ozone levels. In a multivariate analysis controlling for temperature and humidity the picture was less clear, with consistent negative associations only for FVC, despite the high ozone levels (1-h maximum of 60 to 500 $\mu\text{g} \cdot \text{m}^{-3}$). The

Regression coefficients for function on ozone				Remarks
FVC (ml · µg ⁻¹ · m ⁻³)	FEV _{1.0} (ml · µg ⁻¹ · m ⁻³)	PEFR (ml · s ⁻¹ · µg ⁻¹ · m ⁻³)	FEF ₂₅₋₇₅ (ml · s ⁻¹ · µg ⁻¹ · m ⁻³)	
+0.42*	+0.41*	-0.51(NS)	..	YMCA summer camp, New Jersey (United States), 1988, increased respiratory symptoms with increasing ozone > 240 µg · m ⁻³
+0.65**	+0.45**	-1.18(NS)	..	
..	..	-1.0(NS)	..	Family sample in Tucson, Arizona, (United States) May 1986—November 1988
..	..	-1.2*	..	
..	..	-1.5*	..	
-0.04*	-0.02(NS)	NT	+0.08(NS)	January—June 1988, Mexico City (Mexico) (1 ppb=1.5 µg · m ⁻³), unadjusted estimates
-0.04(NS)	-0.39*	NT	-1.87*	
-0.05*	-0.05*	NT	-0.01(NS)	
-0.02(NS)	+0.02(NS)	NT	+0.17(NS)	
-0.07*	-0.09*	NT	-0.01(NS)	Adjusted for temperature and humidity
-0.05*	+0.05*	NT	+0.15*	
-0.07(NS)	-0.33*	NT	-0.29*	
..	..	*	..	1-week summer asthma camp, 1991, Connecticut (United States)
..	..	NS	..	1-week summer asthma camp, 1992, Connecticut (United States)
..	..	-1.52(NS)	..	Summer 1989, Wageningen, Holland, adjusted for temperature
..	..	-0.67(NS)	..	
..	..	-1.72*	..	Data from children with ozone-temperature Pearson correlations > 0.6 excluded
-0.20*	-0.21*	-1.72*	-0.45*	March—July 1989, three nonindustrial towns in The Netherlands; ozone, SO ₂ , NO ₂ ; ozone frequently above 160
-0.67*	+0.34(NS)	-1.1*	..	May—October 1989, Switzerland, weighted mean regression slopes
..	..	-1.27(NS)	..	Common slope regressions, controlled for day of study
..	..	-2.18*	..	
..	..	-0.08(NS)	..	
..	..	-2.28*	..	Multiple regression model, controlled for day, temperature, relative humidity, study area, gender
..	..	-0.62**	..	Summer 1990, Uniontown, Pennsylvania (United States); weighted by time spent outdoors
..	..	-0.77**	..	

effect appeared stronger for the boys. Larger negative coefficients were determined for the children with a history of chronic phlegm, but not with a history of wheezing.

In contrast, a Dutch study of 533 schoolchildren (53) found significant negative associations of previous-day 1-h maximum ozone levels below 240 µg · m⁻³ with FVC, FEV_{1.0}, PEFR, and MMEF. The presence of chronic respiratory symptoms did not change the results. The effects tended to be more pronounced for the girls.

For 212 Dutch children tested during a summer smog episode (ozone 8-h mean > 120 µg · m⁻³ on two or more

days) (23), small significant decrements overall were observed for FEV_{1.0} and FEF₂₅₋₇₅ and, furthermore, for all four spirometric indices on two of the study days which followed 1 to 2 d after the ozone peaks during the smog episode. This finding is consistent with a negative association between primarily previous-day ozone levels and the spirometry indices.

Summer camp studies

Lippman et al (54) studied 58 children in Pennsylvania in 1980. Spirometry and PEFR were measured daily. The 1-h maximum ozone levels the same day (range 56—

$244 \mu\text{g} \cdot \text{m}^{-3}$) were shown to affect the lung function parameters negatively, with a slightly larger effect on the girls.

The ozone levels ranged higher, up to $380 \mu\text{g} \cdot \text{m}^{-3}$, in New Jersey in 1982 (55), where 39 camp children underwent early afternoon spirometry on a daily basis. Negative associations were found with all the lung function parameters, though the finding was significant only for PEFR. The associations were more pronounced for the girls. An analysis was also made covering only a period after a serious smog episode in an attempt to separate persistent effects from transient ones. Similar regression coefficients were obtained.

In a 1984 New Jersey camp study of 91 children (27), ozone was associated with a decrement in lung function variables which persisted after the ozone levels were limited to below $120 \mu\text{g} \cdot \text{m}^{-3}$, and there was no clear difference between the boys and girls. A reanalysis after outlier values were removed produced very similar regression coefficients; this finding suggests that children with a highly variable response are not generally more sensitive than others to ozone (52). The same group investigated 46 New Jersey camp children in 1988 (26). Negative regression coefficients were obtained for all the lung function measurements, even after the ozone levels were limited to below $240 \mu\text{g} \cdot \text{m}^{-3}$. In yet another New Jersey summer camp, Berry et al (28) studied 14 children. The PEFR showed a clear, though not significant, negative association with ozone, but for FVC and $\text{FEV}_{1.0}$ moderate, positively significant, coefficients were obtained.

In Ontario, Canada, 52 children provided spirometric measurements twice daily (56). A stepwise regression model with ozone, sulfuric acid, fine particulates, and temperature resulted in a slightly negative mean FVC coefficient overall for ozone exposure; the results were more clearly negative for the girls, but still not significant. There was a high correlation between fine particles and ozone in the data set. Raizenne et al (57) studied 112 nonasthmatic girls between the ages of 8 and 14 years, also in an Ontario camp (not in table 2). A methacholine test [see the section on controlled human studies in this issue (41)] was positive for 40.6% of the girls. Comparisons were made between the afternoon lung function on days of a major pollution episode and the mean lung function on a number of preceding control days. The 1-h maximum ozone levels were 156 to $158 \mu\text{g} \cdot \text{m}^{-3}$ for the control periods and 216 to $286 \mu\text{g} \cdot \text{m}^{-3}$ on the episode days. Substantial variations in acid aerosols and sulfate levels were also seen. Maximum decrements of 3.5% for $\text{FEV}_{1.0}$ and 7% for PEFR (corresponding to $-0.63 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ and $-3.0 \text{ ml} \cdot \text{s}^{-1} \text{ per } \mu\text{g} \cdot \text{m}^{-3}$, respectively) were observed in the methacholine group. The results in the group with a negative methacholine test were less consistent across the episodes. The effects of ozone and the

other acid haze components were difficult to separate.

One California summer camp study in 1987 measured lung function in 43 children and found significant lung function decrements related to ozone levels 1 h before the testing. In analyses restricted to tests with 1-h ozone levels during 6 h before the testing with concentrations below $240 \mu\text{g} \cdot \text{m}^{-3}$, in order to test for an effect threshold, negative (but not significant) coefficients resulted only in regressions with the 6-h ozone level of the preceding tests, but not with shorter averaging times (58). For 293 children from a 1988 California camp (59, 60), the morning to evening $\text{FEV}_{1.0}$ decrements were associated with intervening 8-h ozone levels, but not with the ozone level 1 h before the afternoon measurements, and they were more frequent during the week with the highest ozone levels. Of the two measurements, the morning $\text{FEV}_{1.0}$ was positively associated with ozone during the preceding 1, 8 or 24 h; the evening $\text{FEV}_{1.0}$ showed no association.

Thurston et al (40) investigated asthmatic summer camp children in Connecticut during two years. In 1991, significant negative effects of ozone on PEFR were seen at levels up to $320 \mu\text{g} \cdot \text{m}^{-3}$, but in 1992 with ozone levels below $126 \mu\text{g} \cdot \text{m}^{-3}$ no effects were apparent.

Exercise studies

The pulmonary flows and symptoms of 40 children in Mexico City were registered before and after treadmill exercise in the open air (61). The mean ozone levels during exercise were 15 to $480 \mu\text{g} \cdot \text{m}^{-3}$. A dose-response relation was found between the quintile of mean ozone exposure and the change in $\text{FEV}_{1.0}$ during exercise, with an $\text{FEV}_{1.0}$ increase of 1% in the lowest exposure quintile and an $\text{FEV}_{1.0}$ decrease of 2.2% in the highest quintile. The response of children with chronic symptoms did not differ.

In a Dutch study of 83 children in outdoor sports training, 65 children contributed four or more PEFR measurements after training. While the change in PEFR during exercise was not related to the ozone levels during training sessions, the PEFR measured after training was associated with high 1-h maximum ozone levels, both on the previous days and on the same day, although a high correlation with temperature made the results difficult to evaluate (62).

Braun-Fahrlander et al (63) studied 128 children in 1989 before and after 10 min of heavy exercise in Switzerland. The difference in the lung function parameters before and after the exercise was regressed against ozone levels during exercise (all below $157 \mu\text{g} \cdot \text{m}^{-3}$). The PEFR and FVC, but not the $\text{FEV}_{1.0}$, showed a significant negative overall effect for ozone. The PEFR effect was pronounced for the boys, but very small for the girls. A history of wheezing or lower respiratory infections did not change the results.

Summary

Most epidemiologic studies report negative effects of ozone on the lung function of children, the negative regression coefficients ranging to $-1.3 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ for FVC, to $-1.25 \text{ ml per } \mu\text{g} \cdot \text{m}^{-3}$ for $\text{FEV}_{1.0}$, to $-1.35 \text{ ml} \cdot \text{s}^{-1} \text{ per } \mu\text{g} \cdot \text{m}^{-3}$ for FEF_{25-75} , and in the range -0.51 to $-3.49 \text{ ml} \cdot \text{s}^{-1} \text{ per } \mu\text{g} \cdot \text{m}^{-3}$ for PEFR. When ozone effects on the change in PEFR (the difference between an afternoon or postexercise PEFR and a person's own same-day base-line value) are considered, the coefficients are consistently larger, from -1.1 to $-3.36 \text{ ml} \cdot \text{s}^{-1} \text{ per } \mu\text{g} \cdot \text{m}^{-3}$, and suggest a greater effect at higher physical activity or better control for individual variation.

