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Environmental Health Criteria 7

PHOTOCHEMICAL OXIDANTS

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While every effort has been made to present information in the criteria documents as accurately as possible without unduly delaying their publication, mistakes might have occurred and are likely to occur in the future. In the interest of all users of the environmental health criteria documents, readers are kindly requested to communicate any errors found to the Division of Environmental Health, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda which will appear in subsequent volumes.

In addition, experts in any particular field dealt with in the criteria documents are kindly requested to make available to the WHO Secretariat any important published information that may have inadvertently been omitted and which may change the evaluation of health risks from exposure to the environmental agent under examination, so that the information may be considered in the event of updating and re-evaluating the conclusions contained in the criteria documents.

WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR PHOTOCHEMICAL OXIDANTS

Tokyo, 30 August–3 September 1976

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ENVIRONMENTAL HEALTH CRITERIA FOR PHOTOCHEMICAL OXIDANTS

A WHO Task Group on Environmental Health Criteria for Photochemical Oxidants met in Tokyo from 30 August to 3 September 1976. Dr Y. Hasegawa, Medical Officer, Control of Environmental Pollution and Hazards, Division of Environmental Health, WHO, opened the meeting on behalf of the Director-General and expressed the appreciation of the Organization to the Government of Japan for acting as host to the meeting. The Task Group reviewed and revised the second draft criteria document and made an evaluation of the health risks from exposure to photochemical oxidants.

The first and second drafts of the criteria document were prepared by Professor Carl M. Shy of the Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC, USA, and Dr Donald E. Gardner, Chief, Biomedical Research Branch, Clinical Studies Division, Health Effects Research Laboratory, US Environmental Protection Agency, Research Triangle Park, NC, USA. The comments upon which the second draft was based were received from the national focal points for the WHO Environmental Health Criteria Programme in Bulgaria, Canada, Czechoslovakia, the Federal Republic of Germany, Japan, New Zealand, Poland, Sweden, the USA, and the USSR; and from the Food and Agriculture Organization of the United Nations (FAO), Rome, the World Meteorological Organization (WMO), Geneva, and the International Union of Pure and Applied Chemistry (IUPAC). The collaboration of these national institutions and international organizations is gratefully acknowledged.

The Secretariat also wishes to acknowledge the most valuable collaboration in the final phases of the preparation of this document, of Professor Shy, Dr Gardner, Dr R. G. Derwent of the Environmental and Medical Sciences Division, Atomic Energy Research Establishment, Harwell, England, and Professor K. Schaffner of the Institute of Radiation Chemistry at the Max-Planck-Institute for Carbon Research, Mulheim an der Ruhr, Federal Republic of Germany.

This document is based primarily on original publications listed in the reference section. Much valuable information may also be found in other published criteria documents (US Department of Health, Education and Welfare, 1970; North Atlantic Treaty Organization, 1974; National Academy of Sciences, 1977).

Because biological knowledge concerning many components of photochemical air pollution is limited, the Task Group agreed that a

definition of photochemical oxidants should be given early in the criteria document.

Photochemical oxidants can be formed as the result of the sunlight-induced oxidation of precursor pollutants emitted into the atmosphere. These precursor compounds include the oxides of nitrogen and a variety of hydrocarbons with different chemical reactivities with respect to the formation of photochemical oxidants. The principal oxidants are ozone, nitrogen dioxide, and the peroxyacylnitrates. However, until recently, measurement methods specific for each of these oxidants were not available and the most commonly employed methods were affected, to some extent, by interference from other atmospheric pollutants. Thus, when such studies are being considered, it is important to know whether some correction has been made for this interference, particularly in studies related to health effects in man.

Although many other ingredients have been identified in photochemical air pollution, there is little information available at the moment concerning their biological significance, and they have not been referred to in this document. The biological significance of nitrogen dioxide has been reviewed and evaluated in another WHO environmental health criteria document (World Health Organization, 1977).

Details of the WHO Environmental Health Criteria Programme including some terms frequently used in the documents may be found in the general introduction to the Environmental Health Criteria Programme published together with the environmental health criteria document on mercury (Environmental Health Criteria 1—Mercury, Geneva, World Health Organization, 1976).[a]

The following conversion factors have been used in the present document[b]:

carbon monoxide (CO)	1 ppm = 1150 $\mu g/m^3$
nitric oxide (NO)	1 ppm = 1230 $\mu g/m^3$
nitrogen dioxide (NO_2)	1 ppm = 1880 $\mu g/m^3$
nitrous oxide (N_2O)	1 ppm = 1800 $\mu g/m^3$
ozone (O_3)	1 ppm = 2000 $\mu g/m^3$
peroxyacetylnitrate (PAN)	1 ppm = 5000 $\mu g/m^3$
sulfur dioxide (SO_2)	1 ppm = 2600 $\mu g/m^3$

[a] Reprints available from the Division of Environmental Health, World Health Organization, 1211 Geneva 27, Switzerland.

[b] When converting values expressed in ppm to $\mu g/m^3$, the numbers have been rounded up to 2 or, exceptionally 3 significant figures and, in most cases, concentrations higher than 10 000 $\mu g/m^3$, have been expressed in mg/m^3.

1. SUMMARY AND RECOMMENDATIONS FOR FURTHER RESEARCH AND OTHER ACTION

1.1 Summary

1.1.1 Chemistry and analytical methods

In the context of this report, photochemical oxidants are understood to include ozone, nitrogen dioxide, and peroxyacylnitrates. Many other compounds have been proposed as components of photochemical air pollution but, as little information is available concerning their biological significance, these substances have not been discussed in this document. As nitrogen dioxide is an important air pollutant in its own right, it is the subject of a separate document (World Health Organization, 1977). Thus this report deals mainly with ozone and "oxidants" as measured by the neutral buffered potassium iodide method (NBKI).

Ozone and peroxyacylnitrates can be measured specifically by chemiluminescent reactions and by gas chromatography in conjunction with electron-capture detectors. These methods are highly sensitive and are not subject to interference from other atmospheric pollutants. To obtain the most reproducible data, sampling manifolds should be made entirely of teflon or glass as oxidants in the inlet stream may react with plastics or metal.

The terms "oxidant" or "total oxidant" are used to describe the oxidizing property of sampled air as determined by its reaction with neutral phosphate-buffered potassium iodide. Nitrogen dioxide in sampled air enhances the reaction with potassium iodide, while sulfur dioxide inhibits it. The terms "corrected oxidant" or "adjusted oxidant" indicate that measurements have been corrected for the presence of nitrogen dioxide and sulfur dioxide. Interference from these substances is not entirely eliminated by the current systems used for removing them from the inlet stream. The accuracy of the analytical procedure for oxidant measurements also depends on the pH of the buffer solution, reagent concentrations, and other variables.

1.1.2 Sources of photochemical oxidants and their precursors

Ozone, a natural constituent of the stratosphere formed by the photolysis of molecular oxygen, can be transported by atmospheric circulation into the lower atmosphere. Natural hydrocarbons including terpenes from trees and vegetation are also subject to photochemical reactions producing oxidants. These two processes are the natural sources of background ozone concentrations.

Ozone and peroxyacylnitrates are formed in the lower atmosphere by reactions between oxides of nitrogen and an array of photochemically reactive hydrocarbons. The chemical structure and reactivity of each organic hydrocarbon determines its importance in the formation of oxidants. Motor vehicles, space heating, power plants, and industrial processes are major sources of these oxidant precursors.

A balance between oxidizing and reducing agents would be maintained in the atmosphere, thus avoiding accumulation of ozone and other photochemical oxidants, if it were not for the photochemical degradation of hydrocarbons into peroxy radicals. Peroxy radicals rapidly convert nitric oxide to nitrogen dioxide, thus shifting the equilibrium towards ozone production during daylight. At night, emissions of nitric oxide into the atmosphere serve as a sink for ozone.

Welding and the manufacture of hydrogen peroxide are the main sources of occupational exposure to ozone. The use of ultraviolet lamps, electrostatic precipitators, or photocopying machines may also generate ozone.

1.1.3 Environmental concentrations and exposures

Since significant concentrations of oxidants in urban areas are generally restricted to a period of 4–6 h per day, oxidant or ozone data are most often reported in terms of maximum 1-h concentrations or in terms of the number of hours or days recorded with hourly concentrations exceeding a specified value. In isolated places far removed from sources of pollution, maximum hourly ozone concentrations of 100 $\mu g/m^3$ (0.05 ppm) have been recorded. Transport of oxidants from urban areas for distances of 100–700 kilometres appears to be a widespread phenomenon, and 1-h ozone concentrations of 120 $\mu g/m^3$ (0.06 ppm) or more have been observed in rural areas. In some large cities, maximum 1-h oxidant concentrations exceed 200 $\mu g/m^3$ (0.1 ppm) on 5–30% of days, while in Los Angeles it is commonplace for maximum 1-h oxidant values to exceed 200 $\mu g/m^3$ (0.1 ppm) on most days of the month between May and October.

Diurnal patterns in oxidant levels are an important feature of the urban environment and result from hourly changes in solar radiation and pollutant emission intensity. Maximum hourly ozone levels frequently occur around noon and are often preceded by peak concentrations of nitrogen dioxide. Concentrations of peroxyacetylnitrate are typically between 1/50th and 1/100th of those of ozone and, in general, closely follow temporal variations in ozone levels.

On a seasonal basis, oxidant concentrations tend to increase during the

high temperature season, and the frequency of days on which oxidant concentrations exceed 200 μg/m³ (0.1 ppm) is greatest during this period.

Oxidant concentrations indoors tend to be lower than those outdoors, and are reduced by destructive reaction on material surfaces. They are also reduced by activities that generate nitric oxide such as smoking and cooking.

1.1.4 Effects on experimental animals

Ozone concentrations of 2000 μg/m³ (1.0 ppm) or less with exposure periods of up to 24 h produced numerous morphological changes in the lung parenchyma in several animal species. With prolonged exposure (6–10 months), pulmonary damage such as emphysema, atelectasis, focal necrosis, bronchopneumonia, and fibrosis has been reported. The degree of morphological injury seems to be proportional to the product of the concentration and the duration of exposure.

Disturbances in respiratory functions have been noted in experimental animals exposed for 2–5 h to ozone concentrations of 520–2000 μg/m³ (0.26–1.0 ppm).

Biochemical studies that have been conducted to clarify the mechanisms of ozone toxicity at subcellular level have mainly been based on two hypotheses: (a) that oxidation of sulfhydryl groups by ozone causes changes in metabolism that result in toxic effects; and (b) that ozone reacts with unsaturated lipids to produce lipid peroxidation and consequent cell damage. However, the subcellular toxic action of ozone is still not fully understood. In other biochemical studies, changes have been reported in the mitochondrial oxygen consumption, the activities of lysosomal and microsomal enzymes, and in the synthesis of nucleic acids. Studies on the induction of oedema by lung histamine and on the effects of ozone on the surface active substance have been inconclusive.

In small rodents, "tolerance" to oedematigenous effects caused by exposure to ozone concentrations of 2000–8000 μg/m³ (1–4 ppm) has been obtained by pre-exposure to an ozone concentration of at least 600 μg/m³ (0.3 ppm). However, this did not seem to provide protection against effects that impair the phagocytic activity of macrophages.

Resistance to artificially-induced respiratory infection was reduced in several animal species by 3–4 h exposure to ozone concentrations of 160–800 μg/m³ (0.08–0.40 ppm). The effect of the ozone was further enhanced by a third stressor such as cold or exercise. Various mechanisms have been proposed for the enhanced infectivity including inactivation of a protective factor that favours survival of alveolar macrophages, inactivation of

alveolar macrophage secretory enzymes, depression of bactericidal activity, and reduction in the phagocytic activity of alveolar macrophages.

It has been shown that exposure of pregnant mice to ozone at 200–400 μg/m³ (0.1–0.2 ppm) for 7 h per day, for 15 days, increased neonatal mortality and that exposure of mice to ozone at 400 μg/m³ (0.2 ppm) for 6 h and rats to 1000 μg/m³ (0.5 ppm) for 45 min resulted in significant losses in motor activity. However, it is not clear whether these effects are due to the direct action of ozone or oxidizing agents or whether they are secondary reactions to damage in the respiratory system caused by ozone.

Data concerning other extrapulmonary effects and the carcinogenicity and mutagenicity of ozone are inadequate.

Effects produced by exposure to various mixtures of ozone and other air pollutants, and ambient air containing elevated concentrations of oxidants are mainly similar to those seen from exposure to ozone alone. However, one study reported that a single exposure to a mixture of ozone and nitrogen dioxide produced an additive effect in reducing resistance to respiratory infection in mice, and that repeated exposure might give rise to a synergistic effect. Some effects such as those on growth have only been reported with exposure to mixtures of pollutants. A deficiency of vitamin E has also been reported to enhance the toxic effects of ozone.

1.1.5 Effects on man

1.1.5.1 *Controlled exposures*

A large number of sensory effects in man have been studied under controlled conditions. The odour threshold for ozone has been shown to be 15–40 μg/m³ (0.008–0.02 ppm) and the lowest oxidant concentration producing eye irritation has been suggested to be 200 μg/m³ (0.1 ppm). Various measures of visual perception were affected by a 3-h exposure to ozone concentrations of 400–1000 μg/m³ (0.2–0.5 ppm).

Controlled exposure of healthy male subjects to ozone concentrations ranging from 200 to 2000 μg/m³ (0.1–1.0 ppm) has been reported to cause increased airway resistance and decreased ventilatory performance. Effects at the lower end of this dose-range were elicited when test subjects carried out intermittent light exercise during a 2-h exposure period. One investigator failed to observe changes in airway resistance at an ozone exposure of 500 μg/m³ (0.25 ppm) for 2 h. Thus, not all investigators were in agreement concerning the lowest experimental ozone exposures that affect airway resistance; however, three investigators found increased airway resistance at an ozone concentration of 740 μg/m³ (0.37 ppm).

A 2-h exposure to a combination of ozone at 50 μg/m³ (0.025 ppm), nitrogen dioxide at 100 μg/m³ (0.05 ppm) and sulfur dioxide at 260 μg/m³

(0.1 ppm) did not have any effect on airway resistance. However, this combined exposure did enhance the bronchoconstrictor effect of acetylcholine. A combination of an ozone concentration of 740 μg/m³ (0.37 ppm) and a sulfur dioxide concentration of 960 μg/m³ (0.37 ppm) had a potentiated effect on the impairment of ventilatory performance compared with the effects of the same concentration of each gas administered singly. In other studies in which various mixtures of ozone and other air pollutants were used, sulfur dioxide seemed to potentiate the effect of ozone more than nitrogen dioxide.

Studies were also performed on volunteer patients with chronic pulmonary disease. The respiratory function of these patients showed an improvement when they breathed filtered air for 40 h or more compared with unfiltered ambient air with an oxidant concentration of about 400 μg/m³ (0.2 ppm).

Exposures to a peroxyacetylnitrate concentration of 1350 μg/m³ (0.27 ppm) caused minor changes in variables that reflect cardiorespiratory and temperature regulation.

1.1.5.2 *Industrial exposure*

Several cases of severe ozone intoxication have been reported in welders using inert gas-shielded, consumable electrodes which greatly increased the ultraviolet irradiation of the work area. At ozone concentrations of 600–1600 μg/m³ (0.3–0.8 ppm), an increasing number of welders complained of chest constriction and irritation of the throat, while acute symptoms disappeared when ozone levels were reduced to 500 μg/m³ (0.25 ppm) or less.

There are very few studies on long-term industrial exposure to ozone and in most of them the exposure-response relationship has either not been well evaluated or has been confounded by other coexistent pollutants.

1.1.5.3 *Community exposure*

So far, no evidence has been obtained to indicate an association between peak oxidant concentrations and variations in the daily mortality rate of the general population. On the other hand, the association of oxidant levels with eye and respiratory irritation has been well documented, and in one study made on student nurses in Los Angeles, a significant increase in the frequency of cough, eye, and chest discomfort, and headache was demonstrated when maximum hourly oxidant concentrations reached 100–580 μg/m³ (0.05–0.29 ppm). Hourly oxidant levels were also correlated with decreased performance in high school cross-country runners, and the estimate of the lowest concentration at which this effect occurred was an hourly oxidant concentration of 240 μg/m³ (0.12 ppm).

Effects of oxidants on children have been extensively studied. A

correlation was detected between the decreases in airway conductance and ventilatory performance of school children and increase in ozone levels over a range up to 560 $\mu g/m^3$ (0.28 ppm); other pollutants monitored at the same time included nitric oxide, nitrogen dioxide, sulfur dioxide, and particulate matter. Combinations of these pollutants may have been responsible for the observed effects. An attempt was also made to relate the rates of illness in school children during an influenza epidemic to the pollution gradient which existed during the season of peak oxidant concentrations, but there was no significant association. In Japan, a variety of respiratory and systemic symptoms were reported among school children on several smog-alert days. The systemic symptoms appeared to be attributable to a psychosomatic response among the students.

In a study on the incidence of acute respiratory diseases, peak concentrations of oxidants and mean concentrations of sulfur dioxide and nitrogen dioxide were found to be correlated with acute episodes of pharyngitis, bronchitis, and upper respiratory infections among college students in the Los Angeles Basin. However, there was no association between the admissions to a hospital in Los Angeles for cardiovascular conditions and oxidant concentrations.

A few reports are available on the effects of oxidants on patients with pre-existing diseases. One study suggested a relationship between the proportion of asthmatics who experienced asthma attacks and daily peak oxidant levels, but results were confounded by concomitant seasonal changes.

Studies on the effect of long-term exposure to photochemical oxidants are relatively few. To date, urban differences in lung cancer mortality rates in Californian cities do not suggest an influence of oxidant exposure on lung cancer risk. Similarly, studies have not revealed any relationships between the prevalence of chronic respiratory disease and geographical differences in oxidant concentrations.

As in all studies of urban populations, epidemiological studies of oxidant exposure cannot yield results on health effects attributable only to oxidants, since photochemical air pollution typically consists of ozone, nitrogen dioxide, peroxyacylnitrates, nitrate and sulfate particulates, and other components. In general, however, observed health effects were found to be more closely correlated with ozone levels than with levels of other pollutants.

1.1.6 Evaluation of health risks

Although it is known that ozone is only one of a number of photochemical oxidants and that there are many other components of

photochemical air pollution, it is the only substance for which a health protection guideline can be given, based on existing exposure-effect data.

From controlled human and community exposure studies, ozone concentrations at which the first adverse effects in man appear have been reported to be 200–500 $\mu g/m^3$ (0.1–0.25 ppm). Experimental studies on animals support these estimates.