For comparison, for an average healthy 10-year-old child who has a height of 135 cm and who experiences a $100 \mu\text{g} \cdot \text{m}^{-3}$ increase in ozone, an effect estimate using the maximum coefficients obtained would mean a 6.0% decrease in FVC, a 6.4% decrease in $\text{FEV}_{1.0}$, a 5.6% decrease in FEF_{25-75} and a 7.4% decrease in PEFR. Given that some coefficients are derived from studies at a low activity level and growing children have a great degree of heterogeneity that makes it difficult to establish a correct base-line value, even stronger relative effects than was evident in this scenario may occur.

Negative effects have been observed at 1-h maximum ozone levels below $160 \mu\text{g} \cdot \text{m}^{-3}$ in several studies, and no clear threshold level has yet been established. Analyses using longer averaging times for ozone exposure have not shown any consistent advantage over the 1-h maximum or the previous hourly ozone level. Several studies indicate that children with asthma or respiratory symptoms do not experience larger absolute lung function decrements from ozone than do healthy children. They may, nevertheless, be more vulnerable because of lower base-line lung function values. A few studies suggest that girls experience stronger lung function effects than boys, but the evidence is not consistent.

Hospital admissions and outpatient visits

Hospital admissions

Studies in the United States. In a 1955 Los Angeles study (64, 65), the weekly average for the 24-h mean total oxidant level was 59.7 ppb, and the weekly daytime (7-h) average was 104 ppb. Multiple regression of respiratory and cardiovascular admissions to major Los Angeles hospitals on the same- and previous-week oxidant levels, as well as on temperature and humidity, showed that the oxidant levels were the most important variables for both outcome measures. A similar study of about 30 000 admissions in 1961 (66–68) measured oxidants (mean daily level 40.5 ppb), as well as other air pollutants. Univariate and multivariate analyses found the levels of oxidants, ozone, sulfur dioxide and, to some

extent, nitrogen dioxide to be correlated with admissions. The oxidant or ozone levels did not seem to be correlated with the duration of hospital stay, apart from ozone to duration of "highly relevant" (mainly respiratory) admissions. Goldsmith et al (69) used a path analysis to study general emergency room admissions on weekdays in Los Angeles in 1974 to 1975. The average 1-h maximum ozone levels were 226, 196, 74 and $68 \mu\text{g} \cdot \text{m}^{-3}$ in the four areas studied. Only at the highest ozone level did ozone produce a positive path coefficient. In the other locations, a negative path coefficient was obtained, although the correlation coefficients were positive. This finding could be due to the high covariation of ozone with temperature.

Thurston et al (70) investigated the relation between hospital admissions and air pollution. In three New York metropolitan areas an association was found between elevated "summer haze pollution" and total respiratory and asthma admissions, when temperature was controlled for. The daily 1-h maximum ozone level ranged from 296 to $412 \mu\text{g} \cdot \text{m}^{-3}$ in 1988, but was only 222 to $256 \mu\text{g} \cdot \text{m}^{-3}$ in 1989. Ozone had the consistently highest etiologic fraction (regression coefficient times mean pollutant level), being implicated in 5% to 18% of the respiratory admissions and 12% to 24% of the asthma ones in 1988. On the other hand, acid aerosols had the highest relative risk estimates for risk at the maximum pollutant level compared with the mean level. The ozone relative risk for total respiratory admissions was around 1.20, slightly higher for asthma admissions, and significant. No attempts were made to separate in detail the effects of the various components of the pollutant mixture.

In several recent studies in different United States locations (71–73), Schwartz found an increase in hospital admissions for pneumonia at higher ozone levels (RR 1.15, 95% CI 0.97–1.36; RR 1.14, 95% CI 0.94–1.38; RR 1.30, 95% CI 1.14–1.48 for a $100 \mu\text{g} \cdot \text{m}^{-3}$ increase in the 24-h mean ozone level). The averages of the 24-h mean ozone level were 42 to $52 \mu\text{g} \cdot \text{m}^{-3}$, the 90th percentile being 72 to $82 \mu\text{g} \cdot \text{m}^{-3}$. In the latter two studies, hospital admissions for chronic obstructive pulmonary disease were also increased (RR 1.17, 95% CI 0.86–1.60; RR 1.32, 95% CI 1.07–1.61). In the first there was an inverse relation for this outcome. Furthermore, in the third study (Detroit, Michigan), there was an apparent threshold with little effect of the 24-h mean ozone levels below $50 \mu\text{g} \cdot \text{m}^{-3}$ on chronic obstructive pulmonary disease or pneumonia admissions. Analyses using the 1-h maximum ozone level gave results similar to that for the 24-h mean. In addition, in contrast to the respiratory admissions, no relationship between cardiac admissions and ozone was seen (74). In two more locations (75), admissions among persons aged 65 years and above for respiratory diseases were related to ozone (RR 1.06, 95% CI 0.99–1.13; RR 1.21, 95% CI 1.06–1.38) for a

50 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the 24-h mean ozone level), as well as to the PM_{10} and sulfur dioxide level. The latter effects were more significant, partly because of more data, but all the effects were essentially unchanged after control for the other air pollutants.

Studies in Canada. Bates & Sizto (76–79) used daily admission data for January, February, July, and August of 1974 to 1983 for 5.7 million people in southern Ontario, Canada (about 2400 daily admissions, of which 40 to 70 were for respiratory conditions). The mean daily 1-h maximum ozone level from all monitoring stations was 50 to 240 $\mu\text{g} \cdot \text{m}^{-3}$ during July to August of 1974. For all the years, associations were found between respiratory admissions in the summer and the 1-h maximum ozone level, the sulfur dioxide level, and temperature, but in winter the association was with temperature only. Six nonrespiratory conditions showed no associations. There was a 7% increase in respiratory admissions if the ozone level was above 160 $\mu\text{g} \cdot \text{m}^{-3}$. In 1992, Lipfert & Hammerstrom (80) reanalyzed data covering 1979 to 1985, using different methods to account for serial correlation and to include subregional effects. Again, ozone, sulfur dioxide and sulfate aerosol were significant predictors, although the effect of each could not be separate and 20% of the summer (July to August) respiratory admissions were attributed to the air pollutants. In 1994 Burnett et al (81) used poisson regression models on similar data from 1983 to 1989 for all of Ontario (8.7 million people). The 1-h maximum ozone level ranged up to 320 $\mu\text{g} \cdot \text{m}^{-3}$. Significant ozone and sulfate associations were confirmed, ozone being the stronger predictor. During May to August, 5% of the respiratory admissions were estimated to be associated with ozone and 1% with sulfates.

Thurston et al (82) analyzed data restricted to Toronto during 1986 to 1988. The daily 1-h maximum ozone level ranged from 98 to 318 $\mu\text{g} \cdot \text{m}^{-3}$. Asthma admissions were found to be related to the ozone level, and the results remained highly significant even after days with a 1-h maximum above 240 $\mu\text{g} \cdot \text{m}^{-3}$ were excluded. On the average, summertime haze pollution was associated with 24% of all the respiratory admissions (21% with ozone and 3% with hydrogen). However, on peak pollution days, aerosol acidity yielded the highest relative risk in comparison with average days (RR 1.5 at 391 $\text{mmol} \cdot \text{m}^{-3}$ for hydrogen), and summertime haze was associated with roughly half of all the respiratory admissions. Estimates were similar to those of the New York study performed by the same group (70).

In Montreal, Delfino et al (83) found a statistically significant univariate relationship between all respiratory admissions for July and August and the 8-h maximum ozone level, which became nonsignificant when co-regressed with temperature or particulates. Low ozone

levels (1-h maximum: mean 82.5 $\mu\text{g} \cdot \text{m}^{-3}$, 90th percentile 129.1 $\mu\text{g} \cdot \text{m}^{-3}$) and a high correlation with particulates and temperature may explain this finding. An association with the sulfate level was also found. Asthma admissions during May to October were related to the particulate level. Nonrespiratory admissions showed no relation to air pollution.

Other studies. For 1310 croup admissions to a pediatric clinic in Innsbruck, Austria, during 1979 to 1988, the best correlation was for 1988 admissions and 1988 ozone levels (84). The highest ozone 30-min maximum concentration recorded in 1988 was 250 $\mu\text{g} \cdot \text{m}^{-3}$, and the ozone level was reported to have increased steadily from 1979 to 1988. For 1979 to 1987, when ozone measurements were missing, the strongest association was to a change in, and average of, the ratio of nitrogen oxide to nitrogen dioxide (a possible indicator for ozone, with a high correlation with the ozone level in 1988, $r = 0.796$).

Daily asthma admissions were studied (85) in a Helsinki population of approximately 500 000 from 1987 to 1989. The pollutant levels were fairly low, except for total suspended particles, which ranged from 6 to 414 $\mu\text{g} \cdot \text{m}^{-3}$. The mean ozone level was 22.0 $\mu\text{g} \cdot \text{m}^{-3}$ and the range of the daily averages was 0 to 89.9 $\mu\text{g} \cdot \text{m}^{-3}$. The nitric oxide and ozone levels were the most strongly associated with the asthma admissions in a multiple regression analysis. Additional analyses of admissions for chronic bronchitis and emphysema (86), incorporating adequate correction for autocorrelation, revealed an association with sulfur dioxide and nitrogen dioxide, but not with ozone or total suspended particles. The total admissions showed no significant associations.

In Barcelona, the 1985 to 1986 admissions for chronic obstructive disease showed weak, but significant positive correlations to sulfur dioxide, black smoke, and nitrogen dioxide, whereas ozone was negatively correlated (87). The annual mean 1-h maximum ozone level was 63.3 (98th percentile 182.3, range 1.5–253) $\mu\text{g} \cdot \text{m}^{-3}$. The correlations of several pollutants and atmospheric variables make it difficult to assess the influence of any single factor.