The Task Group agreed that a 1-h exposure to ozone of 100–200 $\mu g/m^3$ (0.05–0.10 ppm) (measured by chemiluminescence) should be used as a guideline for the protection of public health and that a safety factor could not be applied because of the relatively high natural concentrations of ozone.

The Group also considered that a maximum 1-h oxidant concentration of 120 $\mu g/m^3$ (0.06 ppm) (measured by the NBKI method), which was recommended as the long-term goal by the WHO Expert Committee in 1972 and is approximately equal to the highest natural background level of oxidants, would be the best estimate of the exposure limit for oxidants for the general population.

In response to the question of whether the proposed guideline was realistic in view of natural exposure levels and the long-distance transport of ozone, the Group expressed the view that every effort should, nevertheless, be made to develop control strategies for achieving the proposed guideline or at least for not exceeding it more than once a month.

1.2 Recommendations for Further Research and Other Action

1.2.1 Health effects research

The WHO Task Group was particularly concerned with the potential for enhanced biological response of combined or sequential exposure of human populations to nitrogen dioxide and ozone. The recommendations listed take into account this concern as well as some other gaps in knowledge concerning the health effects of photochemical oxidants.

(*a*) The following information should be obtained by means of carefully controlled exposure studies on human volunteers:

i. data on the lowest concentration of ozone at which various lung function variables are affected;

ii. effects of sequential exposure to nitrogen dioxide and ozone;

iii. effects of ozone pre-exposure on the sensitivity of airways to bronchoconstrictor agents;

iv. effects on airways of combined exposure to: ozone and sulfur dioxide; ozone and tobacco smoke; ozone and increased temperature or other stressors.

(*b*) Epidemiological studies should be conducted to evaluate:
   i. effects of oxidant exposure on the susceptibility of human populations to respiratory infections;
   ii. comparative effects of exposure of urban populations to combined nitrogen dioxide and ozone (oxidants) versus exposure of rural populations to ozone alone. Measurements of lung function and other variables shown to be affected by photochemical oxidant and nitrogen dioxide peaks may be used for these studies.

(*c*) Experimental animal studies should be conducted to evaluate:
   i. effects of intermittent exposure to ozone, mimicking ambient air exposures;
   ii. effects of the joint action of ozone and other pollutants and/or other environmental stressors;
   iii. mechanism of tolerance to oxidants;
   iv. carcinogenic, cocarcinogenic, and mutagenic effects of ozone;
   v. effects of ozone exposure on humoral and cellular immunity.

1.2.2 Photochemical oxidant control

In order to reduce exposure of the general population to photochemical oxidants, the ratio of reactive hydrocarbons to oxides of nitrogen must be carefully controlled as well as their absolute levels. Unilateral or unbalanced control may result in higher levels of ozone and/or nitrogen dioxide. The Task Group recommended that to achieve a balanced control of both reactive hydrocarbons and oxides of nitrogen, appropriate laboratory and field studies should be conducted to evaluate the effects that both groups of compounds may have on the control of photochemical oxidants.

2. CHEMISTRY AND ANALYTICAL METHODS

2.1 Chemical and Physical Properties

2.1.1 Ozone

Ozone is one of the strongest oxidizing agents; only fluorine, atomic oxygen, and oxygen fluoride (OF_2) have higher redox potentials. Ozone is an important constituent of the upper atmosphere. Although it is present in only small concentrations (a few parts per million), ozone is responsible for shielding the earth from ultraviolet radiation (UV-B) that is biologically harmful. Formation of ozone occurs predominantly at altitudes above

30 km where solar UV radiation with wavelengths of less than 242 nm slowly dissociates molecular oxygen (O_2) into oxygen atoms (O). These oxygen atoms rapidly combine with molecular oxygen to form ozone. Ozone strongly absorbs solar radiation in the wavelength region of 240–320 nm. It is this absorption that shields the earth from harmful UV radiation (see for example National Academy of Sciences, 1977).

Some of the physical properties of ozone, the most abundant ubiquitous atmospheric oxidant, are listed in Table 1.

Table 1. Physical properties of ozone[a]

Chemical formula	O_3
Physical state at NTP[b]	colourless gas
Relative molecular mass	48.0
Melting point	−192.7°C
Boiling point	−111.9°C
Density relative to air	1.658
Vapour density	
at 0°C, 101 kPa (760 mmHg)	2.14 g/litre
at 25°C, 101 kPa (760 mmHg)	1.96 g/litre
Solubility at 0°C, 101 kPa (760 mmHg)	0.494 ml/100 ml water

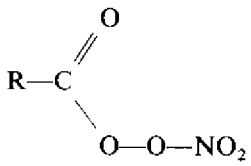
[a] From: US Department of Health, Education and Welfare (1970).

[b] NPT = normal temperature and pressure, i.e. 25°C and 101 kPa (760 mmHg).

The absorption of electromagnetic radiation by ozone in the ultraviolet and infrared regions is used in analytical methods.

2.1.2 Peroxyacylnitrates

Photochemical processes produce other oxidizing species besides ozone. These include peroxyacylnitrates, which have the following general structure:



This class of compounds includes:

$R = CH_3$: peroxyacetylnitrate (PAN)
$R = C_2H_5$: peroxyproprionylnitrate (PPN)
$R = C_6H_5$: peroxybenzoylnitrate (PBzN)

Although each of these species has received some attention, monitoring data are available only for peroxyacetylnitrate. The physical properties of this species are described in Table 2.

Table 2. Physical properties of peroxyacetylnitrate[a]

Chemical formula	$CH_3C(=O)OONO_2$
Physical state at NTP	colourless liquid
Relative molecular mass	121
Boiling point	No true boiling point, compound decomposes before boiling
Vapour pressure at room temperature	About 2 kPa (15 mmHg)

[a] From: US Department of Health, Education and Welfare (1970).

Peroxyacylnitrates have two characteristics that help in their detection at low concentrations i.e., absorption in the infrared region of the spectrum and electron-capturing ability (Stephens, 1969). The second of these characteristics is exploited in the electron-capture detector which, when used in conjunction with gas chromatography, provides the basis of an accepted method for the measurement of peroxyacetylnitrate levels in air.

2.1.3 Other oxidants

Hydrogen peroxide has been identified as a potential photochemical oxidant. However, it is an extremely difficult substance to detect specifically in the atmosphere and, at present, it is not possible to assess its significance as a photochemical air pollutant.

2.2 Atmospheric Chemistry

There are no significant primary emissions of ozone into the atmosphere and all the ozone found has been formed by chemical reactions that occur in the air.

In the upper atmosphere, ozone is mainly formed by the action of solar radiation on molecular oxygen[a]:

$$O_2 + \text{radiation } (\lambda < 175 \text{ nm}) \rightarrow O(^3P) + O(^1D) \quad (1)$$

$$O_2 + \text{radiation } (\lambda < 242 \text{ nm}) \rightarrow 2\,O(^3P) \quad (2)$$

$$O(^3P) + O_2 + M \rightarrow O_3 + M \quad (3)$$

[a] $O(^3P)$ is the symbol for atomic oxygen in its lowest energy state ("triplet oxygen"); $O(^1D)$ represents the next higher energy state of atomic oxygen ("singlet oxygen"); M is another molecule that must be present for the reaction to take place ("third body"), usually oxygen or nitrogen. Square brackets e.g. [NO] represent the concentration of the chemical species inside the brackets.

In the lower atmosphere, ozone-producing processes involve absorption of solar radiation by nitrogen dioxide:

$$NO_2 + \text{radiation } (\lambda < 430 \text{ nm}) \xrightarrow{k_a} NO + O(^3P) \quad (4)$$

$$O(^3P) + O_2 + M \rightarrow O_3 + M \quad (3)$$

$$O_3 + NO \xrightarrow{k_b} NO_2 + O_2 \quad (5)$$

Thus, the mechanism of ozone production during the sunlight irradiation of polluted air is simple in outline (ozone formation by the interaction of molecular oxygen with the photoproducts of nitrogen dioxide) but complex in detail. It is based on reactions (3)–(5) which give the following expression for the ozone concentration:

$$O_3 \approx \frac{k_a[NO_2]}{k_b[NO]} \quad (6)$$

In polluted atmospheres, the most readily observed sink for ozone involves the emission of nitric oxide. At night-time, the equilibrium expressed by equation (6) is displaced by the rapid reaction (5), and continuous nitric oxide emissions rapidly reduce the ozone concentration to undetectable levels. This atmospheric chemical process is supplemented by the destruction of ozone at ground level by contact with soil and vegetation surfaces (Regener & Aldaz, 1969).

In a hydrogen-free atmosphere, a fairly even balance between oxidizing and reducing agents would be maintained. However, peroxy radicals (RO_2) produced by the photochemical degradation of hydrocarbons have the important property of reacting with nitric oxide thereby converting it to nitrogen dioxide. The significance of any process resulting in the conversion of nitric oxide to nitrogen dioxide is that during daylight the equilibrium expressed by equation (6) shifts in favour of ozone production.

There are no significant primary emissions into the atmosphere of peroxyacylnitrates all of which are formed by atmospheric chemical reactions of the general type:

$$RCO(O_2) + NO_2 \rightarrow RCO(O_2)NO_2 \quad (7)$$

2.3 Measurement of Photochemical Oxidant Concentrations

2.3.1 Sampling

Ozone is highly reactive with most materials including plastics, metals, and fabrics. The most reproducible ambient concentration data have been obtained using sampling manifolds made entirely of teflon or glass.

As with oxides of nitrogen, residence time in these sampling manifolds requires specific consideration when sampling air containing nitric oxide, nitrogen dioxide, and ozone during daylight. Since the equilibrium shown in equation (6) is disturbed inside the sampling manifold, ozone concentrations can be underestimated if sampling times exceed 10 seconds (Butcher & Ruff, 1971).

When measuring ozone, the site of sampling should be selected with extreme care as exhaust gases from motor vehicles and central heating appliances readily remove ozone.

2.3.2 Analytical methods

For measurement purposes, oxidants are generally divided into three categories: ozone, total oxidants, and peroxyacylnitrates. Ozone and peroxyacylnitrates can be measured specifically while total oxidants are usually determined as a class of compounds.

The reagent employed to measure the oxidizing property of photochemical oxidants is a solution of neutral-buffered potassium iodide (NBKI). This reagent reacts with ozone, nitrogen dioxide, and peroxyacylnitrates. Reducing agents such as sulfur dioxide have an inhibiting effect on the reagent solution and must be removed from the inlet stream. To this extent, reaction with the potassium iodide reagent is a measure of the net oxidizing capacity of a sample of ambient air.

The terms "oxidant" or "total oxidant" are used to describe the net oxidizing capacity of the sampled air as determined by reaction with NBKI. The terms "corrected oxidant" or "adjusted oxidant" indicate that measurements have been corrected for the presence of reducing agents (sulfur dioxide) or other oxidizing agents (nitrogen dioxide) in the air.

Long-term averaging values are usually meaningless for photochemical oxidants and attention is directed to 1-h values (section 4.3). Thus, the use of continuous instruments with automatic data collection systems has an advantage. Manual methods are available for the determination of photochemical oxidants and these may be automated to a certain extent.

2.3.2.1 *Ozone*

Continuous ozone analysers are usually based on the chemiluminescent reaction of ozone with ethylene (Nederbragt et al., 1965; Warren & Babcock, 1970). A chemiluminescence method based on the reaction of ozone with certain dyes (Regener, 1964) has been further developed in the

Netherlands (Guicherit, 1975). These techniques are not subject to atmospheric interference, are highly sensitive (2 $\mu g/m^3$ or 0.001 ppm), and perform well under field conditions. The methods are not absolute and hence some form of calibration involving potassium iodide or UV absorption spectroscopy is necessary.

Specific determination of ozone may be accomplished by ultraviolet absorption spectroscopy in the 200–300 nm wavelength range (Hodgeson, 1972). The advantages of this method are that it is highly sensitive, does not require cylinders of explosive gases, and that it gives an absolute measurement (De More & Patapoff, 1976). Very high concentrations of certain hydrocarbons or mercury vapour may cause interference.

The dihydroacridine method is a specific, inexpensive, manual method for ozone determination in which samples are taken every 30 min. Interference effects can be eliminated by parallel sampling with an identical system fitted with an ozone scrubber. This is the only suitable method for short-term ozone measurements when a chemiluminescent or UV absorption instrument is not available (World Health Organization, 1976).

2.3.2.2 *Total oxidants*

Total oxidants are generally measured using acid- or neutral-buffered solutions of potassium iodide (Byers & Saltzman, 1958; US Department of Health, Education and Welfare, 1970; World Health Organization, 1976). There are indications that the accuracy of the method depends on the pH of the buffer solution, the concentration of the reagent, and other variables. In addition to these drawbacks, the method is basically unspecific. Any other oxidizing or reducing species can cause interference. Such interference results when sampling air containing sulfur dioxide, chlorine, nitrogen dioxide, or peroxides. Nitrogen dioxide and sulfur dioxide interfere most by giving erroneously high and low values, respectively.

Interference from sulfur dioxide can be eliminated by passing the air sample through glass fibre filters impregnated with a suitable material such as chromium trioxide. Humidity often renders these scrubbers ineffective because under such conditions they oxidize nitric oxide to nitrogen dioxide, thus increasing nitrogen dioxide interference. As there is also ozone loss after extended use, these scrubbers are not entirely satisfactory (World Health Organization, 1976).

Interference from nitrogen dioxide is more difficult to eliminate. Some form of adjustment can be made to the total oxidant reading if continuous nitrogen dioxide measurements are also available. This correction depends to some extent on experimental conditions and, in view of the problems

previously noted with measurements of oxides of nitrogen, may not be easy to make.

Other methods are available for the determination of oxidants but none of them is commonly used. They include the ferrous ammonium sulphate, alkali potassium iodide, and phenolphthalein methods (World Health Organization, 1976).

2.3.2.3 *Peroxyacetylnitrate*

The infrared absorption and electron-capturing properties of peroxyacetylnitrate have been used for its measurement in simulated and real atmospheres (Stephens, 1969). Electron-capture detectors preceded by gas chromatographic separation offer a limit of detection of 0.5 $\mu g/m^3$ (0.0001 ppm) for the automatic determination of ambient concentrations of peroxyacetylnitrate. Water vapours may interfere with the passage of peroxyacetylnitrate along the chromatographic column (Farwell & Rasmussen, 1976) and trace contaminants in cylinder gases can be a problem because of their slow accumulation on chromatography columns used for continuous measurements.

Peroxyacetylnitrate can also be measured by the hydrolysis of peroxyacetylnitrate solution to give nitrite ions that can be determined colorimetrically. However, this method suffers from interference by nitrogen dioxide (Konno & Okita, 1974; Stephens, 1969).

3. SOURCES OF PHOTOCHEMICAL OXIDANTS AND THEIR PRECURSORS

3.1 Natural Sources

As mentioned previously, ozone is a natural constituent of the upper atmosphere. A small amount of ozone, which is formed by the photolysis of molecular oxygen, is carried by atmospheric circulation into the lower atmosphere (section 4). Natural sources of ozone are associated with the passage of cold fronts (Ripperton et al., 1971) and atmospheric electrical phenomena (US Department of Health, Education & Welfare, 1970).

The photochemical oxidation of natural hydrocarbons including terpenes from trees and other vegetation takes place during daylight hours (Rasmussen, 1972; Went, 1966). Generally, these processes are difficult to study because of the low concentrations of the hydrocarbons and their short atmospheric residence. However, hazes often associated with certain

forest regions may well be explained by photochemical aerosol production from natural airborne hydrocarbons (Grimsrud et al., 1975).

3.2 Man-made Sources of Oxidant Precursors

Emissions of oxides of nitrogen from man-made activities include important contributions from both stationary and mobile sources (World Health Organization, 1977).

By comparison, the position with regard to hydrocarbon precursors is much more complex. In this instance, the term "hydrocarbon" refers to a class of pollutants that contain carbon atoms and produce oxidants under irradiation in the presence of oxides of nitrogen. This is a very wide definition that covers many hundreds of different organic compounds emitted into the atmosphere by man-made processes.

Not all organic compounds play an equal role in oxidant formation. The relative importance of each organic compound in this process depends on its chemical structure and reactivity. The chemical structure determines the number of nitric oxide → nitrogen dioxide conversions involved in the atmospheric degradation of each organic compound and ozone may be produced at each of these steps (Calvert, 1976; Demerjian et al., 1974). Reactivity requires special attention because the time scale for ozone or peroxyacetylnitrate production is related to the time scale for hydrocarbon degradation. For the so-called "highly reactive" hydrocarbons this may be 1 h or less. It may take up to 3 h for the less reactive hydrocarbons and require several days for the so-called unreactive hydrocarbons. Even in the presence of reactive hydrocarbons, ozone production may only become significant 10 km downwind from a source, and peak ozone concentrations may be observed over 60 km downwind (White et al., 1976). Thus, the relationship between hydrocarbon precursors and observed oxidant concentrations in large urban areas may be obscured. There may be marked differences in the nature of the oxidants in air sampled during the early morning when peak concentrations of oxidant precursors occur and in that sampled after midday when ozone concentrations are elevated.

In view of this complexity, it is clearly necessary to have some form of rational assessment of hydrocarbon reactivity (Darnall et al., 1976). Complete inventories of individual substances are only available for gasoline-engine exhaust emissions; for the storage, distribution, and use of organic substances such as petroleum products; and for specific industrial processes. An example of total emissions of oxides of nitrogen and hydrocarbons is given in Table 3. These figures are presented merely to show the wide diversity of the processes responsible for oxidant

precursor emissions; it is not sufficient to consider motor vehicles as the only important source.

Table 3. Total emissions of hydrocarbons and oxides of nitrogen in the Federal Republic of Germany in 1971 in thousands of tonnes[a]

Source	Total emission (10^3 tonnes)	
	Hydrocarbons	Oxides of nitrogen
Domestic heating	173	117
Traffic exhaust	325	308
Power plants	14	373
Industrial combustion	53	470
Industrial processes	955	30

[a]From: Federal Republic of Germany, Ministry of Internal Affairs (1974).

3.3 Indoor Sources

The use of ultraviolet lamps, electrostatic precipitators, photocopying machines, and odour control equipment can lead to increases in indoor concentrations of ozone. Welding and the manufacture of hydrogen peroxide are important indoor sources of ozone in industry and pose problems in occupational health (section 6.2).