Tseng et al (88) studied somewhat more long-term effects using age-specific quarterly asthma discharge rates during 1983 to 1989 in Hong Kong (population 6 million) and monthly means of air pollutants. The quarterly average ozone levels varied from 11 to 48 $\mu\text{g} \cdot \text{m}^{-3}$. The most important association was for total suspended particles and asthma in the 1- to 4-year-olds. Ozone was of some importance, especially for the 0- to 1-year-olds.

Outpatient visits

Visits to physicians, usually for asthma or some other respiratory disease, is a measure that can be expected to

reflect less serious disease or disease exacerbation than hospital admission. Many studies have been made on the exacerbation of asthma in children, which often leads to an emergency hospital visit and treatment.

Children. Odom & Taylor (89) studied 50 pediatric patients and extracted data on asthma attack rates during the previous year from the health records of two military hospitals. The monthly ozone averages during the study period were from 16 to 180 $\mu\text{g} \cdot \text{m}^{-3}$. In a stepwise regression analysis, pollen count during September was the only significant predictor of asthma attacks. Monthly ozone was related to increased asthma attacks, but not significantly.

During a six-month study between 1979 and 1980 in Los Angeles (90), the monthly average ozone levels were 60 to 280 $\mu\text{g} \cdot \text{m}^{-3}$. Both the number of pediatric asthma visits ($N = 1975$) and the number of pediatric asthma admissions ($N = 277$) showed significant positive correlations to the nitrogen oxide level, the coefficient of haze (a particulate measure), hydrocarbons, wind, and airborne allergens, but the correlations were negative for ozone, sulfur dioxide, temperature, and humidity. On the basis of 4766 asthma visits among children over a period of two years in Melbourne, Australia, in 1989 (91), particulate pollution was associated with visits for asthma, but ozone was not. During the study period 17 d were above the 1-h maximum ozone level of 240 $\mu\text{g} \cdot \text{m}^{-3}$.

In contrast, White et al (92) studied emergency clinic visits for asthma or reactive airway disease during the summer of 1990 in children up to 16 years of age in a predominantly black community in the state of Georgia in the United States. The ozone levels were 30 to 326 $\mu\text{g} \cdot \text{m}^{-3}$. They found an increased number of visits after high ozone days ($> 220 \mu\text{g} \cdot \text{m}^{-3}$), with a relative risk of 1.37 (95% CI 1.02–1.73). In a multivariate Poisson analysis a statistically unstable dose-response relationship was suggested, as were effects of ozone levels as low as 180 $\mu\text{g} \cdot \text{m}^{-3}$.

Similarly, a Poisson regression analysis of emergency visits for asthma to a major Mexico City pediatric hospital (93) in 1990 by children of the same age group gave a rate ratio of 1.43 (95% CI 1.23–1.65) for a 75 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the ozone level. The ozone levels ranged from 15 to 375 (mean 135) $\mu\text{g} \cdot \text{m}^{-3}$. The total number of visits for respiratory complaints was similarly related.

Many German studies have been performed on the incidence of the croup syndrome and obstructive bronchitis in children. The ozone data are often few or missing. In general, all new cases identified both at outpatient visits and hospital admissions in an area were registered. Wichmann et al (94) presented an evaluation of several such studies performed in Baden-Württemberg and North Rhine Westphalia. While several associations with other

air pollutants were apparent (94, 95), the analysis of data for the summer period (when the ozone levels are high) did not reveal an increase in disease frequency for the croup syndrome or obstructive bronchitis on days with high ozone concentrations (94).

Adults. Using correlation techniques, Durham & Arbor (96) investigated student visits to university health clinics. Peak oxidant level, followed by the mean sulfur dioxide and mean nitrogen dioxide levels, consistently had the strongest associations with certain respiratory illnesses. Factor analysis revealed that colds and eye irritation increased after days of high oxidant levels. In another early study (97), the proportion of patient visits to a family practice clinic for "pollution-related" diseases increased with increasing particulate and smoke or fog levels, whereas the opposite was true for ozone (which was negatively correlated with particulates).

Bates et al (98) investigated data on daily emergency visits of one million people in the Vancouver region of Canada, which is served by nine acute care hospitals with 25 000 visits per month, of which 2.7% were for respiratory conditions. The daily 1-h maximum ozone level, averaged over measuring stations, ranged up to 170 $\mu\text{g} \cdot \text{m}^{-3}$ during the study period. Summer respiratory visits were not related to temperature or the ozone or nitrogen dioxide level, but they were associated with the sulfur dioxide and sulfate level in the 15- to 60-year age group.

In New Jersey, in the United States, summer asthma emergency visits to nine hospitals for patients of all ages were investigated (99). The annual ozone means were 110 $\mu\text{g} \cdot \text{m}^{-3}$ in 1988 and 86 $\mu\text{g} \cdot \text{m}^{-3}$ in 1989 with 34 and 8 d, respectively, of the year above the 1-h maximum level of 240 $\mu\text{g} \cdot \text{m}^{-3}$. In a multiple regression analysis, the ozone levels for 1000 in the morning and 1500 in the afternoon were significant; they explained 7% to 9% of the variability of the visits due to asthma. These data were extended to cover 1986 to 1990 (100). Between 3% and 16% of the variance in asthma visits was explained by the mean ozone levels between 1000 and 1500 in different years, and visits over the full period occurred, on the average, 26% more frequently when the mean ozone level was above 120 $\mu\text{g} \cdot \text{m}^{-3}$ than when it was below this value ($P < 0.0001$).

At lower ozone levels, a study of asthma emergency visits in Paris (101) indicated significant negative associations primarily with sulfate, ozone, and air pressure in bivariate analyses. The daily mean ozone levels were 4 to 112 $\mu\text{g} \cdot \text{m}^{-3}$, and the highest 1-h maximum level was 165 $\mu\text{g} \cdot \text{m}^{-3}$. Factors apart from air pollution and pollen were not evaluated, and simple correlation analysis was used.

In Toronto, Canada, there was a significant relationship between previous weekly mean ozone levels and

weekly asthma emergency visits at a large hospital when the weekly ozone levels were between 4 and 62 $\mu\text{g} \cdot \text{m}^{-3}$ (102).

Summary

The studies on hospital admissions generally show an association with ozone levels. This observation is further strengthened by the fact that, in most instances, there is an association with respiratory-related diseases, but not with other diseases. The effect does not appear to be due to temperature, but generally persists in multivariate analyses controlling for temperature and other covariates. An effect was seen in several studies in which the ozone 1-h maximum level was below approximately 240 $\mu\text{g} \cdot \text{m}^{-3}$ during the whole study period. One study indicated an effect threshold for increased chronic obstructive pulmonary disease and pneumonia admissions at a 24-h mean ozone level of 50 $\mu\text{g} \cdot \text{m}^{-3}$. It is, however, often difficult to separate the effect of ozone from that of other air pollutants. Relative risk point estimates are generally in the range of 1.01 to 1.03 for a 10 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the 24-h mean ozone level or a 30 $\mu\text{g} \cdot \text{m}^{-3}$ increase in the 1-h maximum ozone level, and ozone has been calculated to account for 7% to 21% of hospital admissions due to respiratory problems in different North American settings. For emergency visits, the data are somewhat less clear. However, positive relationships have been reported lately for both adults and children, especially in studies with reasonable numbers of days with high ozone levels (1-h maximum above 220–240 $\mu\text{g} \cdot \text{m}^{-3}$).

Daily mortality

Mills (103) used respiratory and cardiac mortality data for Los Angeles County during the period 1947 to 1949, as well as 1953 to 1956, and found a positive and significant association to days with 1-h maximum total oxidant level of above 200 ppb after adjustment for seasonal variations. In a similar analysis of mortality data for 1956 to 1958 Mills found excess daily deaths on days with a 1-h maximum for the total oxidant level of above 150 ppb, in winter above 100 ppb. The relation was valid at temperatures below 36°C despite a high correlation between temperature and oxidant level. Hechter & Goldsmith (104) reanalyzed the same data and concluded that, after correction for autocorrelation and seasonal variation, no significant association remained.

Shumway et al (105) focused on long-wave periodicities, using Los Angeles weekly mortality data from 1970 to 1979, and found strong associations with temperature combined with the carbon monoxide, hydrocarbon or particulate level. Ozone and other variables were nonsignificant contributors. The actual ozone val-

ues were not reported. Kinney & Özkaynak (106) used daily mortality data for the same period and oxidant rather than ozone levels. The annual oxidant levels were 69 to 90 ppb. In multiple regressions, the total and cardiovascular mortality rates, but remarkably not the respiratory mortality rate, were strongly associated with the previous-day 1-h maximum oxidant levels. They estimated a regression coefficient of 0.15 excess deaths per 5 ppb ($P = 0.005$) for total mortality or an approximate increase of 1% in mortality for 50 ppb. There was also a strong association with the temperature and some association with nitrogen dioxide, carbon monoxide and particulate levels, which were highly correlated. In a sensitivity analysis of the association between mortality and PM_{10} using Los Angeles mortality data from 1985 to 1990 (107), a relative risk of 1.02 (95% CI 1.00–1.05) for a 286 $\mu\text{g} \cdot \text{m}^{-3}$ increase in ozone (from zero to the 95th percentile) was found (ie, a 2% increase). The ozone levels ranged from 6 to 402 $\mu\text{g} \cdot \text{m}^{-3}$. The PM_{10} effect was stronger, and the relative risk for ozone dropped to 1.00 after control for PM_{10} . This finding can imply a weaker or nonexistent effect of ozone. A similar analysis was carried out by the same authors (108) on New York City mortality data for 1971 to 1976. The mean ozone 1-h maximum level for April to September was 224 $\mu\text{g} \cdot \text{m}^{-3}$. A model including temperature, the coefficient of haze (a particulate measure), relative humidity, and previous-day 1-h maximum ozone level explained approximately 10% of the variation in total mortality. The regression slope for ozone was 0.27 deaths per 10 $\mu\text{g} \cdot \text{m}^{-3}$ ($P < 0.001$).

Biersteker & Evendijk (109) presented an analysis of daily mortality data for Rotterdam during July and August in 1974 and 1975. The daily 1-h maximum for ozone was 21 to 165 $\mu\text{g} \cdot \text{m}^{-3}$ in 1974 and 6 to 250 $\mu\text{g} \cdot \text{m}^{-3}$ in 1975, the temperature maximum ranging from 16.4 to 28.4°C and from 17.8 to 35.0°C, respectively. They compared days with ozone levels above 100 $\mu\text{g} \cdot \text{m}^{-3}$ to days with lower levels and found no difference in 1974, but a difference in 1975, which seemed to be attributable to the high correlation of ozone and temperature during heat waves that particular year. In a Greek study as well (110), no main effect of ozone on mortality was seen, but an interaction with temperature was suggested.

Two United States studies showed no relationship. Daily mortality data from Detroit during 1973 to 1982 were analyzed in relation to the ozone, sulfur dioxide, and total suspended particulate levels and temperature and humidity (111). Both the 1-h peak and the daily mean concentrations of ozone proved to be insignificant as a predictor of daily mortality, whereas the level of total suspended particles was highly significant. The actual measured ozone levels were not reported. Similarly, the daily total mortality in St Louis and Tennessee dur-

ing 1985 to 1986 did not show an association with the daily averages of ozone or other gaseous pollutants (112). The daily average ozone levels were 0 to 128 (mean 22.5) $\mu\text{g} \cdot \text{m}^{-3}$ and 0 to 98 (mean 23.0) $\mu\text{g} \cdot \text{m}^{-3}$, respectively. The 1-h national standard of 240 $\mu\text{g} \cdot \text{m}^{-3}$ was exceeded on 5 out of 325 days in St Louis and not at all in Tennessee.

In Sao Paolo, Brazil, Saldiva et al (113) found a strong, statistically significant association between respiratory mortality among children under 5 years of age and oxides of nitrogen, but not with ozone (regression coefficient 1.048 deaths per day for 150 $\mu\text{g} \cdot \text{m}^{-3}$, $P = 0.673$). The daily mean ozone level was 24 (SD 10) $\mu\text{g} \cdot \text{m}^{-3}$.

In summary, the evidence that short-term variations in mortality are associated with ambient ozone levels is, at present, ambiguous. On the other hand, an accumulating number of studies has indicated an effect of particulate air pollution (114). Given the heterogeneity of the underlying, often multiple, adverse health conditions, studies on mortality in relation to variations in air pollution levels are particularly difficult to interpret in conjunction with the high correlations between several pollutants, which may affect different conditions. A mortality effect of ozone would be expected to be due primarily to respiratory conditions and may thus be difficult to discern in total mortality. The possibility of an ozone effect needs to be evaluated together with the effect of particulate matter in large data sets.

Miscellaneous health effects

Athletic performance, school absenteeism and restricted activity or performance

Wayne et al (115) studied high school runners at athletics meets in Los Angeles during the 1959 to 1964 seasons. The athletic performance of the boys at a particular meet was determined by the percentage of boys who failed to improve on their previous time on the same course. Hourly oxidant levels were 20 to 300 ppb, and the levels of total suspended particles and carbon monoxide were also measured. The highest correlation was to oxidants in the hour before the race ($r = 0.88$). When separate analyses were carried out for 1959 to 1961 and 1962 to 1964, because the periods seemed to differ, both periods showed a correlation of 0.945 to oxidants. The level of total suspended particulates showed a much lower correlation, whereas those of carbon monoxide and nitrogen oxides (daily average) and temperature and relative humidity showed no relationship at all.

Daily school absenteeism in 1962 to 1963 was investigated among 1500 children in Los Angeles (115). The 5-h (1000 to 1500) mean ranged from 20 to 480 $\mu\text{g} \cdot \text{m}^{-3}$. After adjusting for a day-of-the-week effect, no clear

relationship was evident. Pearlman et al (116) studied daily school absenteeism and epidemic influenza morbidity, as determined by parent questionnaires and serology, among 3500 schoolchildren in five communities in California during 1968 to 1969. The five communities were classed in terms of low, intermediate, or high chronic oxidant exposure. The oxidant levels, as seasonal peaks, were 80 to 90, 120, and 190 to 230 ppb, respectively, in the three areas (preepidemic: 40, 80, and 80 to 140 ppb; epidemic: 50, 50, and 50 to 60 ppb). They found no significant morbidity differences corresponding to the pollution gradient by any of the effect measures. On the other hand, a study in Mexico City (117) examined the incidence of respiratory-related school absenteeism among 111 preschool children between the ages of three and seven years. The daily 1-h maximum ozone levels varied from 60 to 510 (mean 270) $\mu\text{g} \cdot \text{m}^{-3}$ during January to March 1990. One preceding day with a 1-h maximum above 195 $\mu\text{g} \cdot \text{m}^{-3}$ was associated with an odds ratio of 1.14 (95% CI 1.10–1.18), and two days with an odds ratio of 1.21 (95% CI 1.11–1.32) for respiratory-related absenteeism. In addition, there was an interaction between ozone and temperature. Age, gender, passive smoking, and temperature were controlled for in the analysis.

Portney & Mullahy (118) drew data from the national 1979 Health Interview Survey in the United States; 110 000 persons nationwide responded to questions on acute illnesses during the previous fortnight. A subsample of adults within 10 miles (16 km) of the nearest air-pollutant monitoring station was used. The data were controlled for smoking. The number of "restricted activity days" for each person were significantly related to the same-day 1-h maximum ozone levels. This finding was influenced mainly by minor restrictions in activity, since more serious work loss or bed disability conditions were not related. The association persisted in an analysis restricted to ozone levels below 100 $\mu\text{g} \cdot \text{m}^{-3}$ and therefore indicated no threshold. An association to sulfate could not be ruled out. The average daily 1-h maximum ozone level during the two-week recall period was 86 $\mu\text{g} \cdot \text{m}^{-3}$ and the average 24-h mean sulfate level was 10.9 $\mu\text{g} \cdot \text{m}^{-3}$. For children, no association between either acute or chronic illness and ozone or sulfate was found.

Ostro & Rothschild (119) used data from the Health Interview Survey from the years 1976 to 1981 for similar analyses, using fine particulates and ozone as air-pollutant exposure variables. The number of restricted activity days was found to be related to the particulate level but not to the ozone level. For the minor restrictions in activity, ozone showed a weak association. However, the measurement error associated with using a two-week averaged value for ozone limits the usefulness of the data to analyze the health effects of ozone. The two-week averages of daily 1-h maximum values during 1976 to 1981

were reported to range from 41.11 to 48.61 $\mu\text{g} \cdot \text{m}^{-3}$ (119).

Miscellaneous biological effects

Frischer et al (120) investigated upper airway inflammation after days of high and low ozone concentrations by performing nasal lavage on 44 third and fourth grade primary schoolchildren in a small town in Germany during May to October 1991. Ozone was low (daily 30-min maximum 15–140 $\mu\text{g} \cdot \text{m}^{-3}$, mean 86 $\mu\text{g} \cdot \text{m}^{-3}$) on 10 days and high (daily 30-min maximum 180–263 $\mu\text{g} \cdot \text{m}^{-3}$, mean 230 $\mu\text{g} \cdot \text{m}^{-3}$) on 14 days. The nasal lavage cell counts and some biochemical markers of inflammation were significantly increased on the days after high exposure to ozone. Concomitant with the autumn decline in ozone levels, the white blood cell count in nasal lavage also decreased; this finding indicates that ozone causes reversible inflammatory changes in the nasal mucosa of children.

A Mexican study of 76 subjects found histopathological changes, mainly dysplasias, in the nasal mucosa of male subjects exposed to the ambient air in Mexico City, characterized by high levels of ozone (monthly average 59–420 $\mu\text{g} \cdot \text{m}^{-3}$ in 1984 to 1990, with 19 to 177 h per month above 200 $\mu\text{g} \cdot \text{m}^{-3}$ in 1987 to 1990), but also high levels of particles, nitrogen oxides, sulfur dioxide, benzo[a]pyrene, benzo[k]fluoranthene, aldehydes, and heavy metals (121, 122). The changes were mild for short exposure (less than 30 days in Mexico City), and severe for long exposure (more than 60 days), as compared with those of referents continually resident in a low-ozone area.

Summary

Reduction in athletic performance has been reported for hourly oxidant levels up to 300 ppb. Two studies have failed to find increased school absenteeism related to rather high levels of short-term ozone exposure (daytime mean up to 480 $\mu\text{g} \cdot \text{m}^{-3}$) or long-term oxidant exposure (area seasonal peak 80 to 230 ppb), but one recent study found a clear relationship for preschool children. Some studies have examined restricted activity days (ie, all restrictions in normal activity) or minor restricted activity days, which exclude and more serious restrictions with loss of work or bed disability may then be considered more akin to experiencing symptoms. For the restricted activity days the data are equivocal, whereas for the minor restricted activity days the reports point to an association with the two-week mean of the daily 1-h maximum ozone levels below 100 $\mu\text{g} \cdot \text{m}^{-3}$. Inflammatory changes in nasal mucosa have been found in children after days of ozone exposure with a 30-min maximum of >180 $\mu\text{g} \cdot \text{m}^{-3}$ compared with days with a corresponding value of <140 $\mu\text{g} \cdot \text{m}^{-3}$. Exposure to polluted atmosphere with an ozone monthly average of 60 to 420

$\mu\text{g} \cdot \text{m}^{-3}$ (21 to 177 h per month above 150 $\mu\text{g} \cdot \text{m}^{-3}$) has been associated with dysplasia of the nasal mucosa.