However, other indoor activities such as smoking and cooking with gas stoves tend to produce elevated nitric oxide concentrations that destroy ozone and peroxyacylnitrates (Schuck & Stephens, 1969).

3.4 Oxidant-precursor Relationships

While potential synergistic health effects of oxidants and nitrogen dioxide are recognized, programmes for the control of these two pollutants are usually developed independently of one another. From a control point of view, however, such an approach is not justified because of the complexity of the photochemical reaction system. The only predictable way to control the formation of photochemical oxidants is to reduce the initial components in incremental steps. Decreasing the primary emission of oxides of nitrogen without reducing hydrocarbon emissions will lead to an increase in oxidant levels.

A further example of the uncoordinated approach to air pollution abatement can be illustrated by examination of the current methods applied to hydrocarbon control. These methods have focused on increasing combustion efficiency, an action which does reduce the hydrocarbon concentrations in exhaust but has the side effect of causing an increase in

atmospheric concentrations of nitrogen dioxide. As expected, changing the ratio of oxidant precursors has a complex effect on the formation of photochemical oxidants. For example, the effect of this change in ratio in the south coast air basin of California has been to produce substantial reductions in the concentrations of oxidants in Los Angeles town centre. However, the reductions become smaller at downwind sites and at 80 kilometres downwind, an increase in maximum daily values was observed (Dimitriades, 1976).

4. ENVIRONMENTAL CONCENTRATIONS AND EXPOSURES

4.1 Background Concentrations

Ozone concentrations in places far removed from sources of pollution show fairly constant values with some seasonal variations interspersed with irregular maxima due to specific meteorological events. Monthly mean concentrations of ozone, which vary considerably with both latitude and the month of the year, are illustrated in Fig. 1. The reported values range from 10 to 80 $\mu g/m^3$ (0.005–0.04 ppm).

Many observations indicate that hourly values range from 10 to 100 $\mu g/m^3$ (0.005–0.05 ppm) (Berry, 1964; Haagen-Smit, 1952; US Department of Health, Education and Welfare, 1970). However, higher values have been observed on isolated occasions. In a study at Chalk River, Ontario, Canada, a maximum value was observed of 120 $\mu g/m^3$ (0.06 ppm) for 4 h (US Department of Health, Education and Welfare, 1970).

4.2 Rural Areas

Polluted air masses from urban and industrial areas can affect suburban and rural areas in the direction of the prevailing wind for considerable distances. Elevated oxidant concentrations have been measured in a number of downwind rural locations where local sources of oxidant precursors were insignificant. It has been suggested that long-distance atmospheric transport might be responsible for many cases of high oxidant concentrations found over rural areas and some specific examples are given in Table 4.

A Midwest study in the USA in 1974 showed elevated ozone levels over an extensive rural area (radius of over 240 km) due to the combined effects of a number of urban areas. High ozone levels from one particular

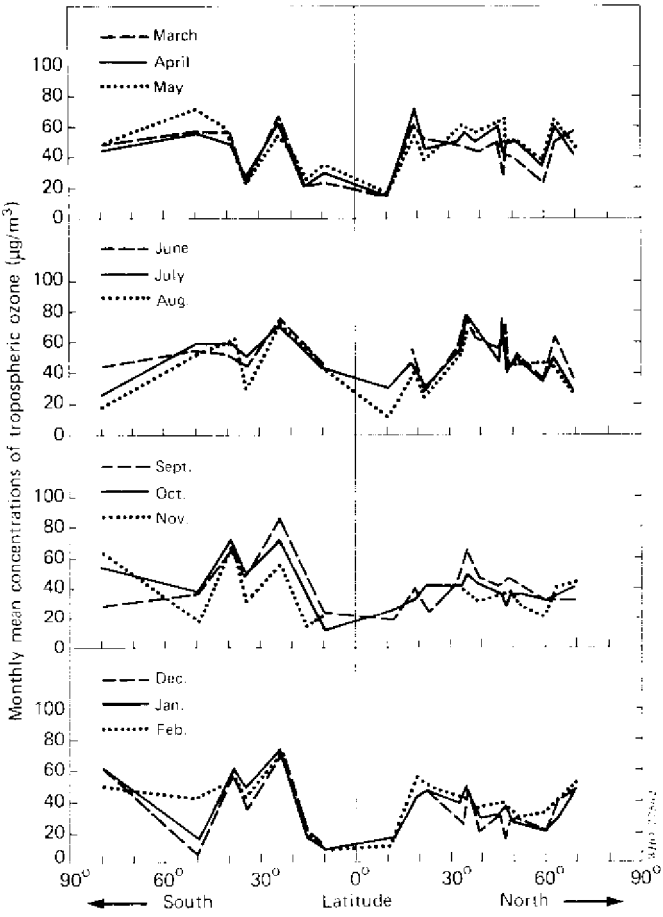


Fig. 1. Global distribution of background ozone concentrations (Fabian & Pruchniewicz, 1973).

Table 4. Long-distance transport of photochemical oxidants

Rural region	Possible source	Trajectory length (km)	Reference
Mineral King Valley, California, USA	Fresno, California	< 100	Miller et al. (1972)
Garrett County, Maryland, USA	New York, Philadelphia, Baltimore, Washington DC or Pittsburgh	> 100	US Environmental Protection Agency (1973)
Southern Eire & Southern UK	Continental Europe	100–700	Cox et al. (1975)
New York State, USA	Buffalo, New York	100–300	Stasiuk & Coffey (1974)
Tochigi and Gunma Prefectures, Japan	Tokyo	< 100	Environment Agency (1976)
Midwest, USA	St. Louis	> 150	White et al. (1976)

urban area extended as far as 48–80 kilometres downwind (US Environmental Protection Agency, 1976).

Observations of a 1-h concentration of 120 μg/m³ (0.06 ppm) in rural areas can generally be associated with the transport of man-made oxidants from distant sources.

4.3 Urban Areas

Since photochemical oxidants are the products of sunlight-induced photochemical reactions, elevated concentrations of oxidants in urban areas are generally restricted to a 4- to 6-h period within a day, representing only 15–25% of the 24-h interval. For this reason, the reporting of oxidant or ozone data as daily, monthly, or yearly means can be misleading when evaluating trends or comparing oxidant concentrations in different cities. Thus, oxidant or ozone data are usually reported in terms of highest 1-h concentrations or in terms of the number of days with hourly concentrations exceeding a specified value or the number of hours when a given range of concentrations occurred within a year. However, they may also be given as instantaneous or five minute peak concentrations or frequency distributions.

Table 5. Highest 1-h concentrations of ozone or total oxidants observed at selected sites in 1974

City	Concentration		Method
	μg/m³	(ppm)	
Bonn, Federal Republic of Germany[1]	290	(0.145)	chemiluminescence
Eindhoven, Netherlands[2]	420	(0.210)	chemiluminescence
London, UK[3a]	294	(0.147)	chemiluminescence
Los Angeles, USA[4]	548	(0.274)	NBKI
Osaka, Japan[5]	320	(0.160)	NBKI
Riverside, USA[4]	744	(0.372)	NBKI
Tokyo, Japan[5]	380	(0.190)	NBKI
Washington, USA[4]	312	(0.156)	chemiluminescence

From: [1] Becker & Schurath (1975).
[2] Guicherit (1975).
[3] Ball (1976).
[4] US Environmental Protection Agency (1976).
[5] Environment Agency (1975b).
[a] Data for 1975.

As shown in Table 5, the highest 1-h concentrations at 8 locations were of the order of 300–800 μg/m³ (0.15–0.40 ppm). It is important to recognize that the data presented are only for one site in each of the cities and do not necessarily represent the maximum levels occurring in these urban areas or provide a good indication of human exposure levels. For this reason,

frequency distributions or reporting of the number of days or hours when a given concentration was exceeded are helpful. Such data are presented in Tables 6, 7, and 8 for selected monitoring stations in Tokyo, Washington DC, and Delft, respectively, to illustrate the distribution of the concentrations recorded at these monitoring stations. These tables are not intended to indicate long-term trends since meteorological patterns that greatly influence the ambient concentrations of oxidants can vary considerably from year to year.

Table 6. Number of days and hours when hourly oxidant concentrations were in the range of indicated levels at a National Air Sampling Network Station, Tokyo, Japan[a]

Concentration[b]		Number of days (hours)			
μg/m³	(ppm)	1971	1972	1973	1974
0–100	(0 –0.05)	113 (7032)	185 (7863)	60 (7248)	184 (8122)
120–180	(0.06–0.09)	135 (798)	111 (379)	199 (1050)	134 (495)
200–280	(0.10–0.14)	60 (251)	31 (81)	73 (298)	40 (107)
300–380	(0.15–0.19)	18 (42)	8 (14)	21 (59)	5 (10)
400–480	(0.20–0.24)	6 (8)	1 (2)	7 (14)	0 (0)
>500	(>0.25)	0 (0)	0 (0)	1 (2)	0 (0)
Total		332 (8131)	336 (8339)	361 (8671)	363 (8734)

[a] From: Tokyo Metropolitan Government (1971–1974) (unpublished data).
[b] Measured by NBKI method.

The data for Tokyo show both the number of days when a given concentration was exceeded and the total number of hours in which concentrations falling within the specified ranges were observed. As shown in Table 6, an hourly concentration of 200 μg/m³ (0.1 ppm) was exceeded on 10–30% of days in these years, and in approximately 1–4% of all the hours in the year.

Table 7. Number of days when at least one hourly oxidant concentration was in the range of indicated levels in Washington, DC, USA[a]

Concentration[b]		Number of days		
μg/m³	(ppm)	1970	1971	1972
0–100	(0 –0.05)	72	155	134
120–200	(0.06–0.10)	85	127	39
220–300	(0.11–0.15)	8	17	6
>300	(>0.15)	2	0	0
Total		167	299	179

[a] From: US Environmental Protection Agency (1964–1973).
[b] Measured by the NBKI method.

In Washington, DC, a concentration of 200 μg/m³ (0.1 ppm) was exceeded, on average, on about 5% of the days, with most of the days having maximum 1-h concentrations of about 100 μg/m³ (0.05 ppm).

Table 8. Number of hours when hourly ozone concentrations were in the range of indicated levels in Delft, Netherlands[a]

Concentration[b]		Number of hours			
μg/m³	(ppm)	1971	1972	1973	1974
0–100	(0 –0.05)	7799	8370	8364	6907
120–150	(0.06 –0.075)	647	325	267	401
152–200	(0.076–0.10)	130	34	81	39
>200	(>0.10)	48	8	30	8
Total		8624	8737	8742	7355

[a] From: Guicherit (1975).

[b] Measured by the chemiluminescence method.

The data for Delft, Netherlands, show the number of hours in the year when given concentrations were observed. More than 90% of the hours exhibited concentrations of less than 100 μg/m³ (0.05 ppm). However, in 1971 and 1973, there were 48 and 30 hours, respectively, when concentrations exceeded 200 μg/m³ (0.1 ppm).

When the maximum concentration for a given averaging time, e.g., 1 h, is known, maximum values for other averaging times can be estimated from the averaging time-concentration model of Larsen (1974). The concentrations for various averaging periods, calculated from the base data of 1-h concentrations of 300 and 800 μg/m³, are shown in Table 9.

These calculations are based on geometric standard deviations reported for several cities in the USA (Larsen, 1969).

Table 9. Estimated ozone concentrations for various averaging periods[a]

Averaging period	Maximum concentration (μg/m³)	
1-h (base data)	300	800
3-h	190–215	435–520
1 day	85–120	225–310
1 year	15–30	35–80

[a] Adapted from: Larsen (1969).

Caution should be exercised when applying Larsen's model. Although it is not applicable to averaging times of less than 1 h, the model is generally accurate when using observed annual means to predict concentrations between 1 h and 1 day. For periods longer than 1 day it is applicable only if the observed data closely follow a log-normal distribution.

Seasonal and diurnal variations in oxidant values are important characteristics of the urban pattern of environmental concentrations of this group of pollutants. These temporal variations result from: (*a*) variations in oxidant precursors; (*b*) variations in atmospheric transport

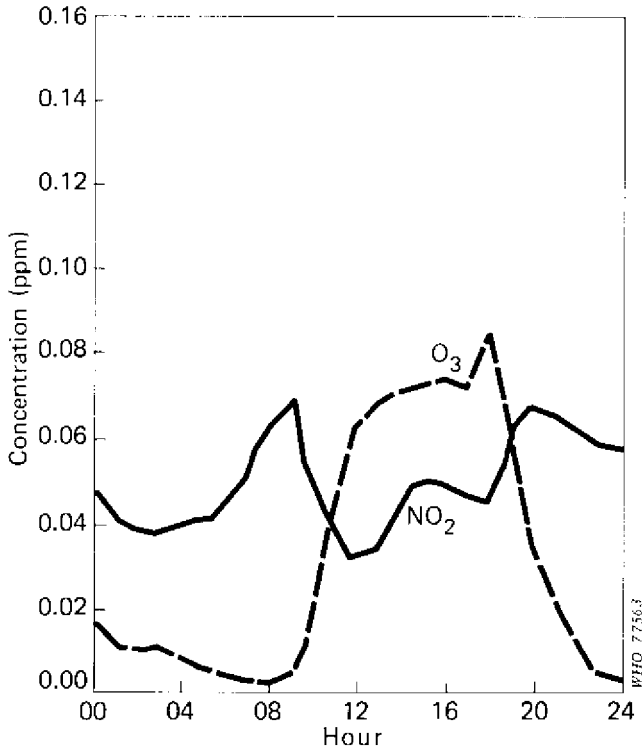


Fig. 2. Daily variations in ozone and nitrogen dioxide concentrations, 12 July 1972, London. Nitrogen dioxide, 1 ppm = 1880 μg/m³; ozone, 1 ppm = 2000 μg/m³ (Derwent & Stewart, 1973).

and dilution of pollutants, and (*c*) variations in meteorological conditions and other atmospheric variables involved in the photochemical reaction process. Because of diurnal variations in intensity of both solar ultraviolet radiation and of precursor emission rates, maximum daily ozone concentrations frequently occur around noon. However, such maxima have also been observed during the morning or afternoon hours, mostly in suburban areas.

Examples of diurnal patterns of oxidants or ozone and nitrogen dioxide concentrations are shown in Figs. 2, 3, and 4. The close relationship between oxidants and nitrogen dioxide quite frequently leads to oxidant peaks following nitrogen dioxide peaks. However, this is not always, as shown in Fig. 3.

Seasonal variations in oxidant concentrations are manifested by increases in the diurnal maxima and by increases in the number of days per month that exhibit elevated oxidant values. For example, Fig. 5 shows the number of days per month in which the oxidant concentration in Los

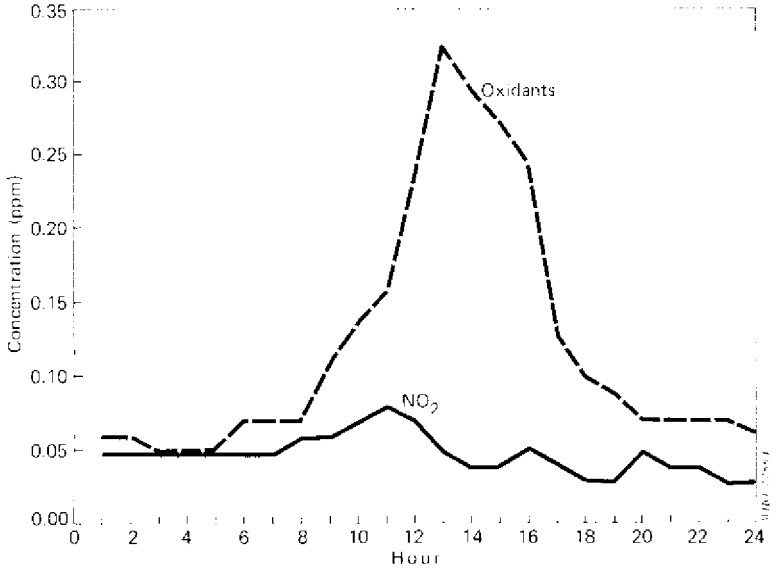


Fig. 3. Daily variations in oxidant and nitrogen dioxide concentrations, 18 May 1974, Chiba, Japan. Nitrogen dioxide, 1 ppm = 1880 µg/m³; oxidants, 1 ppm = 2000 µg/m³ (Environment Agency, 1975a).

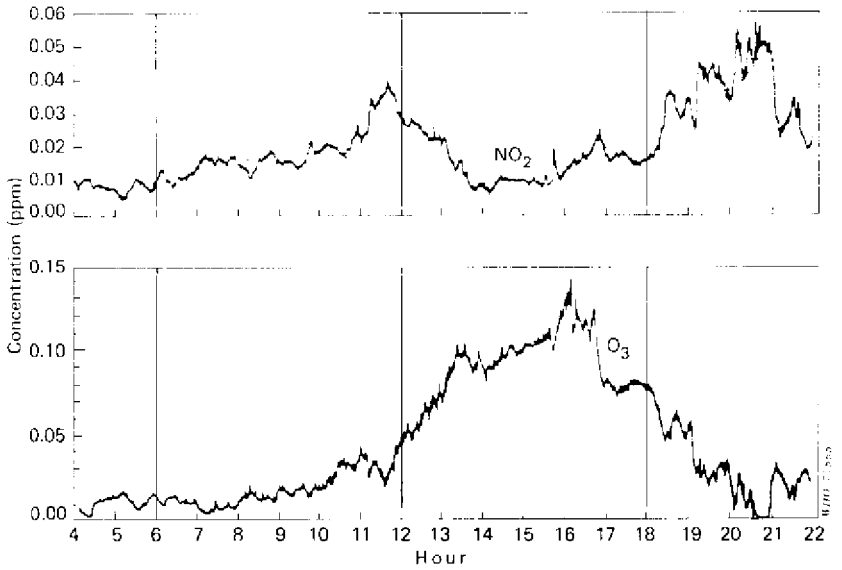


Fig. 4. Daily variations in ozone and nitrogen dioxide concentrations, 24 August 1974, Bonn, Federal Republic of Germany. Nitrogen dioxide, 1 ppm = 1880 µg/m³; ozone, 1 ppm = 2000 µg/m³ (Becker & Schurath, 1975).