Effects of long-term exposure

This section reviews both cross-sectional and longitudinal studies. Cross-sectional studies are included for two reasons. First, some longitudinal studies start with a cross-sectional design. Second, the aim in cross-sectional studies is often to investigate long-term effects on health, although observed effects may be due to both short-term and long-term exposure. In addition, the prevalence of an outcome is affected by both its incidence and its duration. Long-term exposure may have effects on the human organism that are manifested as either chronic or acute diseases. The longitudinal studies, including their initial cross-sectional phase, are presented first, followed by the purely cross-sectional studies.

Studies of the University of California at Los Angeles

The UCLA population studies of chronic obstructive pulmonary disease formed a comprehensive research effort aimed at comparing populations from areas in California with different levels of air pollution. The first reports (123, 124) presented 1973 to 1975 prevalence figures for various symptoms and a history of respiratory disease, as well as lung function measurements (spirometry, whole body plethysmography and single-breath nitrogen test), for subjects from Long Beach (low oxidant level; high levels of sulfur dioxide, particles and hydrocarbons), Lancaster (moderate oxidant level and low other pollutant levels) and Burbank (high oxidant pollution). The 1972 to 1977 averages of the daily 1-h maximum oxidant levels were 37, 65, and 90 ppb, respectively. The prevalence of respiratory disease was similar in the areas. Symptoms showed no clear relation to air pollution. Among never-smokers, Burbank residents significantly reported the least respiratory symptoms. Among smokers, Lancaster residents significantly reported the most symptoms. For lung function tests, the Burbank residents (high oxidants) had particularly poor results for thoracic gas volume, airway resistance, specific airway resistance, and, along with the Long Beach residents, other spirometric measurements, whereas the participants from Lancaster generally had better results.

Base-line results of the lung function tests and the symptom prevalence were compared in Lancaster and a fourth area, Glendora (very high oxidant level and relatively high nitrogen oxide, sulfate and particles), and a five-year follow-up was made of the nonsmokers in the cohorts (125, 126). The averages of the daily 1-h maximum oxidant levels were 70 ppb and 110 ppb, respectively. For Glendora (the more-polluted area), the lung

function test results were significantly worse for both the men and the women at the base line, and there was more rapid deterioration in the follow-up. The incidence for developing or losing symptoms was similar in the two areas. In a Long Beach (oxidant level 40 ppb, high for other pollutants) follow-up, every difference of consequence indicated greater deterioration in lung function than in Lancaster (127).

Seventh-day Adventist studies

Many investigations have also involved a cohort of Seventh-day Adventist nonsmokers in California who had resided within 8 km of their 1977 residence for at least 10 years and who completed respiratory symptom questionnaires of the National Heart and Lung Institute in 1977 and 1987. In a comparison of 4379 nonsmokers from a high-pollution area with 2287 nonsmokers from low-pollution areas, the risk increase for the prevalence of symptoms of chronic obstructive pulmonary disease was 15% for the high-pollution area ($P = 0.03$) (128). Further cross-sectional analyses with estimated individual 11-year cumulative exposures to pollutants above four thresholds, using residence zip codes and interpolated dosages from state air-monitoring stations, were presented for sulfur dioxide and total suspended particles (129), and oxidants and nitrogen dioxide, as well as combined analyses of all four pollutants and interactions (130). The oxidant thresholds were 100, 150, 200, and 250 ppb. The prevalence of chronic symptoms showed relative risks of 1.20 for 750 h per year of exposure above 100 ppb of oxidants and 1.13 for 500 h per year ($P < 0.004$). Associations were also statistically significant for the total suspended particle level above $200 \mu\text{g} \cdot \text{m}^{-3}$ and for sulfur dioxide above 40 ppb. In a multiple regression using all three variables, the total suspended particle level remained significant ($P < 0.01$). The authors concluded that exposure involving total suspended particles was either more strongly associated with the prevalence of symptoms of chronic obstructive pulmonary disease or was the best surrogate representing the mix of pollutants, which were highly correlated.

The monitoring of ozone gradually replaced the monitoring of oxidants during 1973 to 1980. The correlations were 0.90 to 0.98 for 435 station-months with simultaneous measurements, and they were highly significant. This finding justified combining oxidant and ozone measurements in further analyses (131).

The incidence of pulmonary cancer, based on only 17 cases, was related in separate analyses to both total suspended particles (RR 1.72 for 1000 h per year in excess of $200 \mu\text{g} \cdot \text{m}^{-3}$, 95% CI 0.81–3.65) and ozone (RR 2.25 for 500 h per year in excess of $200 \mu\text{g} \cdot \text{m}^{-3}$, 95% CI 0.96–5.31), controlled for gender, smoking, and education (132, 133). In multipollutant analyses, ozone entered the model with a P-value of 0.055, but the

total suspended particle level failed to enter. Myocardial infarction, incidence of all malignant neoplasms, and total natural-cause mortality did not show any relationship to ozone level (134).

In 1987, 3914 subjects received a 10-year follow-up questionnaire on new symptoms. Whereas bronchitis and total chronic obstructive pulmonary disease were associated chiefly with the level of total suspended particles, the development of asthma showed an association with ozone (RR 1.40 for 500 h per year in excess of $200 \mu\text{g} \cdot \text{m}^{-3}$ in 1977 to 1987, 95% CI 0.99–2.34) (135). However, it was similarly associated with the total suspended particle level (RR 1.74 for 1000 h per year in excess of $200 \mu\text{g} \cdot \text{m}^{-3}$ in 1973 to 1977, 95% CI 1.11–2.72). In multipollutant analyses, the ozone level and the total suspended particle level entered different models, and, once one was entered, the other factors were not significant. Interestingly, the increased risk of asthma in relation to ozone level seemed to be restricted to men (RR 3.12, 95% CI 1.61–5.85), possibly because they spend more time outdoors than women (136).

Cross-sectional studies

For 429 male Seventh-day Adventists from San Gabriel Valley and San Diego, no significant or consistent differences in symptoms or pulmonary function were found between the areas (137). The peak oxidant values were higher in the San Gabriel Valley, with a mean daily 1-h maximum of 138 to 150 versus 74 ppb in San Diego and 90th percentile hourly means at 120 to 140 versus 80 ppb, although the average concentrations over the whole period were about equal (44 to 49 versus 38 ppb); other pollutant levels were low and similar.

Likewise, Linn et al (138) compared the symptom prevalence and pulmonary function of 644 office workers in Los Angeles (1969 mean 1-h maximum level for oxidants in 1965–1972 being 70 ppb), and San Francisco (20 ppb), with 10% of the daily maximum above 150 ppb in Los Angeles and above 30 ppb in San Francisco. The levels of nitrogen dioxide, carbon monoxide, and total suspended particles were also somewhat more elevated in Los Angeles. Most of the results of the forced expiratory tests, single-breath nitrogen tests, and questionnaire interviews did not differ significantly, but the Los Angeles women reported nonpersistent cough and phlegm more often than San Francisco women.

Many German cross-sectional studies have been performed on the prevalence of croup syndrome and obstructive bronchitis among children, but most present few data on actual ozone levels. Wichmann et al (94) have presented an evaluation of several such studies performed in Baden-Württemberg and North Rhine Westphalia. In Stuttgart, where part of the investigation was performed and annual averages for ozone were 20 to $40 \mu\text{g} \cdot \text{m}^{-3}$, with 98th percentile values (annual peaks)

of 70 to 130 $\mu\text{g} \cdot \text{m}^{-3}$, there was a significantly elevated relative risk of 5.40 for obstructive bronchitis when the highest and lowest annual average ozone exposure tertiles were compared. Taken together, however, the cross-sectional studies of Baden-Württemberg and North Rhine Westphalia do not present consistent evidence for clear relationships between air pollutants and the prevalence of croup syndrome or obstructive bronchitis.

A Canadian investigation of respiratory symptoms and pulmonary function (139) compared children aged from 7 to 12 years from two towns. Tillsonburg, Ontario, had higher average ozone levels annually than Portage la Prairie, Manitoba, and, although the annual maximums were similar (272 versus 260 $\mu\text{g} \cdot \text{m}^{-3}$), the number of days per year in 1983 to 1984 exceeding 160 $\mu\text{g} \cdot \text{m}^{-3}$ were 23 to 30 versus 2, and the annual mean ozone levels were 53.4 versus 38.0 $\mu\text{g} \cdot \text{m}^{-3}$. The Tillsonburg levels of sulfur dioxide, sulfate, and nitrogen oxide were also significantly higher. Except for more frequent inhalant allergies in Tillsonburg, the prevalence of symptoms and illnesses was similar for the two communities. However, the Tillsonburg children's pulmonary function levels were significantly lower by 2% for FVC and 1.7% for $\text{FEV}_{1.0}$. Although such an effect may also have been due to acute air pollution, pulmonary tests did not show a relation to air pollution variables on the day of testing or preceding days. The role of individual pollutants was difficult to evaluate. A later study by the same group (140) on five rural communities in Ontario and five in Saskatchewan (the 90th percentile of the daily 1-h maximum ozone level being 160.0 versus 94.0 $\mu\text{g} \cdot \text{m}^{-3}$ and the annual mean ozone level being 62.8 versus 48.0 $\mu\text{g} \cdot \text{m}^{-3}$) also found no difference in respiratory illness or symptoms, but there were statistically significant decrements of 1.7% in FVC and 1.3% in $\text{FEV}_{1.0}$ for the Ontario children.

In a cross-sectional assessment of air pollution and the chronic respiratory health of children in the Harvard study of six cities (141), the annual mean ozone levels in the cities ranged from 36.0 to 75.6 $\mu\text{g} \cdot \text{m}^{-3}$. In analyses of each air pollutant separately, the asthma and hay fever rates were positively associated with the annual mean ozone levels when controlled for gender, age, gas stove, and maternal smoking. The relative odds were 1.88 (95% CI 1.03–3.43) and 1.62 (95% CI 0.44–6.0) between the most and least polluted cities. Bronchitis, chronic cough, and chest illness were associated with particulate pollution and, to some extent, other gaseous pollutants. No associations were found between the air pollutants and pulmonary function measures.

Spirometric measurements and questionnaire data from 4300 people aged from 6 to 24 years in 44 cities from the NHANES II national health survey in the United States (1976 to 1980) were analyzed against the annual mean levels of the preceding year for sulfur dioxide,

nitrogen dioxide, total suspended particles, and ozone (daily averages for 1100 in the morning to 1700 in the afternoon, and daily 1-h maximum) (142). Due to insufficient overlap of the pollution monitoring stations, each pollutant was considered in separate regressions. Ozone analyses used 1005 subjects and 24 sampling units. The 10th percentile of the annual mean ozone level for the average 1100 to 1700 (5-h) levels was 46 $\mu\text{g} \cdot \text{m}^{-3}$, the median was 64 $\mu\text{g} \cdot \text{m}^{-3}$, and the 90th percentile was 80 $\mu\text{g} \cdot \text{m}^{-3}$. The total suspended particle level and the ozone and nitrogen dioxide levels were all significantly associated with the FVC, $\text{FEV}_{1.0}$ and PEF values in regression models. A nonparametric smoothing function of the FVC percentage difference from the predicted indicated a threshold for effect at about 80 $\mu\text{g} \cdot \text{m}^{-3}$, reaching 10% difference at about 160 $\mu\text{g} \cdot \text{m}^{-3}$.

Zwick et al (143) performed a cross-sectional study of 499 children aged from 10 to 14 years from a high-ozone and a low-ozone area in Austria in 1989. The maximum ozone concentrations were 376 $\mu\text{g} \cdot \text{m}^{-3}$ and 190 $\mu\text{g} \cdot \text{m}^{-3}$, respectively, during 1987 to 1989, and the percentage of time above the cut-offs of 120, 200 and 240 $\mu\text{g} \cdot \text{m}^{-3}$ were 45%, 9.7%, and 2.5%, respectively, as compared with 0.3%, 0%, and 0%. Subjective complaints showed no differences, nor did the lung function parameters, except bronchial hyperresponsiveness, which was more frequent and more severe in the exposed group (29.4% versus 19.9%, $P < 0.02$; dose required to provoke a 20% decline in $\text{FEV}_{1.0}$ 2100 $\mu\text{g} \cdot \text{m}^{-3}$ versus 2350 μg , $P < 0.05$). The allergic disease prevalence and the results of the skin prick tests and the allergologic blood tests were similar. Although the white blood cell count was the same, the lymphocyte subpopulations differed, with a significantly lower helper/suppressor cell index ($\text{OKT4}^+/\text{OKT8}^+$) and a decreased number of natural killer cells (OKNK^+) among the exposed.

A study of 1626 school-aged Austrian children in different areas in the European alpine region (144) found decreased lung function (significant for $\text{FEV}_{1.0}$, FEF_{50} , FEF_{75}), and a 2.36 times higher asthma prevalence (girls 3.95, boys 1.93), in an area with high ozone levels (mean 30-min maximum 292 $\mu\text{g} \cdot \text{m}^{-3}$, annual mean 104 $\mu\text{g} \cdot \text{m}^{-3}$) when compared with an area with a lower ozone level (mean 30-min maximum 224 $\mu\text{g} \cdot \text{m}^{-3}$, annual mean 52 $\mu\text{g} \cdot \text{m}^{-3}$), but with somewhat higher nitrogen dioxide levels.

Summary

Cross-sectional and cohort studies on the effects of long-term exposure to ozone on the respiratory system present a mixed picture. The UCLA studies from California report several adverse effects, both cross-sectionally and longitudinally, from areas with higher oxidant air pollution with respect to both symptoms and lung function. However, no attempt was made to separate the effects of

various pollutants in the different areas. The Seventh-day Adventist studies, also from California, found an association of chronic obstructive pulmonary disease with ozone level in prevalence data, but the association with total suspended particles seemed stronger. In the follow-up, the total prevalence of chronic obstructive pulmonary disease again seemed mainly to be related to the total suspended particle level, but an association with ozone concentration was suggested for asthma among the men, as well as for pulmonary cancer overall. The analyses used a cut-off of 500 h per year above $200 \mu\text{g} \cdot \text{m}^{-3}$ for exposure versus nonexposure.

Most other studies are cross-sectional in design, and they compare two or more areas with different ozone levels. The effects reported include increased symptom prevalence and decreased lung function. Two early studies on adults report little differences in these outcomes in relation to oxidant levels.

Most later investigations have focused on children and young adults. An increased asthma and hay fever prevalence has been reported for children in the United States for areas with a range of annual average ozone levels between 36 and $75.6 \mu\text{g} \cdot \text{m}^{-3}$, but no difference in the prevalence of bronchitis, chronic cough chest illness, or lung function was noted. German studies (annual average range $40\text{--}80 \mu\text{g} \cdot \text{m}^{-3}$) found some indication of increased obstructive bronchitis in children, but the results were not consistent. In Canada, at annual average ozone levels of $53.4 \mu\text{g} \cdot \text{m}^{-3}$ versus $38 \mu\text{g} \cdot \text{m}^{-3}$, and 23 to 30 versus 2 days with a 1-h maximum above $160 \mu\text{g} \cdot \text{m}^{-3}$, increased inhalant allergies, but no other symptoms, and decreased lung function values were found for the exposed children. A later study confirmed the decreased lung function values at annual average ozone levels of $62.8 \mu\text{g} \cdot \text{m}^{-3}$ versus $48 \mu\text{g} \cdot \text{m}^{-3}$ and 36 versus very few days per year with a 1-h maximum ozone level above $160 \mu\text{g} \cdot \text{m}^{-3}$. In a study from Austria comparing two areas (45% versus 0.3% of time above $120 \mu\text{g} \cdot \text{m}^{-3}$) no difference in children's symptoms or lung function was reported, but an increased bronchial hyperresponsiveness and altered lymphocyte subpopulations were seen. A large study covering 24 cities (annual adjusted average for time between 1100 and 1700, 10th to 90th percentile, being 46 to $80 \mu\text{g} \cdot \text{m}^{-3}$) in the United States found decreased lung function for subjects between 6 and 24 years of age in more exposed areas and indications of a threshold for differences at an ozone level of $80 \mu\text{g} \cdot \text{m}^{-3}$. Likewise, an Austrian study showed a decreased lung function and an increased asthma prevalence for exposed children at annual averages of 104 versus $52 \mu\text{g} \cdot \text{m}^{-3}$.

Thus the data suggest some effects on asthma, obstructive bronchitis, inhalant allergy, and lung function when the average annual ozone level is above 60 to $80 \mu\text{g} \cdot \text{m}^{-3}$. This effect may depend mainly on the length

of time above $120 \mu\text{g} \cdot \text{m}^{-3}$ or higher cut-offs. Levels of other pollutants, such as particulates and nitrogen dioxide, were often increased in the high ozone areas, and it is therefore difficult to evaluate the effect specifically related to ozone, in particular for the cross-sectional studies.

Summary and concluding remarks

Epidemiologic studies on *short-term exposure* to ozone have investigated different end points, such as symptoms, lung function disturbances, hospital admissions, and mortality. A common approach has been to assess adverse health effects in relation to hourly or daily changes in air pollutant levels during periods extending from several weeks to years. It is essential to control for the effects of other factors, including temperature and other air pollutants, as well as seasonal and other long-term variations, to enable an evaluation of the effects of the short-term ozone exposure.

Studies of lung function provide the most consistent evidence of ozone-induced effects. In adults decrements of various lung function parameters, including FVC, $\text{FEV}_{1.0}$, FEF_{25-75} , and PEF, have been observed after physical exercise at ozone levels below $160 \mu\text{g} \cdot \text{m}^{-3}$ in several studies. Lung function effects were also seen in children below this level, and no threshold has been established. For healthy children and 30-year-old adults the absolute effects correspond to a mean decrease of up to 2.7% to 7.5% for the various lung function parameters following a $100 \mu\text{g} \cdot \text{m}^{-3}$ increase in the 1-h maximum ozone concentration. However, these groups represent sections of the population with optimal lung function. It is not clear whether subjects with asthma or respiratory symptoms are more sensitive to the lung function effects of ozone. However, if the absolute effect is similar to that on healthy children and adults with their lower baseline values, they may risk decrements of up to 15% to 20% for the different lung function parameters. Likewise, since lung function decreases with age, elderly people may risk larger relative decrements. Notwithstanding, these groups are more vulnerable to clinical symptoms at any level of lung function decrement.

Studies with children and adults, including subjects with asthma or other respiratory conditions, show that symptoms can appear at 1-h maximum ozone concentrations of about $300 \mu\text{g} \cdot \text{m}^{-3}$, and with heavy exercise at levels of 160 to $200 \mu\text{g} \cdot \text{m}^{-3}$, although the evidence is not consistent. Hospital admissions have been found to correlate with ozone levels in several studies in which the ozone levels ranged up to $240 \mu\text{g} \cdot \text{m}^{-3}$. The results have been calculated to account for 7% to 21% of respiratory admissions in different North American settings.

Studies on daily mortality in relation to ozone in ambient air provide no clear evidence of an effect.

Investigations on effects following *long-term exposure* to ozone have primarily used a cross-sectional design. With this design it is difficult to differentiate between effects caused by long-term and short-term exposure. Increased rates of bronchial hyperreactivity, inhalant allergies, diseases of the lower respiratory tract, including asthma, and decreased lung function have been reported in areas with annual mean ozone levels of about 60 to 80 $\mu\text{g} \cdot \text{m}^{-3}$. Some studies suggest that this effect may depend mainly on the length of time above 120 $\mu\text{g} \cdot \text{m}^{-3}$ or higher cut-offs. In most studies, levels were also increased for other air pollutants, such as sulfur dioxide, nitrogen dioxide or particulates, and it is difficult to assess the role of ozone separately. Follow-up studies in a group of Seventh-day Adventists have suggested an increased incidence of asthma and pulmonary cancer for exposures of more than 500 h per year in excess of 200 $\mu\text{g} \cdot \text{m}^{-3}$, but these data should also be interpreted cautiously due to the high correlation with other air pollutants, especially particulates.