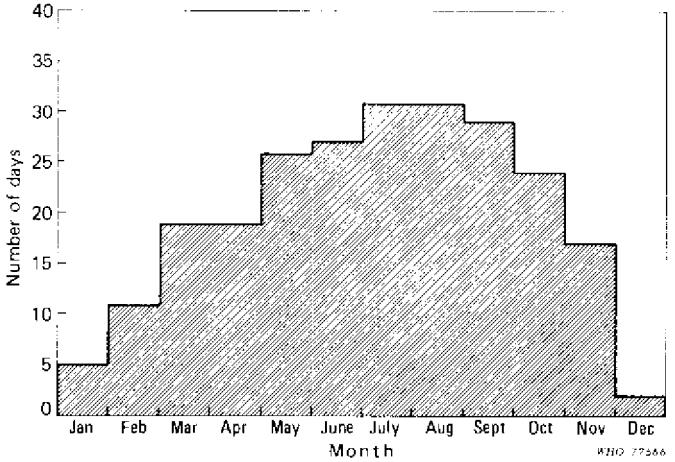


Fig. 5. Number of days in which 1-h oxidant concentrations equalled or exceeded 200 μg/m³ (0.10 ppm) in Los Angeles, 1970 (Barsky & Birakos, ed., 1971).

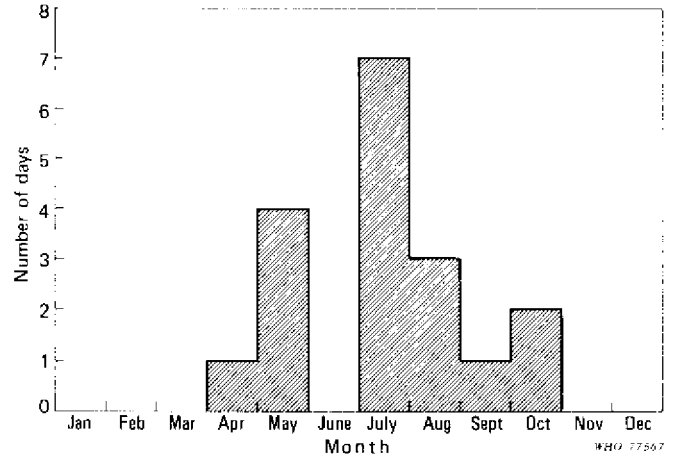


Fig. 6. Number of days in which 1-h ozone concentrations equalled or exceeded 200 μg/m³ (0.10 ppm) in Delft, 1971 (Guicherit, 1975).

Angeles, USA, equalled or exceeded 200 μg/m³ (0.1 ppm). Similarly, Fig. 6 shows the number of days per month in Delft, Netherlands, that also exhibited ozone concentrations equal to or above 200 μg/m³ (0.1 ppm).

Peroxyacetylnitrate is generally formed simultaneously with ozone. However, comparatively few measurements have been made of peroxyacetylnitrate in the ambient atmosphere. The ratio of peroxyacetylnitrate to ozone observed at a maximum concentration of peroxyacetylnitrate was about 1:100 in rural England (Sandalls et al., 1974) and about 1:50 in

Delft, Netherlands (Nieboer & Van Ham, 1976). Variations in concentrations of peroxyacetylnitrate often follow those of ozone, as shown in Fig. 7 (Sandalls et al., 1974). However, this is not always the case (Stephens, 1976).

4.4 Indoor Concentrations

Oxidant concentrations inside buildings tend to be lower than those outdoors because of destructive reactions that occur on most surfaces (Mueller et al., 1973; Sabersky et al., 1973). However, certain indoor sources of ozone (section 3.3) may increase indoor concentrations. Indoor concentrations at places of work are discussed in section 6.2.

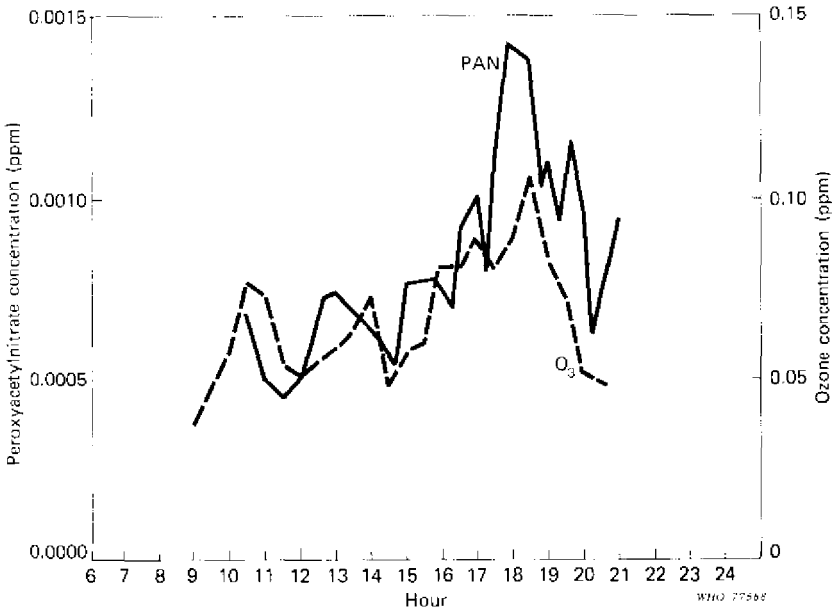


Fig. 7. Daily variations in peroxyacetylnitrate and ozone, 9 September 1973, Harwell, England. Peroxyacetylnitrate, 1 ppm = 5000 µg/m³; ozone, 1 ppm = 2000 µg/m³ (Sandalls et al., 1974).

5. EFFECTS ON EXPERIMENTAL ANIMALS

There is considerable evidence to show that even short exposure to high concentrations of ozone may endanger the health of experimental

animals. In reviewing this evidence, emphasis has been placed on studies in which animals were exposed to concentrations of oxidants of 2000 $\mu g/m^3$ (1.0 ppm) or less, since these studies are more relevant for predicting the health risk to man. However, some experiments conducted at higher concentrations have also been discussed when it was considered that they would contribute to a better understanding of the mechanism of the biological action of oxidants.

5.1 Absorption of Ozone

A number of factors can influence the transport and removal of ozone in the upper airways such as: (*a*) nasal morphology; (*b*) route, rate, and depth of breathing; and (*c*) biochemical composition and amount of mucus. The decomposition of ozone within the upper airways may protect the lower part of the respiratory tract against the irritant gas. Various attempts to determine or model the respiratory absorption of ozone in the upper airways have been made (McJilton et al., 1972; Vaughan et al., 1969; Yokoyama & Frank, 1972). This work has recently been reviewed by Miller (1977) who developed a mathematical model for the transport and removal of ozone in the respiratory tract of guineapigs, rabbits, and man, and predicted that whatever the initial concentration, the respiratory bronchioles would receive the highest dose of ozone. This agrees well with various experimental studies in animals. For all three species the relationship between respiratory bronchiolar concentration and the inhaled ozone concentration at the tracheal level is linear on a log-log scale at concentrations greater than 100 $\mu g/m^3$ (0.05 ppm), the respiratory bronchiolar dose for rabbits being 80% of that for man and twice that for guineapigs.

5.2 Effects on the Respiratory System

5.2.1 Morphological changes

5.2.1.1 *Short-term exposure (24 h or less)*

The primary target of ozone is the respiratory tract and particularly the pulmonary parenchyma. In small laboratory animals, exposure to ozone at acutely toxic concentrations results in pulmonary oedema, haemorrhage, and death. The LD_{50} is about 12 mg/m^3 (6.0 ppm). At lower concentrations in the range of 400–2000 $\mu g/m^3$ (0.2–1.0 ppm), ozone causes numerous changes in both the epithelial and endothelial cells of the

lung and ultrastructural effect indicate that the primary lesions are in the epithelial lining of the terminal bronchioles and the proximal alveoli.

The sequence of degeneration, desquamation, and destruction of type I alveolar cells in rats following exposure to an ozone concentration of 400 $\mu g/m^3$ (0.2 ppm) for 2 h was demonstrated in electron microscopic studies by Stephens et al. (1974). The type II epithelial cells appeared to be more resistant. Freeman et al. (1974) also reported significant histological changes in type I epithelial cells after 4 h exposure to a concentration of 1800 $\mu g/m^3$. The loss of ciliated epithelium throughout the upper respiratory tract, swelling and denudation of type I cells, erythrocyte lysis within alveolar capillaries, and breakdown of capillary endothelium has been reported in cats exposed to ozone concentrations of 520, 1000, and 2000 $\mu g/m^3$ (0.26, 0.5, and 1.0 ppm) for 4.7–6.6 h (Boatman et al., 1974). These effects appeared to be dose-related. Similar effects were noted by Bils (1970) in mice after a 7-h exposure to ozone at concentrations of 1200 and 2600 $\mu g/m^3$ (0.6 and 1.3 ppm).

Cell renewal rate within the alveoli was studied by Evans et al. (1971) by injecting tritiated thymidine into 18 to 20-month-old mice before exposing them for 6 h to ozone concentrations of 1000, 2400, 5000, or 7000 $\mu g/m^3$ (0.5, 1.2, 2.5, and 3.5 ppm). Immediately following exposure, the number of labelled alveolar cells (those synthesizing DNA) was significantly lower in all exposed groups compared with controls.

Similar morphological studies have also been performed using complex mixtures containing oxidants. Ultrastructural alterations consisting of disrupted cytoplasm and abnormal mitochondria were seen in the alveolar tissue of mice exposed for 2–3 h to Los Angeles air that had a total oxidant concentration of 800 $\mu g/m^3$ (0.4 ppm) (Bils, 1966). Exposure of mice for 3 h to an irradiated synthetic atmosphere that contained propylene, nitric oxide, carbon monoxide, and water vapour and simulated a heavy smog, gave rise to a similar pattern of ultrastructural changes (Bils & Romanovsky, 1967). Rats continuously exposed for 24 h to a mixture of ozone at a concentration of 500 $\mu g/m^3$ (0.25 ppm) and nitrogen dioxide at a concentration of 4700 $\mu g/m^3$ (2.5 ppm) exhibited increases in the numbers of alveolar macrophages and of free cells in the lung resembling desquamated type I cells, and hypertrophy of the epithelium were noted in rats continuously exposed for 24 h to a mixture of ozone at a concentration of 500 $\mu g/m^3$ (0.25 ppm) and nitrogen dioxide at a concentration of 4700 $\mu g/m^3$ (2.5 ppm) (Freeman et al., 1974).

5.2.1.2 *Prolonged and repeated exposures*

Prolonged exposure to low levels of ozone causes more extensive and irreparable damage to the lung than the oedematigenous and acute

inflammatory reactions observed following short exposure to high concentrations. Emphysema, atelectasis, focal necrosis, bronchopneumonia, and fibrosis have been reported, often accompanied by a variety of cellular alterations. The degree of morphological injury appears to be proportional to the concentration and time of exposure.

Freeman et al. (1974) described a number of pathomorphological changes in rats continuously exposed to ozone at concentrations of 1100 and 1800 $\mu g/m^3$ (0.54 and 0.88 ppm) for as long as 6 months. After 48 h of exposure to the lower concentration, an influx of macrophages and an increase in mitotic figures were observed, and the alveolar ducts became demarcated by hypertrophic alveolar epithelium. Most of these changes became more obvious with increasing length of exposure with the exception of the terminal bronchioles which appeared to recover and return to normal. After exposure to the higher concentration of 1800 $\mu g/m^3$ (0.88 ppm) for 48 h, the bronchiolar epithelium exhibited both metaplasia and fibrosis. Adenoma-like structures containing large numbers of macrophages and fibrotic lesions appeared after 6 days and after 3 weeks of exposure, half of the rats had died and were found to have emphysema-like lesions.

Using scanning electron microscopy, Ikematsu et al. (1976) found that continuous exposure to an ozone concentration of 2000 $\mu g/m^3$ (1.0 ppm) for 10 days resulted not only in desquamation of epithelial cells but also in the appearance of inflammatory cells in the tonsils of rabbits. Similar pathological effects were observed with intermittent exposure indicating that the animals were unable to recover sufficiently during the periods of exposure to clean air. Emphysematous and vascular lesions in the lung of the rabbit described by P'an et al. (1972), were the result of repeated exposure to 800 $\mu g/m^3$ (0.4 ppm) for 6 h per day, 5 days per week, for 10 months. Stokinger et al. (1957) also reported fibrotic changes and chronic bronchial and bronchiolar emphysema in the lungs of mice, rats, guineapigs, and hamsters exposed to an ozone concentration of 2000 $\mu g/m^3$ (1.0 ppm) for 6 h per day, 5 days per week, for 433 days. However, under the same conditions of exposure, the effects in dogs were limited to the trachea and large bronchi. When dogs were exposed to ozone concentrations ranging from 2000 to 6000 $\mu g/m^3$ (1–3 ppm) for 8, 16, or 24 h daily for 18 months, the morphological damage was roughly proportional to the product of the concentration and the time of exposure, the epithelial lining of the terminal airways and proximal alveoli being most adversely affected (Freeman et al., 1973; Stephens et al., 1973). Similarly, Castleman et al. (1973a) found that the walls of the terminal airways and the interalveolar septa of rat lung were most affected by continuous exposure to an ozone concentration of 1600 $\mu g/m^3$ for 7 days.

Intermittent exposure for 8 h per day, for 7 days to an ozone concentration of 400 μg/m³ (0.2 ppm) produced damage to the respiratory bronchioles in bonnet monkeys. In rats fed a normal diet, similar effects were seen at the same concentration of ozone, but, in vitamin E-deficient rats, an equivalent effect was caused by ozone at 200 μg/m³ (0.1 ppm) (Dungworth et al., 1975; Dungworth, 1976).

Loosli et al. (1972) measured the cell turnover rate in mice and found it to be significantly higher in animals exposed to synthetic photochemical air pollution for 8–12 months than in control animals breathing filtered air. The synthetic air pollution contained ozone at 600–840 μg/m³ (0.30–0.42 ppm), carbon monoxide at 3.5–12 mg/m³ (3–10 ppm), nitrogen dioxide at 1300–1600 μg/m³ (0.70–0.85 ppm), and sulfur dioxide at 5700–6000 μg/m³ (2.2–2.3 ppm). The effects were similar to those seen with exposure to pure ozone.

Rats exposed for 1 month to a mixture of ozone at 1800 μg/m³ (0.9 ppm) and nitrogen dioxide at 1700 μg/m³ (0.9 ppm), developed enlarged alveolar spaces (Freeman et al., 1974). In rats exposed for 2 weeks to a combination of ozone at 500 μg/m³ (0.25 ppm), and nitrogen dioxide at 4700 μg/m³ (2.5 ppm), the bronchiolar epithelium became cuboidal. However, after 6 months of exposure, the tissue appeared to be normal (Freeman et al., 1974).

Slightly inflammatory and proliferative changes were observed by Nakajima et al. (1972) in the bronchial membranes of mice exposed to irradiated auto exhaust that contained oxidant concentrations of 200–300 μg/m³ (0.1–0.15 ppm), for 2–3 h per day, 5 days per week, for 30 days.

5.2.2 Functional changes

5.2.2.1 *Short-term exposure (24 h or less)*

The first abnormal sign observed in various animal species during exposure to ozone is an irregular respiratory pattern. This usually appears within the first few minutes of exposure and, in most cases, the animal returns to normal, when allowed to recover in the clean air.

Functional changes in the respiratory system have been observed in several species of animals at ozone concentrations of less than 2000 μg/m³ (1.0 ppm). Yokoyama (1972a) studied the ventilatory function of guinea-pigs before, during, and after a 2-h exposure to an ozone concentration of 1000 μg/m³ (0.5 ppm). He reported increases in the frequency of respiration and airway resistance, but a decrease in tidal volume. In another investigation, Yokoyama (1973) exposed only the right lung of rabbits to ozone at 2000 μg/m³ (1 ppm) for 3 h and used the left lung as a

control. The exposed lung of rabbits killed 1 and 3 days after exposure, had a reduced vital capacity. However, the reduction in vital capacity in those killed 7 days after exposure was only slight and was not significant. Other studies on lung function in guineapigs exposed to ozone for 2 h indicated that pulmonary flow resistance was not altered by exposure to concentrations of 700 and 1400 $\mu g/m^3$ (0.34 and 0.68 ppm), but that it increased significantly at exposure levels of 2000 and 2700 $\mu g/m^3$ (1.08 and 1.35 ppm) (Murphy et al., 1964a).

Scheel et al. (1959) exposed rats to an ozone concentration of 4000 $\mu g/m^3$ (2 ppm) for 3 h. Decreases in minute ventilation, tidal volume, and oxygen uptake, that occurred immediately after exposure, reached minimum recorded values 8 h later. All measurements returned to normal levels 20 h after exposure.

When cats were exposed for an average of 4.6 h to ozone concentrations of 520, 1000, and 2000 $\mu g/m^3$ (0.26, 0.5, and 1 ppm), Watanabe et al. (1973b) found that pulmonary flow resistance increased with increasing ozone concentrations. Dynamic compliance was reduced, but to a lesser extent, and vital capacity was unaffected. The proportion of animals that showed a reduction in diffusion capacity appeared to increase with increasing ozone concentrations.

There are a few studies in which animals were exposed to ambient air and irradiated auto exhaust containing high concentrations of oxidants, including those of Swann & Balchum (1966) who measured the total expiratory flow resistance in guineapigs on days of unusual weather and smog conditions in Los Angeles. When the resistance was compared with routine monthly measurements on the same animals, significant increases were found at oxidant levels of approximately 600 $\mu g/m^3$ (0.30 ppm) or more. Substantial increases in resistance were also observed, when relatively high concentrations of nitrogen dioxide (1700 $\mu g/m^3$; 0.92 ppm), carbon monoxide (30 mg/m^3; 26 ppm), and hydrocarbons (16 ppm) were present, but the oxidant level (80 $\mu g/m^3$; 0.04 ppm) was relatively low.

The effects on guineapigs of a 4-h exposure to diluted, irradiated, or nonirradiated exhaust atmospheres were reported by Murphy et al. (1963). Marked, rapid increases in total expiratory flow resistance accompanied by a decrease in respiratory rate and a small increase in tidal volume occurred during exposure to irradiated exhaust. The reaction to nonirradiated exhaust was comparatively slight. Ranges of concentrations of the main pollutants in the exhaust-contaminated air used in this study were: for irradiated exhaust gases: total oxidants, 660–1640 $\mu g/m^3$ (0.33–0.82 ppm); nitrogen dioxide, 800–10 000 $\mu g/m^3$ (0.43–5.5 ppm); and carbon monoxide, 39–360 mg/m^3 (34–310 ppm): for nonirradiated exhaust gases: total oxidants, less than 40 $\mu g/m^3$ (0.02 ppm); nitrogen dioxide,

710–3000 μg/m³ (0.38–1.58 ppm); and carbon monoxide, 98–345 mg/m³ (85–300 ppm). In addition, increased levels of formaldehyde, acrolein, and olefin were present.

5.2.2.2 *Prolonged and repeated exposures*

The few available pulmonary function studies on animals exposed to ozone for extended periods of time include a study by Bartlett et al. (1975) who reported that continuous exposure of rats to an ozone concentration of 400 μg/m³ (0.2 ppm) for 30 days caused a 16% increase in lung volume and an increase in alveolar dimension. A reduction in lung elasticity that was also reported was possibly an effect of ozone on collagen. Yokoyama (1974) exposed rabbits to an ozone concentration of 4000 μg/m³ (2 ppm), for 6 h per day, for 3–4 days, and found that the pulmonary flow resistance was greater, and compliance lower, than in the controls. In animals exposed to 2000 μg/m³ (1 ppm), for 6 h per day, for 7–8 days, the values were between those of the controls and of the group treated with 4000 μg/m³ (2 ppm).

5.2.3 Biochemical changes

5.2.3.1 *Effects indicating possible mechanisms of action*

The actual mechanism of ozone toxicity at the subcellular level is still obscure. Studies of the biochemical effect of ozone have mainly been based on two hypotheses: (*a*) that ozone interacts with readily oxidizable substances thus altering the course of metabolism and producing a toxic effect; and (*b*) that ozone interacts with unsaturated lipids to produce lipid peroxidation and consequent cell damage.

Sulfhydryl systems appear in the cell not only as reducing substances but also as functional constituents of a variety of enzymes and proteins. Ozone is capable of oxidizing these substances causing inactivation of enzymes and alterations in the structure and function of the cell membrane.

In studies on ozone oxidation of glutathione *in vitro*, Mudd et al. (1969) showed that both fast and slow oxidation occurred. It has also been shown that the oxidation of reduced glutathione (GSH) results in oxidized glutathione (GSSG), though some of the sulfhydryl groups form higher oxidation products that are not reduced by the reductases available in the cells (Menzel, 1971). Mountain (1963) demonstrated *in vivo* oxidation of reduced glutathione and a decrease in the sulfur-containing enzyme succinic dehydrogenase (1.3.99.1) activity in the lungs of mice. King (1961) also found a decrease in sulfhydryl content and of enzymatic function of partially purified glyceraldehyde-3-phosphate dehydrogenase

(1.2.1.12) in rat lungs following exposure to an ozone concentration of 2400 μg/m³ (1.2 ppm) for 4 weeks.

When rats were exposed to an ozone concentration of 4000 μg/m³ (2.0 ppm) for 4–8h, the concentrations of both the protein and nonprotein sulfhydryls in the lungs decreased. The activities of lung enzymes containing sulfhydryl also decreased including those of glucose-6-phosphate dehydrogenase (1.1.1.49), glutathione reductase (1.6.4.2), and cytochrome *c* reductase related to succinate and reduced nicotinamide adenine dinucleotide (NADH) (DeLucia et al., 1972). Expanding these studies, DeLucia et al. (1975) showed that the magnitude of the decrease in the nonprotein sulfhydryl groups in rats was dependent on the duration of exposure and the concentration of ozone. Significant decreases were not observed at an ozone concentration of 1600 μg/m³ (0.8 ppm) for 24 h.

Further studies have shown that exposure to ozone tends to increase the activity of enzymes that protect against intracellular oxidation. When rats were continuously exposed to an ozone concentration of 1500 μg/m³ (0.75 ppm) for 1, 3, 10, or 29 days, it was noted that the activities of glutathione peroxidase (1.11.1.9), glutathione reductase (1.6.4.2), glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase (1.1.1.43), and pyruvate kinase (2.7.1.40) were lower than those in the controls after 1 day but higher after, 3, 10, and 29 days of exposure (Chow & Tappel, 1973). In another study by the same investigators, rats exposed to 400 μg/m³ (0.2 ppm) continuously for 8 days or intermittently (8 h per day) for 7 days showed significantly increased glutathione peroxidase activity in the lung. Fukase et al. (1975a, 1975b) exposed mice to ozone concentrations of 400, 1000, or 2000 μg/m³ (0.2, 0.5 or 1.0 ppm) for 4 h per day, for 30 days, and found progressive increases in the levels of glutathione and vitamin C in the lung with increasing ozone concentration. The authors also reported significant increases in the activities of glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase in the lungs of mice. Exposure of both rhesus and bonnet monkeys to levels of ozone ranging from 400 to 1600 μg/m³ (0.2–0.8 ppm) for 8 h per day for 7 days, resulted in increased activities of succinate oxidase and glutathione peroxidase in the lungs. Linear regression analysis showed a significant correlation between ozone concentration and the augmentation in activity of these enzymes (Dungworth et al., 1975).

Many investigators have attributed the biological effect of ozone to lipid peroxidation (Fournier, 1973; Goldstein et al., 1969; Menzel, 1970; Roehm et al., 1971b). Oxidation of unsaturated fatty acids by ozone has been demonstrated both *in vivo* and *in vitro*. The mechanism of such action

is based on the proclivity of ozone to react with the ethylene groups of the acid to form peroxides. Their decomposition results in the further formation of free radicals capable of initiating peroxidation of other unsaturated fatty acids. The breakdown products (peroxides, carbonyl compounds) may themselves be cytotoxic.

Evidence of lung lipid peroxidation during ozone exposure was suggested by Goldstein et al. (1969) who found conjugated diene bonds in an extract of lungs of mice exposed to 800–1400 $\mu g/m^3$ (0.4–0.7 ppm) for 4 h. Another index of lipid peroxidation is an increase in malonaldehyde. In studies by Chow & Tappel (1972), the malonaldehyde concentration increased in the lungs of rats exposed continuously to 1400–1600 $\mu g/m^3$ (0.7–0.8 ppm) for 5 and 7 days.

Further evidence of the role of peroxidation in ozone toxicity is the fact that animals deficient in vitamin E are more susceptible to ozone. This is discussed in detail in section 5.2.7.

5.2.3.2 *Biochemical effects at the subcellular level*

In morphological and ultrastructural studies, swelling and degenerative changes in lung mitochondria have frequently been reported following ozone exposure. Mitochondrial functions are critical to the cellular terminal substrate oxidation and energy production. These organelles in lung cells may be the target for ozone since many mitochondrial enzyme activities are sulfhydryl-dependent and mitochondrial membranes contain abundant unsaturated phospholipids. It has therefore been suggested that the lung mitochondria may be a sensitive test system for detecting and evaluating ozone toxicity.

Mustafa et al. (1973) found a 45% increase in pulmonary mitochondrial oxygen consumption in rats continuously exposed to 1600 $\mu g/m^3$ (0.8 ppm) for 10–20 days. A 3-fold increase in the number of type II cells was also reported. These cells are rich in mitochondria and may have been responsible for the increase in oxygen consumption. A 17% increase in oxygen consumption was noted with continuous exposure to an ozone concentration of 400 $\mu g/m^3$ (0.2 ppm) for 7 days. This effect appeared to be dose-related.

Lysosomes are vitally important in the intra- and possibly extracellular destruction of inhaled matter. The hydrolytic enzyme system of lysosomes in the alveolar machrophage is crucial to maintain the sterility of the lung against inhaled microbes. Any inactivation of these enzymes would be expected to increase the risk of respiratory disease.

Hurst et al. (1970) reported that, when rabbits were exposed for 3 h to an ozone concentration of 500 $\mu g/m^3$ (0.25 ppm), there was a reduction in the activity of lysosomal hydrolases, i.e., acid phosphatase (3.1.3.2),

lysozyme (3.2.1.17), and β-glucuronidase (3.2.1.31). *In vitro* exposure of rabbit alveolar macrophages produced a similar decrease in lysosomal hydrolases (Hurst & Coffin, 1971).

Ozone may also induce increases in the concentrations of these enzymes which may be related to eventual chronic lung disease.

When the specific activities of a number of lysosomal hydrolases were measured in whole lung homogenates of rats, there was a significant increase in activities after the animals had been continuously exposed to an ozone concentration of 1400–1600 μg/m³ (0.7–0.8 ppm) for 5–7 days (Dillard et al., 1972). This increase could be attributed to an inflammatory reaction induced by the ozone (Coffin et al., 1968b).

Castleman et al. (1973b) exposed rats continuously for 7 days to ozone concentrations of 1400–1600 μg/m³ (0.7–0.8 ppm). Using histo- and cytochemical techniques, they observed increased acid phosphatase activity but no change in β-glucuronidase activity.

One of the structural alterations reported following exposure to ozone is a change in the appearance of the endoplasmic reticulum. Biochemical evidence of the effects of ozone on microsomal enzymes was reported by Palmer et al. (1971, 1972). After a 3-h exposure to ozone at 1500 μg/m³ (0.75 ppm), the lung tissue of hamsters and the tracheobronchial mucosa of rabbits showed reductions of 33% and 53%, respectively, in the activity of benzopyrene hydroxylase (1.14.14.2), a mixed function oxidase that depends on cytochrome P-450 and is located in the endoplasmic reticulum. Similar results were obtained by Goldstein et al. (1975) who exposed rabbits for 90 min to 2000 μg/m³ (1.0 ppm) and demonstrated a decrease in the rabbit lung cytochrome P-450 concentration. It is of interest that the maximum effect was observed a few days after exposure.

Very little information is available describing alterations in nucleic acids related to ozone exposure. Most of the studies have been conducted at much higher concentrations than those found in the environment and have yielded conflicting results. Since DNA synthesis, cell division, and growth are closely linked, further studies clarifying the potential hazard would be of value.

In studies on mice exposed to an ozone concentration of 5000 μg/m³ (2.5 ppm) for 2 h per day, for 120 days, Werthamer et al. (1974) noted reductions in both DNA and RNA syntheses and a concomitant increase in protein synthesis. Evans et al. (1971) exposed aging mice for 6 h to 1000–7000 μg/m³ (0.5–3.5 ppm) and found that, regardless of the ozone concentration, there was inhibition of DNA synthesis. The authors believed that this indicated a reduction in the ability of the alveoli to act as a source of new cells and to maintain the integrity of lung tissue during ozone exposure.

5.2.3.3. *Extracellular effects*

Since the primary cause of death from high concentrations of ozone is pulmonary oedema, investigators have attempted to determine the role of histamine in the pulmonary toxicity of ozone. The lung is rich in histamine – containing mast cells and among the many effects of histamine are oedematigenous alterations in vascular capillaries. Oedema, whatever the cause, reduces the number of alveoli participating in gas exchange, and produces conditions favourable for bacterial growth. The exact concentration of ozone required to produce oedema depends on the animal species. It is generally believed that gross oedema is probably not elicited in any species exposed to ozone at the concentrations found in the ambient air.

Alpert et al. (1971a) used a radio labelled albumen technique to detect the presence of pulmonary oedema in rats. A significant increase in albumen levels in pulmonary lavage fluid appeared after 6 h exposure to 1000 $\mu g/m^3$ (0.5 ppm).

The available data on lung histamine are conflicting. Dixon & Mountain (1965) reported that, following a single exposure of mice to an ozone concentration of 2000 $\mu g/m^3$ (1.0 ppm) for 5 h, there was a release of histamine from the lungs that persisted for at least 4 days. Pretreatment with an antihistamine (promethazine) reduced the amount of oedema following exposure to a sublethal dose of ozone. It should be noted that the antihistamine used is a phenothiazine derivative the action of which might stabilize membranes and trap free radicals. In contrast, Easton & Murphy (1967) were unable to demonstrate any reduction of lung histamine in guineapigs exposed to ozone concentrations of 10–12 mg/m^3 (5–6 ppm) for 2 h. Cronin & Giri (1974) also failed to observe any differences in the lung histamine level in rats exposed to an ozone concentration of 8000 $\mu g/m^3$ (4.0 ppm) for 4 h, although pulmonary oedema was evident.

Surface tension is an important contributor to the elastic properties of the lung. Any alteration of the normal surface tension in the alveoli may be implicated in the development of chronic lung disease. Consequently, several investigators have examined the effect of ozone on pulmonary surface activity. Gardner et al. (1971) exposed rabbits to ozone levels as high as 20 mg/m^3 (10 ppm) for 2.5 h and then isolated the surface active material by pulmonary lavage. They found that ozone did not alter the surface tension of this material and that *in vitro* exposure did not affect the properties of dipalmitoyl lecithin, a principal component of this surface active substance. Similar results were reported by Huber et al. (1971) who showed that a 3-h exposure of rabbits to ozone at 10 mg/m^3 (5 ppm) did not alter surface activity in the lavaged alveolar lining material nor in extracts of the whole lung. On the other hand Yokoyama (1972a) reported

that *in vitro* exposure of guineapig lung extracts to ozone concentrations of 1000 to 24 000 μg/m³ (0.5–12 ppm) for 25–60 min resulted in a rapid increase in surface tension. However, he, too, did not find any change when dipalmitoyl lecithin was exposed to ozone.

The effect of ozone on the appearance of lung tissue lipids in saline lavage fluid was studied by Kyei-Aboagye et al. (1973). They proposed that ozone affected the lung by decreasing lecithin formation while simultaneously stimulating the release of surfactant lecithins (palmitoyl and oleyl).

In the lung, there is a ground substance between the basement membrane of the alveolar epithelium and the capillary endothelium. Any destruction of the integrity of this substance could affect the elasticity of the lung. Buell et al. (1965) fractionated the lung tissue of rabbits, after a 1-h exposure to an ozone concentration of 2000 μg/m³ (1.0 ppm), into soluble, lipid, and protein fractions. The isolation of aldehydes and ketones from the protein fraction indicated structural changes. It was suggested that, once these compounds were formed, they might affect the intra-and intermolecular crosslinking of elastic protein molecules which would in turn cause a reduction in the elasticity of the lung.

5.2.4 Carcinogenicity

The possibility that ozone might be carcinogenic has been studied in experimental animals.

Exposure to ozonized gasoline with ozone concentrations of 2000–7600 μg/m³ (1.0–3.8 ppm) for 52 weeks caused an increased incidence of lung tumours in strain A mice (350 in each experimental group). After 40 weeks, tumours were found in 21% of animals in the control group and in 63% in the test group. After 52 weeks, the incidence of tumour-bearing animals in the control group exposed to washed air was 41% compared with 80% in the test group (Kotin & Falk, 1956). Additional studies using the same atmosphere of ozonized gasoline were conducted on C57BL mice (405 in each experimental group) (Kotin et al., 1958). After 92 weeks, the incidence of tumour-bearing animals in the control group was 1.6% compared with 9.6% in the exposed group.

Although these studies indicate that ozone may be tumorigenic further work is necessary to confirm these results.

A number of studies including that of Penha et al. (1972) have not been considered in this document because information concerning the numbers of animals tested, control groups, etc. was inadequate.

5.2.5 Tolerance to ozone

The term "tolerance" refers to the fact that exposure to a nonlethal

dose of a specific toxic substance protects the host against subsequent exposure to higher dose of the same chemical or of different agents with similar toxicological properties (cross-tolerance). Stokinger et al. (1956) reported such a protective effect for ozone. Tolerance to ozone has been reviewed by Fairchild (1967) and has also been reported by many other investigators (Henschler, 1960; Matzen, 1957; Mendenhall & Stokinger, 1959). In small rodents, tolerance can be initiated by a concentration as low as 600 μg/m³ (0.3 ppm), maximum protection being obtained with concentrations within the range of 2000–8000 μg/m³ (1–4 ppm) (Stokinger & Scheel, 1962). A single exposure to ozone can also induce a cross-tolerance against subsequent lethal doses of X-ray irradiation (Hattori et al., 1963), nitrogen dioxide, hydrogen peroxide, carbonic dichloride (phosgene), ethanone (ketene), nitrosyl chloride, or hydrogen sulfide (Fairchild, 1967).

Although the mechanism of tolerance is still not well understood, several possibilities have been envisaged. Fairchild (1967) observed that the level of reduced glutathione was maintained in ozone-tolerant animals but not in the nontolerant group. This could be brought about either by directly blocking the oxidation of reduced glutathione or through some enzymatic pathway that would stimulate production of reduced glutathione in response to a second exposure to ozone. However, it is possible that the maintenance of these thiols in the tolerant animal may be a result of the tolerance rather than the cause. The studies of Chow & Tappel (1972, 1973) provide biochemical support for this hypothesis. In addition, it has been shown (Mountain et al., 1960) that the activities of serum alkaline phosphatase (3.1.3.1), adrenal succinate dehydrogenase (1.3.99.1), and glucose-6-phosphatase (3.1.3.9) either remain normal or are only slightly altered in tolerant animals.

Fukase et al. (1975a) reported tolerance in mice to an ozone concentration of 20–58 mg/m³ (10–29 ppm) after pre-exposure to concentrations of 400–2000 μg/m³ (0.2–1.0 ppm) and proposed that the mechanism involved an increase in reduced glutathione and the elevation of the activity of the peroxidative metabolic pathway. Physical swelling of the intra-alveolar septa has also been proposed by Henschler et al. (1964) as a mechanism of protection against the oedematigenous effects of ozone.

Unilateral lung exposure models showed that, in order to induce protection in a particular lung, the tissue must have actually come into contact with ozone (Alpert & Lewis, 1971; Frank et al., 1970a). There was no significant crossover effect from contralateral lung, suggesting that there was no basis for assuming a circulating humoral factor, and that the phenomenon was purely local. While reduction in oedema development could be induced by pre-exposure to ozone, no protection was obtained

against the influx of polymorphonuclear leukocytes into the lung (Gardner et al., 1972). It was also apparent that tolerance did not influence the number of recoverable cells in pulmonary lavage, their phagocytic capability, or the loss of macrophage hydrolytic enzyme activity. This indicated the possibility that tolerance protects only against pulmonary oedema and not against other more subtle reactions.

As previously mentioned, tolerance is initiated in small rodents at approximately 600 $\mu g/m^3$ (0.3 ppm) (Stokinger & Scheel, 1962). According to Alpert et al. (1971a) this approximates the lowest level at which oedema can be demonstrated in rats by means of recovery of labelled serum albumen via lung lavage. Thus, Coffin & Gardner (1972b) postulated that it is probably necessary to produce a minimal oedematigenous response before tolerance develops.