In conclusion, it appears that slight acute decrements in pulmonary function are associated with exposure to ozone at 1-h maximum levels as low as 160 $\mu\text{g} \cdot \text{m}^{-3}$ or lower. The clinical significance of these effects at rest is not clear, but they may result in impaired performance during exercise. At higher concentrations, symptoms and exacerbation of respiratory disease can develop, leading to emergency visits and admissions to a hospital. Long-term exposure to ozone at annual concentrations of about 60 to 80 $\mu\text{g} \cdot \text{m}^{-3}$ has been associated with increased rates of diseases of the respiratory tract and lung function, but the specific role of ozone for these effects is uncertain, mainly due to possible contributions from other air pollutants.

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Health risk evaluation and recommended guideline values for ozone

Summary and health risk evaluation

Ozone is an atmospheric pollutant occurring on a regional scale. Elevated concentrations of ground-level ozone are mainly due to chemical reactions of emissions of volatile organic compounds and nitrogen oxides. The concentrations of ground-level ozone occurring in non-urban areas in western Europe are generally in the range of 40 to 70 $\mu\text{g} \cdot \text{m}^{-3}$. The background ozone levels at the end of the 19th century were about 20 $\mu\text{g} \cdot \text{m}^{-3}$. In summer the ozone concentrations are higher than in winter, with a level of 120 $\mu\text{g} \cdot \text{m}^{-3}$ exceeded 20 to 150 h and those above 150 $\mu\text{g} \cdot \text{m}^{-3}$ exceeded 2 to 20 h in southern Sweden and considerably more often in central and western Europe. The ground-level ozone concentration has a strong daily cycle with a peak in the afternoon and a minimum in the early morning.

The indoor to outdoor ratio of the concentration of ozone varies strongly with the ventilation rate and the building materials, the ratio probably being in the range of 0.3 to 0.8 $\mu\text{g} \cdot \text{m}^{-3}$ for Swedish conditions.

Ozone levels are reduced in urbanized areas, as compared with the nonurban surroundings because of the reaction between ozone and the emission of nitrogen monoxide from traffic. However, photochemical reactions of nitrogen oxides and volatile organic compounds give rise to ozone pollution downwind of urban areas.

The exposure of humans to ozone depends on the outdoor and indoor concentrations and also on the pattern of activity of the population, such as travel patterns, dwellings and workplaces. Most of our outdoor activities take place during the summer and during daytime, when the concentration of ozone reaches its maximum values. The ozone level is also high in nonurban areas and suburbs to larger cities, areas where a large section of the population resides.

Metabolism

Upon inhalation, a large proportion (30 to 40%) of ozone is absorbed within the nasal or oral airways. As much as 90% of inhaled ozone is absorbed in the entire respiratory tract. During exercise proportionately more of the gas is absorbed by the lower respiratory airways. It is evident that the efficiency of uptake is relatively insensitive to the concentration of ozone inhaled. Due to the chemical reactivity of ozone, ozone probably does not penetrate the epithelial lining fluid of the airways in an intact form, except in areas of minimal lining fluid thickness or a lack

of surfactant. Consequently, ozone is not appreciably absorbed through the pulmonary epithelium into blood circulation. Instead, ozone reacts readily with biomolecules, such as lipids, forming more stable, but none the less reactive molecules, which often possess potent biological properties. Reactive oxygen metabolites, such as hydrogen peroxide and the hydroxyl radical, are also formed as ozone dissolves into the aqueous phase of the epithelial lining fluid. Accordingly, the toxicity of ozone is mainly restricted to the lung and probably involves a multitude of secondary biochemical mediators. Ozone reacts readily with antioxidants such as glutathione and ascorbate, both intracellularly and intercellularly, and the availability of such molecules strongly dictates the balance between the detoxification of the gas and its reaction with other biological molecules. One of the major macromolecular targets for ozone and derived reactive species are the phospholipids constitutive of biomembranes. Ozone readily reacts with polyunsaturated fatty acids in *in vitro* systems, yielding lipid hydroperoxides, other hydroperoxides, and reactive aldehydes. Such metabolites, as well as molecules such as arachidonic acid, may play a central role in the cytotoxicity of ozone. Similarly, the direct interaction of ozone and derived reactive species with cellular proteins may detrimentally alter their activities and also contribute to the induction of toxicity in the lungs.

Animal studies

There are numerous studies on the toxic effects of ozone in animals. Studies have shown that the area of the lung most affected is the centriacinar region (the junction of the bronchioles and alveoli). When damaged by ozone, the ciliated epithelial cells of the bronchi and the type I cells of the alveoli are replaced by more resistant non-ciliated cells and type II cells, respectively. The epithelial cells may also form hyperplasia, and bronchiolar cells replace alveolar cells. Thickening of the epithelial-interstitial layers and collagen deposition are early signs of an interstitial fibrosis, which increases gradually over time in long-term exposure studies. The lowest concentration of ozone studied that has caused morphological alterations in primates is 300 $\mu\text{g} \cdot \text{m}^{-3}$, 8 h per day for 90 d.

The morphological changes are the most marked during acute exposure to the gas, especially in macrophage cell number, the increase of which is subsequently attenuated. However, a definitive increase in the number of bronchiolar Clara cells is still at hand in primates after

90 d. There is still overgrowth of Clara cells into alveoli (metaplasia) in rats after a two-year exposure, and in mice after life-long exposure to an ozone concentration of $233 \mu\text{g} \cdot \text{m}^{-3}$. At $1000 \mu\text{g} \cdot \text{m}^{-3}$ in these two species the numbers of pulmonary macrophages and pulmonary fibrosis increase at the end of exposure.

Ozone affects host-defense mechanisms in animals. Mucociliary clearance is affected at $200 \mu\text{g} \cdot \text{m}^{-3}$, at which the function of alveolar macrophages has been shown to be changed. Perhaps as a consequence of these effects, ozone enhances the susceptibility to bacterial lung infections from $200 \mu\text{g} \cdot \text{m}^{-3}$ and above in mice.

A major effect of ozone inhalation is the induction of pulmonary inflammation and the resultant increase in airway permeability, particularly after acute exposures. Inflammatory mediators are released from macrophages, epithelial cells, and endothelial cells, and many of these mediators participate in the induction of a situation of localized inflammation by acting as chemoattractants to circulatory leukocytes and by increasing endothelial and epithelial layer permeability to circulatory and interstitial fluid, protein, and cells. This phenomenon often results in the accumulation of fluid, protein, and leukocytes within the airways. Acute or subchronic exposures to ozone concentrations as low as $200 \mu\text{g} \cdot \text{m}^{-3}$ for up to 24 h induces transient inflammatory changes in the lungs of several experimental animals. These changes eventually subside leaving few signs of permanent change to lung structure and function. Repeated exposures initially cause similar responses to acute exposure. However, as the length of continuous exposure increases, an adaptive response occurs which instigates tolerance. Thus chronic and continuous exposure to $400 \mu\text{g} \cdot \text{m}^{-3}$ is required to induce proinflammatory effects in rodents. However, several studies indicate the importance of the pattern of exposure. Thus chronic exposure to $120 \mu\text{g} \cdot \text{m}^{-3}$, interspersed by spikes of $500 \mu\text{g} \cdot \text{m}^{-3}$, induce definite signs of inflammation. These short periods of elevated exposure seem, therefore, to dictate the chronic effects of the gas. As this pattern of exposure closely mimics those occurring in the ambient environment, consideration of the duration of elevated levels of ozone during chronic environmental exposure may be critical for a correct appraisal of risks to health.

Ozone also causes transient effects on lung function. Acute exposure causes an increased breathing frequency and a decreased tidal volume (rapid, shallow breathing). In animal experiments, effects on compliance and resistance have been found, as is also a decreased lung volume.

Ozone enhances the sensitization to inhaled allergens in animals. After four intermittent 4-d exposures to an ozone concentration of $260 \mu\text{g} \cdot \text{m}^{-3}$, combined with short periods of allergen exposure, mice were significantly more allergic and a larger number of animals in the exposed group were allergic. In addition, primates be-

come significantly more sensitized to allergen when exposed in the presence of an ozone concentration of $2000 \mu\text{g} \cdot \text{m}^{-3}$. However, data on primate exposure to lower concentrations are not available. Ozone can also enhance the bronchial response to allergens and bronchoconstricting agents, the effect being due to the airway inflammation caused by ozone.

Controlled human studies

Ozone-induced effects on the respiratory system have been investigated in a large number of controlled human exposure studies. Some of the studied effects are qualitatively similar to those observed in animal experiments, for example, inflammatory effects and effects on lung function. The duration of the exposure has varied from 40 min to 6.6 h, and the ozone concentration from 160 to $1500 \mu\text{g} \cdot \text{m}^{-3}$. Decrements in lung function measured as a decreased forced vital capacity (FVC), forced expiratory volume in 1 s ($\text{FEV}_{1.0}$), forced expiratory flow between 25% and 75% of vital capacity (FEF_{25-75}), or increased airway resistance (SR_{aw}) occur in a dose-dependent fashion and, in some studies, even at the lowest dose tested, $160 \mu\text{g} \cdot \text{m}^{-3}$ (6 h, exercise). The lowest concentration affecting lung function at rest and during shorter duration (2 h) is about $1000 \mu\text{g} \cdot \text{m}^{-3}$. These effects on lung function are often accompanied by subjective respiratory symptoms such as cough, substernal pain, and shortness of breath. Peak exposures seem to be more important than steady-state exposure. The sensitivity to ozone varies greatly between individual persons but the effect seems to be fairly well reproducible for the same subject. In contrast to the effect of nitrogen dioxide, the effects of ozone on lung function and bronchial responsiveness are generally similar in patients with asthma as in normal persons. However, recent reports on inflammatory reactions in the airways suggest that asthmatic subjects are more sensitive to ozone than normal persons are. Furthermore, patients with asthma or chronic obstructive pulmonary disease often possess reduced lung function. Thus, even if the ozone-induced decrement in lung function among these groups is comparable with that of normal persons, the clinical importance of the change is much greater for these patients.