Experiments designed to test the susceptibility to bacterial infection of "tolerant" animals have been conducted by Coffin & Gardner (1972b). These studies illustrated that the "tolerant" animals were only partially protected against the joint effects of ozone and a viable microorganism. Partial protection was afforded by tolerance only when the initiating dose of ozone was 600 $\mu g/m^3$ (0.3 ppm) for 3 h (Coffin & Gardner, 1975). The authors considered that the tolerance to infection seen at levels of ozone above 600 $\mu g/m^3$ (0.3 ppm) could be due to the prevention of the formation of oedema and that tolerance was only partial because tolerance to the action of ozone on cellular and noncellular specific defence systems could not be induced. This was shown by Gardner et al. (1972) who reported that there was no tolerance to ozone damage in the alveolar macrophages, which are the prime defence against infectious disease in the lower part of the respiratory tract.

In their study on the effects of ozone on laboratory-induced allergic respiratory disease in guineapigs, Matsumura et al. (1972) found that animals that had received pretreatment with ozone at 2000 $\mu g/m^3$ (1 ppm) for 1 h before a challenging exposure of 4000 $\mu g/m^3$ (2 ppm) showed only slight respiratory reaction to acetylcholine inhalation compared with those in which tolerance had not been induced.

A variety of neurohumoral factors resulting from exposure to ozone have been described by Fairchild (1963), who reported that thyroidectomy, adrenalectomy, and hypophysectomy would increase the resistance of rats to otherwise lethal doses of ozone by preventing pulmonary oedema.

While the mechanism of tolerance in relation to ozone-induced oedema is still not well understood, it has been suggested that the thymus might play a role. Thymectomized mice were unable to develop tolerance to ozone although the sham-operated animals exhibited tolerance under the same experimental conditions (Gregory et al., 1967).

5.2.6 Effects on the host defence system

Animals that are exposed to ozone have an increased susceptibility to disease-producing biological agents, which can result in an increased incidence of pulmonary infectious disease and death. Animal models have been developed by several investigators to examine this phenomenon experimentally by exposing animals to the pollutants and to aerosolized viable microorganisms. The high sensitivity of this model system is probably due to the fact that it reflects a summation of all the subtle effects that ozone has on the lung. Any alteration in cellular defence and ciliary activity, oedema, and immunosuppression would allow the viable organism to multiply and cause disease. In mice exposed to ozone at concentrations ranging from 2600 to 8800 $\mu g/m^3$ (1.3 to 4.4 ppm) for 3 h, or 1700 $\mu g/m^3$ (0.84 ppm) for 4 h, per day, 5 days per week, for 2 weeks, and subsequently challenged with *Klebsiella pneumoniae*, resistance to respiratory infection was significantly reduced (Miller & Ehrlich, 1958). Similar results were obtained with hamsters. In a study to elucidate a relationship between the time of exposure to ozone and the time of challenge with the infectious agent, Purvis et al. (1961) found a decrease in resistance to infection in mice treated with the bacterial aerosol within 19 h of exposure to the gas or 27 h before exposure. A subsequent study by Coffin and co-workers (1968a) showed that the lowest concentration of ozone necessary to produce this effect in mice was 160 $\mu g/m^3$ (0.08 ppm) for 3 h; Coffin & Gardner (1972) established a dose-response relationship.

Further enhancement of toxicity could be demonstrated when a third interactant such as cold or exercise was added to this infectivity model. Housing mice at 6°–9°C for 3 h prior to exposure to ozone at 1400–1800 $\mu g/m^3$ (0.7–0.9 ppm) for 2 h and to the microorganisms increased the mortality rate compared with that in animals housed at room temperature (Coffin & Blommer, 1965). Mice subjected to physical activity while being exposed to ozone at 200–600 $\mu g/m^3$ (0.1–0.3 ppm) were less resistant to infectious agents than animals that were at rest during exposure (Gardner et al., 1974b).

The effects of low concentrations of ozone on mice with induced silicosis were investigated by Goldstein et al. (1972). Starting at an ozone concentration of 800 $\mu g/m^3$ (0.4 ppm) for 4 h, a progressive decrease in pulmonary bactericidal activity occurred with exposure to increasing concentrations of ozone. Silicosis itself did not inhibit bactericidal activity.

Further research has sought to delineate the mechanisms by which ozone reduces the resistance of animals to bacterial infection. It appears that a number of factors may be responsible.

The number of organisms initially deposited within the lung does not

play a major role in the enhancement of mortality since the pulmonary deposition of the microorganisms is less in ozone-treated animals. However, regardless of the initial deposition, ozone-treated animals subsequently have more organisms within the lungs, mainly because of reduced bactericidal ability and the subsequent multiplication of the inhaled organism (Coffin & Gardner, 1972a). Goldstein et al. (1971b) found that the magnitude of the increase in bacterial numbers was correlated with an increase in ozone concentration up to 5200 $\mu g/m^3$ (2.58 ppm). In rabbits exposed to ozone at a concentration of 1000 $\mu g/m^3$ (0.5 ppm), for 16 h per day, for 7 months, Friberg et al. (1972) did not find any effect on the physical removal of inhaled particles or on the number of macrophages but, under the same conditions, a significant decrease was found in the clearance of viable *Escherichia coli* in the lungs of guinea-pigs.

The effect of ozone on antibacterial activity in the mouse lung was determined *in vivo* by investigating the removal of bacteria by mucociliary activity and by bactericidal activity simultaneously (Goldstein, 1971a, 1971b). Mice were exposed to various concentrations or ozone including 1200, 1400, 1600, and 2200 $\mu g/m^3$ (0.62, 0.70, 0.80, and 1.1 ppm), for 17 h prior to, or 4 h after, infection with aerosols of radiolabelled *Staphylococcus aureus*. Inhibition of pulmonary bactericidal activity was shown at an ozone concentration as low as 1200 $\mu g/m^3$ (0.62 ppm) and activity decreased progressively with increasing levels of ozone. The authors proposed that the bactericidal defect was due to dysfunction of the alveolar macrophage.

Rabbits exposed to an ozone concentration of 600 $\mu g/m^3$ (0.3 ppm) for 3 h showed an impairment of the phagocytic properties of the pulmonary alveolar macrophages (Coffin et al., 1968b). Furthermore, exposure to ozone at 10 mg/m^3 (5 ppm) for 3 h resulted in a reduction in the total number of macrophages with a concomitant influx of polymorphonuclear leukocytes into the lower respiratory tract (Coffin & Gardner, 1972a). Enzyme activity was reduced in macrophages recovered by lavage after exposure of rabbits to ozone at levels as low as 500 $\mu g/m^3$ (0.25 ppm) for 3 h (Hurst et al., 1970). The enzymes studied were lysozyme (3.2.1.17), acid phosphatase, and β-glucuronidase and since these enzymes are involved in the intracellular degradation of ingested bacteria, their reduction could contribute to the poor bactericidal effect seen in the studies mentioned previously.

Other effects on the alveolar macrophage have also been reported. For example, alveolar macrophages from rabbits exposed to 4000 $\mu g/m^3$ (2.0 ppm) for 8 h per day for 7 days exhibited an increased membrane fragility (Dowell et al., 1970) and a dose-related reduction in interferon

was observed in the alveolar macrophages of rabbits exposed to ozone concentrations of 2000, 6000, and 10 000 $\mu g/m^3$ (1, 3, and 5 ppm) for 3 h (Shingu et al., 1972).

Morphological changes seen in rabbit alveolar macrophages after exposure to ozone at a concentration of 10 mg/m^3 (5 ppm) for 3 h included dilatation of the endoplasmic reticulum and perinuclear envelope, swelling of the mitochondria, intracellular vacuolization, cell lysis, and the formation of myelin figures and autophagic vacuoles (Huber et al., 1971). The ultrastructure of pulmonary macrophages was also examined *in situ* in the lung tissue of rats exposed to ozone at 6000 $\mu g/m^3$ (3 ppm) for 4 h (Plopper et al., 1973b). Immediately after exposure, the cells resembled those of unexposed animals, but 12 h later there were twice as many macrophages. Many of these had large granular cytoplasmic inclusions that indicated increased phagocytic activity.

There is evidence that the effect seen on the alveolar macrophage may be mediated through the noncellular milieu of the lung. Within the lung, the macrophages are located close to the so-called pulmonary surfactant contained in the extracellular lining of the epithelial surface of the lung alveoli. It is possible that ozone might also react with this lining film, which in turn, might be deleterious to the cell. Gardner (1971) and Gardner et al. (1971) conducted experiments on lavage fluid containing this surface-active substance and found that, when isolated from rabbits exposed to ozone at 20 mg/m^3 (10 ppm) for 2.5 h, it could adversely affect the stability of the alveolar macrophage *in vitro*. A similar effect was noted when ozone was bubbled through the lavage fluid *in vitro*. The effect on the lavage fluid could be seen at levels as low as 200 $\mu g/m^3$ (0.1 ppm) for a 2.5-h, *in vivo* exposure or after only 30-min *in vitro* exposure. Since no significant alteration in the surface tension of the lavage fluid was produced by ozone exposure, it is suggested that the effect might be on a nonlipid component, possibly a protein. The activity found in the cell-free pulmonary lavage fluid has been called the "protective factor".

Ozone may also inactivate some opsinogenic factor within the lung since Holzman et al. (1968) showed that it has a protein-degrading property. Exposure of mice and rabbits to a concentration of 10 mg/m^3 (5 ppm) for 3 h reduced the activity of active lysozyme, obtained by bronchopulmonary lavage, by approximately 30%.

Various components of the endogenous defence mechanism of the lung were studied through a unilateral lung exposure model (Alpert et al., 1971b). Exposure to ozone at concentrations ranging from 1000 to 6000 $\mu g/m^3$ (0.5 to 3 ppm) for 3 h decreased cellular viability, depressed the intracellular hydrolytic enzymes and increased the absolute number of polymorphonuclear leucocytes in the pulmonary lavage fluid. All effects

were dose-related and were found only in the lung under treatment and not in the contralateral lung that breathed ambient air.

Heuter et al. (1966) reported that exposure to an irradiated automobile exhaust atmosphere for 15 months increased the susceptibility of mice to pulmonary infection during the latter half of the animal's lifetime. The oxidant concentrations in the irradiated exposure chamber ranged from 400 to 2000 $\mu g/m^3$ (0.2–1.0 ppm). In a similar study using irradiated automobile exhaust, enhancement of mortality compared with that of control animals kept in filtered air was noted with exposure to total oxidants at 300 $\mu g/m^3$ (0.15 ppm) and carbon monoxide at 29 mg/m^3 (25 ppm) for 4 h. Coexistent concentrations of nitrogen dioxide ranged from a trace to 1900 $\mu g/m^3$ (1.0 ppm) (Coffin & Blommer, 1967).

Goldstein et al. (1974) studied the bactericidal effect when mice were exposed to a combination of nitrogen dioxide and ozone, and concluded that the combined pollutants caused bactericidal dysfunction at concentrations that were approximately the same as the lowest concentrations that caused similar dysfunction when the animals were exposed to the gases individually.

Erlich et al. (1977) observed that a single joint exposure of mice to nitrogen dioxide and ozone resulted in an addition of effects and that a synergistic action might result from repeated exposures to the mixture.

The animals were exposed for 3 h to 16 different combinations of nitrogen dioxide at levels of 0, 2800, 3800, 6600, and 9400 $\mu g/m^3$ (0, 1.5, 2.0, 3.5, and 5.0 ppm) and ozone at 0, 100, 200, and 1000 $\mu g/m^3$(0, 0.05, 0.1, and 0.5 ppm). Within 1 h of termination of exposure to the pollutants, the mice were infected with *Streptococcus pyogenes*. Excess mortality rates due to exposure to the mixture of the two gases were approximately equivalent to the sum of those induced by the inhalation of each individual pollutant. In mice exposed repeatedly for 3 h per day, for 20 days, to a mixture of nitrogen dioxide and ozone at concentrations of 3800 $\mu g/m^3$ (2.0 ppm) and 100 $\mu g/m^3$ (0.05 ppm), respectively, and challenged with *Streptococcus* aerosol, the number of deaths was significantly higher than in the control group. On the other hand, repeated daily exposure to either nitrogen dioxide or ozone at the above-mentioned concentrations did not show any major effect on the mortality rate. The authors considered that this result might suggest a synergistic action of the two pollutants making them more effective in reducing resistance to respiratory infection.

5.2.7 Interaction of ozone with bronchoactive and other chemicals

The effects of pre-exposure to ozone on the sensitivity of guineapigs to

inhaled acetylcholine were studied by Matsumura et al. (1972). Animals pre-exposed to an ozone concentration of 4000 μg/m³ (2 ppm) for 30 min manifested severe difficulty in breathing and many died. This effect persisted for as long as 2 h after exposure. In a similar study, Matsumura (1970a) sensitized guineapigs to albumen and found that repeated 30 min pre-exposures to an ozone concentration of 10 mg/m³ (5 ppm) enhanced the sensitization. An ozone concentration of 2000 μg/m³ (1 ppm) had no such effect.

Ozone-exposed guineapigs were also more susceptible to the toxic action of injected histamine (Easton & Murphy, 1967). A 2-h exposure to ozone at 10 mg/m³ (5 ppm) caused severe lung function changes and increased mortality. This increased susceptibility to histamine was detectable for as long as 12 h after the end of the exposure.

In order to study the effects of ozone on susceptibility to serotonin, Suzuki & Nagaoka (1973) injected the compound into the abdominal cavity of rats after exposing the animals to ozone at concentrations ranging from 2000 to 12 000 μg/m³ (1–6 ppm) for 3 h. Mortality increased with increasing levels of ozone, but fatalities did not occur in a control group injected with serotonin and breathing filtered air.

A report by Goldstein et al. (1970) that a deficiency of vitamin E increased the toxicity of ozone in the rat has been supported by the studies of Roehm et al. (1971b, 1972) and Menzel et al. (1972). These investigators observed a significantly shorter 50% lethal time and more pronounced signs of respiratory distress in ozone-exposed rats (2000 μg/m³ (1.0 ppm) for 9 days continuously) that were fed vitamin E-depleted diets in comparison with those fed vitamin E-supplemented diets. When the ozone concentration was reduced to 1000 μg/m³ (0.5 ppm), pulmonary oedema became evident and mortality rates increased in animals fed vitamin E-deficient diets after 6 weeks of exposure. In rats continuously exposed to toxic levels of ozone ranging from 1400 to 1600 μg/m³ (0.7–0.8 ppm), protection by dietary vitamin E against lung lipid peroxidation was proportional to the logarithm of the concentration of the vitamin in the diet (Fletcher & Tappel, 1973). In further studies by Mustafa (1975), rats were fed a basal diet containing vitamin E at either 66 mg/kg or 11 mg/kg for 5 weeks, and then exposed continuously to an ozone concentration of 200 μg/m³ (0.1 ppm), for 7 days. Oxygen consumption was measured in lung homogenate using succinate as a substrate. Animals receiving the higher concentration of vitamin E (66 mg/kg) were relatively insensitive to this level of ozone, i.e., there was no significant increase in oxygen consumption, but those receiving the lower concentration of vitamin E (11 mg/kg) showed a significant increase in oxygen consumption.

5.3 Systemic Reactions and other Effects

The number of studies on the effects of ozone on a wide range of biological phenomena including growth, reproduction, and behaviour is increasing but many of these studies need further confirmation. Furthermore, even when a correlation is found between ozone exposure and effects, it is difficult to decide whether the effects are due to the direct oxidizing action of ozone or are secondary reactions to pulmonary injury caused by ozone.

5.3.1 Effects on growth

There does not seem to be any convincing study which shows that ozone, at the concentrations found in ambient air, has any detrimental effect on body growth. Nevskaja & Kočetkova (1961) who exposed rats to a mixture of ozone at 800 $\mu g/m^3$ (0.4 ppm) and sulfuric acid at 7000 $\mu g/m^3$ for 5 h per day, 6 days per week, for 100 days, and Loosli et al. (1972) who exposed mice to synthetic photochemical air pollution containing ozone at 600–840 $\mu g/m^3$ (0.30–0.42 ppm) reported that exposed animals weighed less than the controls. However, Emik et al. (1971) did not find any significant differences between the growth of guineapigs breathing ambient air containing oxidants at a mean concentration of 110 $\mu g/m^3$ (0.057 ppm) for over two years, and that of the controls breathing filtered air.

5.3.2 Haematological effects

It is still questionable whether the haematological effects noted in ozone exposure are due to the direct action of ozone on the cellular and acellular components of the blood as it passes through the lung capillaries or whether they are caused by oxidizing intermediates, such as ozonides or peroxides, that might penetrate the alveolar basement membrane and enter the pulmonary circulation. A third possibility would be that these effects are secondary reactions induced by ozone perhaps causing the release of some mediating substance, yet to be identified.

5.3.2.1 *Short-term exposure (24 h or less)*

Short-term exposure to high concentrations of ozone ranging from 6000 to 16 000 $\mu g/m^3$ (3.0–8.0 ppm) has been reported to cause increases in neutrophil-lymphocyte ratios in rats (Bobb & Fairchild, 1967), and in the number of erythrocytes and leukocyte indices in mice (Kusumoto et al., 1976). Within this range of concentrations, Goldstein et al. (1968) and

Goldstein (1973) found a reduction in acetylcholinesterase (3.1.1.7) in the erythrocytes of mice and induction of hydrogen peroxide formation in the circulating erythrocytes of rats and mice. Veninga (1970, unpublished data)[a] reported doubling in the number of binucleated lymphocytes and increased levels of serum glutamic pyruvic transaminase (2.6.1.2) but no changes in blood catalase (1.11.1.6) in mice exposed for 2 h to an ozone concentration of 400 μg/m³ (0.2 ppm).

Increased resistance to haemolysis of erythrocytes was reported in mice exposed to an ozone concentration of 2000 μg/m³ (1 ppm) for 30 min (Mizoguchi et al., 1973). Menzel et al. (1975) presented evidence that fatty acid ozonides produced Heinz bodies in erythrocytes in mice exposed to ozone at 1700 μg/m³ (0.85 ppm) for 4 h. Further exposure for 3 days resulted in a decline in the number of Heinz body positive cells. It is of interest to note that these bodies were not produced with ozone in the absence of serum containing unsaturated lipids. The authors postulated that this indicated an oxidation of the erythrocyte membrane and suggested that fatty acid ozonides might be the toxic intermediaries. At lower concentrations, Brinkman et al. (1964) noted increased sphering of erythrocytes of mice, rabbits, and rats, after an *in vitro* exposure to ozone at 400 μg/m³ (0.2 ppm) for 1–2 h.

A 3-h exposure to irradiated automobile exhaust containing an oxidant concentration of 740–1200 μg/m³ (0.37–0.58 ppm) was found to increase the number of leukocytes in the blood of mice and reduce the level of serum alkaline phosphatase (3.1.3.1) (Kusumoto et al., 1976).

5.3.2.2 *Prolonged and repeated exposures*

Increasing the length of exposure provided further evidence for the oxidizing effect of ozone on the blood of rabbits and rats. After continuous exposure for 8 days to ozone at 1600 μg/m³ (0.8 ppm), the lysozyme activity in the plasma and soluble fraction of lung of rats significantly increased (Chow et al., 1974). Continuous exposure of rats to an ozone concentration of 110 μg/m³ (0.06 ppm) for 93 days resulted in a decrease in blood cholinesterase (3.1.1.8) activity which returned to normal 12 days after exposure ceased (Eglite, 1968). More recently, Jegier & P'an (1973) and P'an & Jegier (1972) reported a rise in serum trypsin protein esterase in rabbits exposed to 800 μg/m³ (0.4 ppm) for 6 h per day, 5 days per week, for 10 months.

Long-term, combined exposure to ozone and carbon monoxide

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[a] Veninga, T. S. Ozone-induced alterations in murine blood and liver. Paper presented at the 2nd International Clean Air Congress, Washington, DC, 6–11 December 1970 (No. MB 15E).

produced a reduced level of serum glutamic oxaloacetic transaminase (2.6.1.1) in rabbits. The animals were exposed for 1000 days to urban air containing oxidants and carbon monoxide at mean concentrations over 2 years of 110 $\mu g/m^3$ (0.057 ppm) and 2000 $\mu g/m^3$ (1.7 ppm) respectively, (Emik et al., 1971).

5.3.3 Effects on reproduction

A few studies have been reported concerning the possible effects of ozone and photochemical oxidants on reproduction. The data indicate that the newborn animals may be more susceptible to exposure to oxidants than the parents.

Brinkman et al. (1964) and Veninga (1967) exposed pregnant mice for 7 h per day, 5 days per week, for 3 weeks to ozone concentrations of 200–400 $\mu g/m^3$ (0.1–0.2 ppm). They found a 4-fold increase in neonatal mortality. The second author also observed increases in the incidence of both incisor growth and blepharophimosis in the new born.

Similar studies using irradiated automobile exhaust were performed by Hueter et al. (1966) who found that mice exposed for 13 months before, during, and after gestation showed a marked decrease in the number and frequency of litters, the survival of infants, and the total number of pups born. Nonirradiated automobile exhaust did not produce any significant effects. The oxidant concentrations in the irradiated exposure chamber ranged from 400 to 2000 $\mu g/m^3$ (0.2 to 1.0 ppm). In a follow-up study, Lewis et al. (1967) found that the number of female mice that did not become pregnant after mating with pretreated males was twice that found in mice mated with untreated controls.

5.3.4 Behavioural and related changes

5.3.4.1 *Short-term exposure (24 h or less)*

Behavioural changes in response to acute ozone exposures have not been studied very extensively. The major effect that has been noted is a reduction in spontaneous activity. Murphy et al. (1964a) and Konigsberg & Bachman (1970) found significant losses in motor activity in mice exposed to 400 $\mu g/m^3$ (0.2 ppm) for 6 h and in rats exposed to 1000 $\mu g/m^3$ (0.5 ppm) for 45 min, respectively.

Using an evoked response technique, Xintaras et al. (1966) measured a reduction in the amplitude of response to a flash in the specific visual cortex and in the superior colliculus in rats exposed to ozone at 1000–2000 $\mu g/m^3$ (0.5–1.0 ppm) for 1 h. In studies on rhesus monkeys, Reynolds &

Chaffee (1970) reported increases in both simple and choice reaction times after a 30-min exposure to an ozone concentration of 1000 μg/m³ (0.5 ppm).

Gardner et al. (1974) showed that the pentobarbital-induced sleeping time of mice significantly increased after 2 and 3 exposures each of 3 h, to an ozone concentration of 2000 μg/m³ (1 ppm). After the third exposure, there was no change in the sleeping time compared with the controls unless in subsequent exposure the ozone concentration was raised to 10 mg/m³ (5 ppm), when the sleeping time again differed significantly. The authors suggested that ozone might be deactivating a liver microsomal enzyme that was responsible for the detoxification of the drug.

5.3.4.2 *Prolonged and repeated exposures*

Additional studies on the effects of ozone on the behaviour of animals have been conducted with longer exposures and complex mixtures of air pollutants. It appears that the reduction of voluntary running time may be an extremely sensitive indicator of ozone toxicity. The mechanism of this change remains obscure.

Continuous exposure for 1 week to an ozone concentration of 2000 μg/m³ (1.0 ppm) caused an 84% reduction in voluntary activity in rats (Fletcher & Tappel, 1973). Decreased running activity has also been observed in mice exposed continuously to urban air with elevated oxidant levels and to irradiated motor vehicle exhaust (Emik et al., 1971; Heuter et al., 1966).

According to Litt et al. (1968), ozone-exposed rats required a longer time to learn a specific task; learning was unstable and they exhibited a reduced ability for temporal discrimination. The animals were exposed intermittently for 2 months to ozone concentrations of 600–1000 μg/m³ (0.3–0.5 ppm). Nevskaja & Kočetkova (1961) exposed rats to a mixture of ozone at 800 μg/m³ (0.4 ppm) and sulfuric acid at 7000 μg/m³ for 5 h per day, 6 days per week, for 100 days, and found that a conditioned reflex was retarded.

5.3.5 Miscellaneous systemic reactions to lung damage

Other changes have been reported which indicate biochemical, physiological, and structural effects at sites distant from the lung that are possibly related to the inhalation of ozone. In most of these studies, the levels of ozone employed greatly exceeded those in the ambient air. After exposing rabbits to an ozone concentration of 1500 μg/m³ (0.75 ppm) for 4–8 h, Atwal & Wilson (1974) found histological and ultrastructural changes in the parathyroid glands. They reported hyperplasia of the chief

cells, large numbers of secretion granules, proliferation and hypertrophy of the rough endoplasmic reticulum, Golgi complex, mitochondria, lipid bodies, and free ribosomes. These effects were seen up to 66 h after exposure. Atwal et al. (1975) expanded their short-term exposure study by exposing rabbits to 1500 μg/m³ (0.75 ppm) for 48 h and found morphological changes in the parathyroid gland similar to those that they had reported for 4–8 h of exposure. The data suggested that ozone might trigger off an immune reaction which caused inflammatory injury to the parathyroid gland.

It was demonstrated by Brinkman et al. (1964) that exposure to an ozone concentration of 400 μg/m³ (0.2 ppm), for 5 h per day, for 3 weeks, resulted in the rupture of nuclear envelopes and the extrusion of the contents of myocardial muscle fibres in rabbits and mice. This effect became reversible one month after the exposure.

There are some data which suggest that ozone might accelerate the aging process. Stokinger (1965) reported premature aging in rabbits after 1 year of weekly 1-h exposures to ozone. The major changes found in the exposed animals were premature calcification of the sternocostal cartilage, severe depletion of body fat, dull cornea, sagging conjunctivae, and a general appearance of not thriving.

5.4 Mutagenicity

Chromosome aberrations (anaphase bridges) were found in 42% of root meristem cells of *Vicia faba* exposed to an ozone concentration of 8000 mg/m³ (4000 ppm) for 1 h, but none were found in cells exposed to clean air (Fetner, 1958). When chick embryonic fibroblasts were exposed to ozone concentrations of 5000–10 000 mg/m³ (2500–5000 ppm) for 24 h, a small number of cells were found with chromosome bridges in anaphase and telophase and with nuclear fragments (Sachsenmaier et al., 1965). Using a much lower concentration, Pace et al. (1969) exposed strain L cells continuously to an ozone concentration of 8000 μg/m³ (4 ppm) for 30 h, and found a significant reduction in cell survival compared with that in the control group. The authors considered that this was due to ozone interference with mitotic activity.

In vivo exposure studies on mammals by Zelac et al. (1971a, 1971b) are of special interest. Female Chinese hamsters were exposed to an ozone concentration of 400 μg/m³ (0.2 ppm) for 5 h and the number of circulating blood lymphocytes with chromosomal breaks was measured immediately after exposure and 6, and 15.5 days later. A significant increase in

chromosomal breaks compared with pre-exposure values was still observed 15 days after exposure.

Although further confirmation is required, these data and the results of studies on chromosomal changes in human tissues (see section 6.1.1) seem to indicate that ozone might be a mutagenic agent.

5.5 Summary Table

Table 10 is a summary of experimental animal studies that provide quantitative information useful for the establishment of guidelines for the protection of public health with respect to exposure to ozone at concentrations of up to 2000 $\mu g/m^3$ (1.0 ppm).

Table 10. Experimental animal studies
I. Local effects on the respiratory system
1. *Morphological changes*

Ozone concentration		Length of exposure		Effects	Response[a]	Species	Number of animals	Reference
µg/m³	(ppm)	number of days	h/day					
1800	(0.88)	180	24	Epithelial injury seen as early as 4 h after the beginning of exposure; after 3 weeks half of animals died and emphysema-like lesions observed.	n.a.[b]	rat	n.a.	Freeman et al. (1974)
1600	(0.8)	7	24	Walls and interalveolar septa of terminal airways thickened and infiltrated by mononuclear cells.	—	rat	8 (8)	Castleman et al. (1973a)
1200	(0.6)	1	7	Swelling of epithelial alveolar lining cells & endothelium cells with occasional breaks in basement membrane.	—	mouse	32 (13)	Bils (1970)
1100	(0.54)	180	24	Progressive changes in the airway epithelium after 6 days.	—	rat	n.a.	Freeman et al. (1974)
1000	(0.5)	1	6	Immediately after the exposure the number of alveolar cells significantly decreased.	—	mouse	16 (12)	Evans et al. (1971)
800	(0.4)	5 per week × 10 months	6	Emphysematous & vascular-type lesions.	—	rabbit	6 (6)	P'an et al. (1972)
520–2000	(0.26–1.0)	1	4.7–6.6	Dose-related loss of ciliated epithelium.	—	cat	14 (3)	Boatman et al. (1974)
400	(0.2)	1	2	Degenerative changes in type I cells.	—	rat	n.a.	Stephens et al. (1974)
				2. *Functional changes*				
1400	(0.68)	1	2	No significant increase in flow resistance.	—	guineapig	10 (10)	Murphy et al. (1964a)
1000	(0.5)	1	2	Increase in airways resistance and breathing frequency with decrease in tidal volume.	—	guineapig	10 (10)	Yokoyama (1972a)
520–1000	(0.26–0.5)	1	4.6	Increased flow resistance.	—	cat	10 (4)	Watanabe et al. (1973b)
400	(0.2)	30	24	Reduction in lung elasticity; increase in lung volume and in alveolar dimensions.	—	rat	44 (44)	Bartlett et al. (1975)

[a] Number of animals showing effects/total number of animals; numbers in brackets refer to control groups.
[b] Not available.

3. *Biochemical changes*

1500	(0.75)	1	3	Reduction in activity of benzopyrene hydroxylase (1.14.14.2).	—	hamster & rabbit	25 (95) 8 (15)	Palmer et al. (1971, 1972)
1400–1600	(0.7–0.8)	7	24	Increased acid phosphatase activity.	—	rat	14 (12)	Castleman et al. (1973b)
1400	(0.7)	5	24	Indication of lipid peroxidation; increase in lysosomal hydrolase activity.	—	rat	33 (20)	Chow & Tappel (1972); Dillard et al. (1972)
1000	(0.5)	1	6	Increased albumen recovery from alveolar spaces.	—	rat	10 (18)	Alpert et al. (1971a)
800–1400	(0.4–0.7)	1	4	Evidence of formation of lipid peroxides in the lung.	n.a.[b]	mouse	n.a.	Goldstein et al. (1969)
500	(0.25)	1	3	Reduced activity of several lysosomal hydrolases.	—	rabbit	6 (6)	Hurst et al. (1970)
400	(0.2)	7	24	Increase in pulmonary mitochondrial oxygen consumption.	—	rat	5–8 (5–8)	Mustafa et al. (1973)

4. *Effects on the host defence system*

1200–1600	(0.62–0.80)	1	17	Inhibition of pulmonary bactericidal activity.	—	mouse	20 (29)	Goldstein et al. (1971b)
1000	(0.5)	210	16	No effect on physical clearance of inhaled particles or on the number of macrophages.	—	rabbit	8 (8)	Friberg et al. (1972)
1000	(0.5)	60	16	Decrease in the clearance of viable *Escherichia coli*.	—	guineapig	18 (18)	Friberg et al. (1972)
800	(0.4)	1	4	Inhibition of bactericidal activity; no additive role of the induced silicosis.	—	mouse	38 (37)	Goldstein et al. (1972)
600	(0.3)	1	3	Impairment of phagocytic properties of pulmonary alveolar macrophages.	—	rabbit	n.a.[b]	Coffin et al. (1968b)
500	(0.25)	1	3	Diminished enzyme activities of alveolar macrophages.	—	rabbit	6 (6)	Hurst et al. (1970)
160	(0.08)	1	3	Increased susceptibility to *Streptococcus*.	15/40 (6/40)	mouse	40 (40)	Coffin et al. (1968a)

[a] Number of animals showing effects/total number of animals; numbers in brackets refer to control groups.

[b] Not available.

Table 10. Experimental animal studies—*continued*

II. Systemic reactions and other effects

1. *Haematological effects*

Ozone concentration		Length of exposure		Effects	Response[a]	Species	Number of animals	Reference
µg/m³	(ppm)	number of days	h/day					
1700	(0.85)	1	4	Formation of Heinz bodies in red cells.	n.a.[b]	mouse	n.a.	Menzel et al. (1975)
1600	(0.8)	8	24	Increase of lysozyme activity in plasma and soluble fraction of lung.	—	rat	8 (8)	Chow et al. (1974)
800	(0.4)	5 per week × 10 months	6	Increase in serum trypsin protein esterase.	—	rabbit	6 (6)	P'an & Jegier (1972); Jegier & P'an (1973)
600	(0.3)	1	1	Inhibition of acetylcholine esterase (3.1.1.7) activity.	—	ox (*in vitro*)	—	P'an & Jegier (1970)
400	(0.2)	1	1–2	Increased sphering of red blood cells.	—	mouse, rabbit, rat (*in vitro*)	—	Brinkman et al. (1964)
400	(0.2)	1	2	Doubling in number of binucleated lymphocytes.	—	mouse	n.a.	Veninga (1970, unpublished)
110	(0.06)	93	24	Decrease in blood choline esterase activity.	—	rat	15 (15)	Eglite (1968)

2. *Effects on reproduction*

400	(0.2)	5 per week × gestation period + 1st 3 weeks of life	7	Increase in neonatal mortality.	n.a.	mouse	n.a.	Veninga (1967)
200–400	(0.1–0.2)	5 per week × 3 weeks	7	Increase in neonatal mortality.	n.a.	mouse	n.a.	Brinkman et al. (1964)

[a] Number of animals showing effects/total number of animals; numbers in brackets refer to control groups.

[b] Not available.

3. Behavioural changes

1000–2000	(0.5 –1.0)	1	1	Decrease in the amplitude of evoked response to flash.	—	rat	3 (3)	Xintaras et al. (1966)
1000	(0.5)	1	0.5	Increase in simple and choice reaction time.	—	rhesus monkey	4 (4)	Reynolds & Chaffee (1970)
1000	(0.5)	1	0.75	Significantly reduced motor activity.	—	rat	12 (12)	Konigsberg & Bachman (1970)
600–1000	(0.3 –0.5)	60	intermittently (variable intervals)	Increase in time to learn specific tasks.	—	rat	6 (6)	Litt et al. (1968)
400	(0.2)	1	6	Reduction in spontaneous running activity.	—	mouse	9 (9)	Murphy et al. (1964a)

4. Miscellaneous extrapulmonary changes

1500	(0.75)	1–2	4–8, 24	Histological changes in parathyroid gland.	—	rabbit	16 (16)	Atwal & Wilson (1974); Atwal et al. (1975)
400	(0.2)	21	5	Structural changes in myocardial muscle fibres.	—	rabbit & mouse	n.a.	Brinkman et al. (1964)
400	(0.2)	1	5	Increase in chromosomal breaks in circulating lymphocytes.	—	hamster	8 (4)	Zelac et al. (1971a, b)

[a] Number of animals showing effects/total number of animals; numbers in brackets refer to control groups.

[b] Not available.

6. EFFECTS ON MAN

6.1 Controlled Exposures

A considerable number of studies have been performed, under controlled conditions, on the effects of ozone on both healthy subjects and patients, with their consent. Some of these studies have provided useful information for the evaluation of exposure-effect relationships. A few *in vitro* studies using human tissues have also helped to clarify the mechanisms of the biological actions of ozone. There have been few human studies on other oxidants.

6.1.1 *In vitro* effects on human tissues

Brinkman et al. (1964) found that *in vitro* exposure of human red blood cells to an ozone concentration of 0.5 mg/m^3 (0.25 ppm) for 30 min accelerated sphering of these cells by X-ray irradiation, compared with unexposed cells. Chromosome breakages in human cell (epidermoid carcinoma cell) cultures exposed to an ozone concentration of 16 mg/m^3 (8 ppm) for 5 or 10 min were equivalent to those produced by X-rays (200R, 250 kV) (Fetner, 1962).

The production of interferon was suppressed when human tonsil lymphocytes were exposed *in vitro* to an ozone concentration of 10 mg/m^3 (5 ppm) for 3 h (Watanabe et al., 1973a).

Goldstein (1976) measured the combined effects of ozone at 4–82 mg/m^3 (2–41 ppm) and nitrogen dioxide at 6.8–190 mg/m^3 (3.6–102 ppm) on human red cells, *in vitro*, and found that the absolute and relative concentrations of the pollutants as well as the sequence of administration could affect the interaction. In general, the effects of the two pollutants on the variables measured (osmotic fragility, acetylcholinesterase (3.1.1.7) activity, lipid peroxidation, reduced glutathione, and methaemoglobin levels) were additive. At lower pollutant doses, a synergistic effect on the increase in lipid peroxides was reported. Ozone also potentiated the formation of methaemoglobin due to the action of nitrogen dioxide.

6.1.2 Sensory effects

The effects of ozone or oxidants on sensory organs have been studied in terms of eye irritation, changes in visual parameters, and olfactory thresholds.