An ozone-induced increase in nonspecific airway responsiveness has been reported for healthy subjects at concentrations as low as $160 \mu\text{g} \cdot \text{m}^{-3}$ (6 h, exercise). Enhanced bronchial responsiveness to inhaled allergen in asthmatic subjects has been reported after ozone exposure to $240 \mu\text{g} \cdot \text{m}^{-3}$ at rest. This finding may indicate that ambient levels of ozone can potentiate the effects of airborne allergens, which should be an effect of great importance. However, whereas another recent report on ozone-allergen interactions also shows an enhancing effect of ozone, two other preliminary reports indicate no effect. Thus no firm conclusion can as yet be drawn as to

whether ozone potentiates the allergic reaction in humans.

A reduction of oxygen uptake and reduced performance during heavy exercise are other adverse effects of ozone, demonstrated at concentrations of about $400 \mu\text{g} \cdot \text{m}^{-3}$, but even down to 120 to $140 \mu\text{g} \cdot \text{m}^{-3}$ in one study.

As in animal experiments, inflammatory reactions have been demonstrated in humans at ozone concentrations down to $160 \mu\text{g} \cdot \text{m}^{-3}$ (6 h, exercise) by the analysis of bronchial lavage fluid.

Repeated exposure to ozone day after day gives an attenuation of the acute symptoms and lung function responses after the second day of exposure. In addition, airway responsiveness and some markers of inflammation seem to attenuate with repeated exposures, while the occurrence of other markers of inflammation do not. This phenomenon of attenuation is not fully understood and therefore complicates the interpretation of the findings from controlled human studies. However, many of the acute effects must be regarded as adverse health effects per se, even if no prediction concerning chronic respiratory effects can be made.

Epidemiologic studies

Epidemiologic studies on short-term exposure to ozone have investigated different end points, such as symptoms, lung function disturbances, hospital admissions, and mortality. A common approach has been to assess adverse health effects in relation to hourly or daily changes in air pollutant levels during periods extending from several weeks to years. It is essential to control for effects of other factors, including temperature and other air pollutants, as well as seasonal and other long-term variations in order to enable an evaluation of the effects of the short-term ozone exposure.

Studies of lung function provide the most consistent evidence of ozone-induced effects. Decrements of various lung function parameters, including $\text{FEV}_{1.0}$, FEF_{25-75} and PEF, have been observed in adults after physical exercise at ozone levels below $160 \mu\text{g} \cdot \text{m}^{-3}$ in several studies. In children, effects on lung function were also found below this level, and no clear threshold has been established. The effects correspond to a mean decrease of about 5% after a $100 \mu\text{g} \cdot \text{m}^{-3}$ increase in ozone concentration. It is not clear whether subjects with asthma or respiratory symptoms are more sensitive to the effects of ozone on lung function. However, subjects with obstructive airway disease may be more vulnerable as their lung function is already compromised.

Studies on children and adults, including subjects with asthma or other respiratory diseases, show that symptoms can appear at 1-h maximum ozone concentrations of about $300 \mu\text{g} \cdot \text{m}^{-3}$, and with heavy exercise at

levels down to 160 to $200 \mu\text{g} \cdot \text{m}^{-3}$, although the evidence is not consistent. Hospital admissions and emergency visits for respiratory conditions have been found to correlate with daily 1-h maximum ozone concentrations ranging up to $240 \mu\text{g} \cdot \text{m}^{-3}$.

Investigations on effects after long-term exposure to ozone have primarily used a cross-sectional design. With this design it is difficult to differentiate between effects caused by long-term and short-term exposure. Increased rates of respiratory symptoms, bronchial hyperreactivity, diseases of the lower respiratory tract, and decreased lung function have been reported for areas with annual mean ozone levels of about 60 to $80 \mu\text{g} \cdot \text{m}^{-3}$. Some studies suggest that this effect depends mainly on the amount of time above $120 \mu\text{g} \cdot \text{m}^{-3}$ or higher cut-offs. In most studies, levels were also increased for other air pollutants, such as sulfur dioxide, nitrogen dioxide and particulates, and it is difficult to assess the role of ozone separately. Follow-up studies have suggested an increased incidence of asthma and respiratory cancer for exposures of more than 500 h per year in excess of $200 \mu\text{g} \cdot \text{m}^{-3}$, but these data should be interpreted cautiously due to the high correlation with other pollutants, especially particles.

Cancer

The genotoxicity and carcinogenicity of ozone have been investigated in different experimental systems. Ozone is genotoxic at high concentrations in vitro as it has caused mutations, chromatid-type chromosome aberrations, sister chromatid exchanges, and neoplastic transformation. There is no convincing evidence of cytogenetic effects on lymphocytes from exposed animals or humans. However, in one study, chromatid-type chromosome aberrations were induced in lung macrophages in rats that were exposed to ozone at $430 \mu\text{g} \cdot \text{m}^{-3}$ for 6 h.

Lung adenomas have been induced in the sensitive strain A/J mouse, and in a recent two-year and life-long inhalation study with 1000 and $2000 \mu\text{g} \cdot \text{m}^{-3}$, both lung adenomas and carcinomas were induced in B6C3F₁ mice at the higher concentration but not in rats. There was an increased frequency of mutations in the *K-ras* oncogene in lung tumors of the ozone-treated female mice. The ability of ozone to act as a tumor promoter or a cocarcinogen has been investigated, but it cannot be substantiated.

Recommended guideline values

Short-term exposure

The best information available on the health effects of ozone comes from relatively short-term peak exposures.

As there are several good chamber studies and epidemiologic studies, the risk assessment for short-term exposure is primarily derived from human data.

Most chamber studies have used exposures of 1- to 6-h duration, while the epidemiologic studies have generally correlated the health effects with 1-h maximum daily values. In the chamber studies, respiratory symptoms and pulmonary effects such as lung function decrements, increased airway responsiveness, and inflammatory reactions have all been demonstrated after exposure to $160 \mu\text{g} \cdot \text{m}^{-3}$ during moderate exercise over 6 h. Concentrations lower than $160 \mu\text{g} \cdot \text{m}^{-3}$ have not been tested. If subjects are resting or the exposure time shortened, higher ozone concentrations may be endured without adverse effects. Reduced lung function at 1-h maximum daily values of $160 \mu\text{g} \cdot \text{m}^{-3}$ has been demonstrated in the epidemiologic studies, and some reports indicate effects at even lower levels. There appears to be a considerable variation in sensitivity to ozone, but it is not clear if subjects with asthma or chronic obstructive pulmonary disease are particularly sensitive.

Ozone is a comparatively well studied air pollutant, and the effects must be regarded as well documented. Potentially sensitive subgroups have been tested, even during exercise. Thus only a small uncertainty factor should be warranted. However, there is a large variation in sensitivity between persons, and effects have been demonstrated even at the lowest concentration repeatedly tested in human experiments ($160 \mu\text{g} \cdot \text{m}^{-3}$). An uncertainty factor of 2 would lead to a recommended guideline value of $80 \mu\text{g} \cdot \text{m}^{-3}$ for short-term exposure. If the guideline is expressed as a 1-h maximum value, there would be an extra safety margin, as some of the controlled studies were performed with longer exposure times.

Long-term exposure

There are no controlled human studies with long-term exposure, and the epidemiologic basis for a risk assessment is much weaker than for short-term exposure. However, in some cross-sectional studies, long-term exposure to ozone, at annual concentrations of about 60 to $80 \mu\text{g} \cdot \text{m}^{-3}$, has been associated with increased rates of various symptoms and diseases of the respiratory tract, although the specific role of ozone for these effects is uncertain. Some studies suggest that the effects depend

mainly on the amount of time above $120 \mu\text{g} \cdot \text{m}^{-3}$ or higher cut offs.

Long-term exposure induces an adaptive attenuation of most pulmonary responses of animals to ozone. However, short-term elevated levels of ozone ($500 \mu\text{g} \cdot \text{m}^{-3}$) during long-term exposure ($120 \mu\text{g} \cdot \text{m}^{-3}$) induce proinflammatory effects in rodents. Morphological effects such as interstitial fibrosis have been induced in the lung tissue of primates after long-term exposure. After 90 d of exposure to $300 \mu\text{g} \cdot \text{m}^{-3}$ the number of Clara cells increase with overgrowth into alveoli, as does the number of macrophages in bronchioli; these increases indicate that an ongoing inflammation is still evident after such prolonged exposure.

The potential carcinogenic effects of ozone must also be considered in the risk assessment of ozone. Lung tumors have been induced in mice after life-long exposure to $2000 \mu\text{g} \cdot \text{m}^{-3}$, although no carcinogenic response has been seen in rats. As mutations in the *K-ras* oncogene were enhanced in lung tumors of the ozone-exposed female mice and as ozone causes mutations and chromosome effects in some in vitro tests, a genotoxic mode of action for the tumor induction is possible. However, considering the reactivity and cytotoxicity of ozone, the cancer risk for mice at low concentrations may not be proportional to the risk at the high concentrations that were applied in the cancer study because ozone is inactivated in the fluid layer that protects the lung epithelium. No lung tumors have been induced in rats and hamsters by ozone. Ozone-induced tumors have been detected only in one species of animals tested, and therefore it is at present difficult to assess the carcinogenicity in humans.

Overall, the epidemiologic and experimental evidence is not considered sufficient as a basis for a long-term guideline for ozone.

In summary, we recommend a 1-h guideline value of $80 \mu\text{g} \cdot \text{m}^{-3}$. This limit is intended to protect against adverse effects of ozone on human health also among the most sensitive part of the population. The proposed guideline level is currently exceeded and should be regarded as a goal for action to avoid adverse health effects. At present the evidence is not considered sufficient as a basis for a long-term guideline. However, the proposed short-term guideline would probably offer some protection against potential effects of ozone following long-term exposure.

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