Eye irritation in two groups of 20 female telephone company employees

working in identical adjacent rooms was evaluated by Richardson & Middleton (1957, 1958) in relation to oxidant concentrations from May to November 1956. Activated-carbon and dummy air-filter media were switched periodically between the two rooms so that the groups were alternately exposed to test and control conditions. In all cases, differences in eye irritation between the activated-carbon filtered and nonfiltered test conditions were highly significant ($p < 0.01$). The scatter diagram (Fig. 8) suggests the existence of a threshold for eye irritation at an oxidant concentration of approximately 200 $\mu g/m^3$ (0.10 ppm).

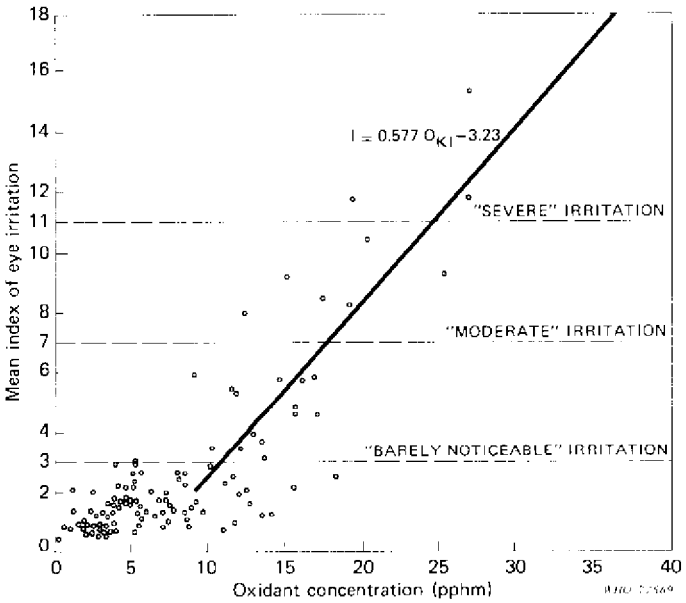

Fig. 8. Mean index of eye irritation versus oxidant concentration. Oxidants, 1 pphm = 20 $\mu g/m^3$ (Adapted from: Richardson & Middleton, 1957).

The index of eye irritation increased progressively as oxidant concentrations exceeded this value. No significant correlations between eye irritation and concentrations of nitrogen dioxide or suspended particulates were observed. However, in interpreting these results, care must be exercised in drawing conclusions regarding cause-effect relationships, as other controlled exposure studies have shown that ozone is not an eye irritant. By comparison, peroxyacylnitrates, acrolein, and peroxybenzoylnitrates have all been shown to be strong eye irritants (Heuss & Glasson, 1968; Schuck & Doyle, 1959; Stephens et al., 1961). Each of these

compounds is a product of the photochemical reaction system and thus is highly correlated in time with measured levels of oxidants or ozone.

Lagerwerff (1963) measured the effect of exposure to ozone on visual parameters in 22 male and 6 female volunteers. The subjects were exposed to ozone at 400, 700, and 1000 μg/m³ (0.20, 0.35, and 0.50 ppm) for 3 h, and again for 6 h, with 10 days rest between exposures. Visual acuity, depth perception, lateral and vertical phoria, divergence and convergence, near vision, and peripheral, colour, and night vision were tested. Considerable decreases in visual acuity in scotopic and mesopic ranges, increases in peripheral vision, changes in extra ocular muscle balance, and decrease in night vision were observed in the majority of subjects. A few subjects also developed a nonproductive cough at the highest ozone concentration, and several complained of difficulties with mental concentration at these levels.

Studies of the olfactory threshold in 10–14 male volunteer test subjects exposed for 30 min to a series of different ozone concentrations were performed by Henschler et al. (1960). The lowest ozone concentration used, 40 μg/m³ (0.02 ppm), was recognized immediately by 9 out of 10 subjects. The subjects reported that the odour diminished rapidly, and that it was no longer perceptible within ½–12 min. When exposed to an ozone concentration of 100 μg/m³ (0.05 ppm), 13 out of 14 subjects indicated that the odour was considerably stronger and that it lasted longer (2–30 min, with an average duration of 13 min). In a study by Eglite (1968), it was found that the threshold of smell for ozone was 15 μg/m³ (0.008 ppm) for the most sensitive subject in a group of 20 persons.

6.1.3 Effects on respiratory function

6.1.3.1 *Exposure to ozone*

A considerable number of controlled studies on exposure to ozone have been reported. However, with one exception, all these studies were concerned with short-term exposures of less than 6 h.

A highly significant fall in steady state diffusion capacity and 0.75-second forced expiratory volume ($FEV_{0.75}$) was noted in each of 16 test replicates on 10 men and 1 woman aged 20–45 years, exposed to ozone concentrations of 1200–1600 μg/m³ (0.6 to 0.8 ppm) for 2 h (Young et al., 1964).

Goldsmith & Nadel (1969) exposed 4 healthy males, for 1 h, to ozone concentrations of 200, 800, 1200, and 2000 μg/m³ (0.1, 0.4, 0.6, and 1.0 ppm). Consistent increases in airway resistance (R_{aw}) were demonstrated only with exposure to 2000 μg/m³ (1 ppm). Lower concentrations caused an increase in R_{aw} in some subjects, but a clear dose-effect

relationship could not be established at levels below 2000 μg/m³ (1 ppm). When exposed to an ozone concentration of 1500 μg/m³ (0.75 ppm) for 2 h, 10 healthy males exhibited significant increases in R_{aw}, reductions in maximum static elastic recoil pressure of the lung, and a fall in maximum flow at 50% of vital capacity (Bates et al., 1972). Exercise on a bicycle ergometer during ozone exposure accentuated these pulmonary function changes, and most subjects complained of substernal soreness and cough at the end of a 2-h period. These observations were extended by Hazucha et al. (1973) to include an ozone concentration of 740 μg/m³ (0.37 ppm) for 2 h and subjects were intermittently exercised during exposure. After exposure to 740 and 1500 μg/m³ (0.37 and 0.75 ppm) for 2 h, both smokers and non-smokers (6 subjects per group) revealed significant decreases in forced vital capacity (FVC), one-second forced expiratory volume ($FEV_{1.0}$), mid-maximal expiratory flow rate (MMFR), and maximum expiratory flow rate at 50% of vital capacity (MEFR 50%), but the effects were greater at 1500 μg/m³ (0.75 ppm). These effects were closely related to the changes measured in the closing volume and indicated an early effect in the small airways.

Seven healthy males, exposed to an ozone concentration of 1000 μg/m³ (0.50 ppm) for 2 h while performing intermittent light exercise, showed decreases in lung function measurements and oxidative changes in erythrocytes; 3 subjects had symptoms such as cough, substernal discomfort, and malaise. When 2 healthy and 3 sensitive subjects (those with a prestudy history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure, but with normal base line pulmonary function studies) were exposed to an ozone concentration of 740 μg/m³ (0.37 ppm) for 2 h under the same conditions of exercise, there was a significant increase in the total respiratory resistance compared with the pre-exposure resistance. Oxidative biochemical changes were detected in the blood of this group, but were not as severe as in those exposed to an ozone concentration of 1000 μg/m³ (0.5 ppm). Three healthy and 3 sensitive subjects exposed to 500 μg/m³ (0.25 ppm) for 2 h did not show any consistent physiological changes attributable to exposure (Hackney et al., 1975). All the subjects of the above study were southern Californians. In order to study possible adaptation of these subjects to long-term ambient ozone exposure, the same investigators compared the effects of ozone exposure on southern Californians with those on Canadians and found that the latter were more responsive (Hackney et al., 1977).

Ohmori (1974) found increased breathing frequency and volume in 4 healthy males who were exposed under exercise to ozone concentrations of 200–500 μg/m³ (0.1–0.25 ppm) for 30 min. Four normal male subjects exposed for 5 min to a combination of ozone at 1800 μg/m³ (0.9 ppm) and

light exercise showed a highly significant decrease in specific airway conductance (Kagawa & Toyama, 1975a). Folinsbee and co-workers (1975) tested the reaction of 28 normal adults (20 males, 8 females) exposed to ozone at concentrations of 740, 1000, or 1500 μg/m³ (0.37, 0.50, or 0.75 ppm) for 2 h: the subjects were either at rest or exercising intermittently with sufficient intensity to increase lung ventilation by a factor of 2.5. There was a gradation of complaints depending on the ozone concentration. Under exercise, increase in respiratory frequency was closely correlated with the total dose of ozone and, at any given concentration, the effect was greater with the subject who had exercised. Statistically significant decreases were found in FVC at ozone concentrations of 1000 μg/m³ (0.50 ppm) or more. Decrease in MEFR occurred in 50% of subjects at concentrations of 740 μg/m³ (0.37 ppm) or more.

The effects of ozone on pulmonary function were studied in 22 young, healthy, male, nonsmokers who were exposed to 800 μg/m³ (0.4 ppm) for 2 and 4 h. Subjects were seated during the exposure except for 2 exercise periods of 15 min (bicycle), beginning after 1 and 3 h of exposure, respectively. Significant changes in FVC, MMFR and R_{aw} occurred after 2 h of exposure. Borderline effects were reported for $FEV_{1.0}$, $\dot{V}_{50}$ (expiratory flow rate at 50% FVC) and $\dot{V}_{25}$ (expiratory flow at 25% FVC). After 4 h of exposure, changes in all of these lung function variables became statistically significant (Rummo et al., 1975, unpublished data).[a] In studies by Kerr et al. (1975), 20 lightly exercising subjects (19 males and 1 female) were exposed to an ozone concentration of 1000 μg/m³ (0.5 ppm) for 6 h. Subjects, particularly nonsmokers, commonly reported dry cough and chest discomfort. Significant changes were produced in airway conductance, pulmonary resistance, and forced expiratory volumes, but not in diffusing capacity.

Changes in the pulmonary function of 11 male subjects, aged 24–38 years, exposed to an ozone concentration of 200 μg/m³ (0.1 ppm) for 2 h, were compared with a 1-h pre- and post-control period without ozone and with a control series without ozone. Under test conditions, which included intermittent light exercise, a significant increase in R_{aw} and in the alveolar to arterial oxygen pressure difference ($AaDO_2$) was observed in 7 out of 11 subjects (von Nieding et al., 1977).

In an attempt to study the effects of long-term repeated exposures to ozone, Bennet (1962) exposed 2 groups of 6 healthy males to 400 μg/m³ (0.2 ppm) or 1000 μg/m³ (0.5 ppm) respectively, for 3 h per day, 6 days per

[a] Rummo, N. J., Knelson, J. H., Lassiter, S., Cram, J. J., & House, D. (1975). Effects of ozone on pulmonary function in healthy young men. Research Triangle Park, NC, US Environmental Protection Agency, 17 pp. (In-house Technical Report).

week for 12 weeks. The 400 μg/m³ (0.2 ppm) exposure group did not experience any symptoms or changes in the forced expiratory volume. While the second group was also asymptomatic, the $FEV_{1.0}$ showed a significant decrease during the last few weeks of exposure.

6.1.3.2 *Exposure to mixtures of ozone and other air pollutants*

Bates & Hazucha (1973) demonstrated a synergistic action of ozone at 740 μg/m³ (0.37) and sulfur dioxide at 960 μg/m³ (0.37 ppm). With 4 normal subjects engaged intermittently in light exercise, a 2-h exposure to sulfur dioxide, only, at 960 μg/m³ (0.37 ppm) did not produce any changes in FVC, $FEV_{1.0}$, MMFR, and MEFR 50%, while exposure to an ozone concentration of 740 μg/m³ (0.37 ppm) caused a 10–15% reduction in these lung function variables. However, exposure to a combination of these gases at the same concentrations resulted in a 20–45% decline in lung function measurements and this effect was even greater than the changes caused by a 2-h exposure to an ozone concentration of 1500 μg/m³ (0.75 ppm). On the other hand, addition of nitrogen dioxide at a concentration of 560 μg/m³ (0.30 ppm) to ozone at 500 μg/m³ (0.25 ppm) did not result in any decrement in the lung functions of 3 healthy and 3 sensitive (section 6.1.3.1) subjects (Hackney et al., 1975a).

In a series of studies already mentioned in section 6.1.3.1, von Nieding et al. (1977) found that the effects of exposure to an ozone concentration of 200 μg/m³ (0.1 ppm) were not enhanced by combination with nitrogen dioxide at 9400 μg/m³ (5 ppm), or by combination with nitrogen dioxide at 9400 μg/m³ (5 ppm) and sulfur dioxide at 13 000 μg/m³ (5 ppm), though recovery time was delayed in the last experiment. Exposure for 2 h to a combination of ozone at 50 μg/m³ (0.025 ppm), nitrogen dioxide at 100 μg/m³ (0.05 ppm), and sulfur dioxide at 260 μg/m³ (0.1 ppm) did not have any effect on R_{aw} or on $AaDO_2$. However, there was a dose-dependent increase in the sensitivity to acetylcholine of the bronchial tree compared with the controls. This effect was observed by measuring the increase in R_{aw} after inhalation of 2% acetylcholine alone and in combination with the previously mentioned mixture of gases at the same concentrations. The effect became more pronounced when the acetylcholine was combined with ozone at 200 μg/m³ (0.1 ppm), nitrogen dioxide at 9400 μg/m³ (5 ppm), and sulfur dioxide at 13 000 μg/m³ (5 ppm).

6.1.3.3 *Exposure to peroxyacetylnitrate alone or in combination with carbon monoxide*

Thirty-two male college students were exposed to a peroxyacetylnitrate concentration of 1500 μg/m³ (0.3 ppm) for 5 min while exercising on a bicycle ergometer (Smith, 1965). A statistically significant increase in

oxygen uptake was observed during exercise, in comparison with that observed when filtered air was breathed. MEFR was significantly decreased by exposure to this gas during the recovery phase following exercise. However, peroxyacetylnitrate did not have any effect when the subjects were at rest.

The metabolic, temperature, and cardiorespiratory reactions of 20 healthy males (10 smokers and 10 nonsmokers) were monitored while working to their maximum and breathing filtered air or 3 different gas mixtures at 25 ± 0.5°C and a relative humidity of 20 ± 2% (Raven et al., 1974). The mixtures were carbon monoxide in filtered air at 57 500 $\mu g/m^3$ (50 ppm), peroxyacetylnitrate in filtered air at 1350 $\mu g/m^3$ (0.27 ppm), and a combination of the two. Maximum aerobic capacity did not decrease significantly in either group during exercise and exposure to any of the pollutant gas mixtures compared with filtered air. For both smokers and nonsmokers exposure to peroxyacetylnitrate and carbon monoxide alone or in combination, while exercising to maximum aerobic capacity, produced minor alterations in cardiorespiratory and temperature regulating variables.

6.1.3.4 *Exposure to irradiated automobile exhaust*

Fourteen college students were exposed on 2 occasions to irradiated automobile exhaust and measurements were made of reaction time, vital capacity, and submaximum work performance on the bicycle ergometer. The individuals were exposed to a mixture of the following gases: carbon monoxide, carbon dioxide, nitric oxide, nitrogen dioxide, hydrocarbons, aldehydes, formaldehyde, and oxidants (Table 12, section IID). It appeared that exposure to this mixture of pollutants had little effect on the types of human motor performance chosen for this study (Holland et al., 1968).

6.1.3.5 *Exposure to ambient air containing elevated concentrations of oxidants*

The effect of oxidant air pollution on patients with chronic obstructive lung disease was studied by Motley et al. (1959). Twenty normal individuals and 46 patients with chronic lung disease were evaluated by measuring lung volumes, forced expiratory volumes, and nitrogen washout before and after removal from ambient Los Angeles air into a room with clean filtered air. No significant changes were detected in the pulmonary function of normal subjects or in patients with chronic lung disease who entered the room on nonsmoggy days. However an improvement in lung function, particularly a decrease in residual lung volume and nitrogen washout time, occurred among patients who entered

the filtered room on smoggy days and remained for 40 or more hours. Simultaneous measurements of oxidants were not obtained at the study site, but during the study, ambient hourly oxidant concentrations at nearby monitoring stations ranged from 400–1400 $\mu g/m^3$ (0.2–0.7 ppm). Subjects with chronic lung disease, who remained in the filtered air for only 18–20 h, did not experience any significant improvement in lung function.

In a similar study, Balchum (1973) observed the pulmonary function of 15 patients with moderately severe chronic obstructive lung disease, who spent one week in a room without air filtration and a second week in clean filtered air. During the first week, when unfiltered ambient air was drawn into the room, 1-h oxidant concentrations averaged 220 $\mu g/m^3$ (0.11 ppm) and ranged up to 400 $\mu g/m^3$ (0.2 ppm). During the second week, when air filters were activated, oxidant exposures ranged from 40 to 60 $\mu g/m^3$ (0.02 to 0.03 ppm). Comparison of the effects of unfiltered and filtered air revealed a decrease in airway resistance and an increase in the arterial pO_2 during the week of filtered air breathing. Changes were observed both at rest and during exercise in about 75% of the subjects. Decreases in airway resistance first became apparent after breathing filtered air for 48 h.

6.1.4 Changes in electroencephalograms

Changes (decrease in the alpha rhythms) in electroencephalograms were studied by Eglite (1968) in relation to exposure to ozone for 3 min. All 3 healthy subjects studied showed changes when exposed to an ozone concentration of 20 $\mu g/m^3$ (0.01 ppm) and 1 subject reacted at a concentration at 10 $\mu g/m^3$ (0.005 ppm).

6.1.5 Chromosomal effects

In a recent study by Merz et al. (1975), 6 human volunteers were exposed to an ozone concentration of 1 mg/m^3 (0.5 ppm) for 6–10 h, and the circulating lymphocytes were examined for chromosomal changes. Although no chromosome aberrations were found, there was a significant increase in the number of minor chromosomal abnormalities (chromatid deletions) compared with pre-exposed lymphocytes.

6.2 Industrial Exposure

Several studies on the effects of industrial exposure have been reported, but in most of them, the effects of ozone have been confounded by the

coexistence of other pollutants and a threshold concentration has not been determined.

Kleinfield et al. (1957) reported several cases of severe ozone intoxication in welders using an inert gas-shielded consumable electrode which greatly increased the ultraviolet radiation. Ozone was measured at the breathing zone in 3 plants using this welding technique. No complaints or clinical findings were associated with ozone concentrations of 500 $\mu g/m^3$ (0.25 ppm) or less. At concentrations of 600–1600 $\mu g/m^3$ (0.3–0.8 ppm), an increasing number of welders complained of chest constriction and irritation of the throat.

Similar findings using this welding technique were reported by Challen et al. (1958), when 11 out of 14 workers complained of respiratory symptoms at ozone concentrations in the range of 1600–3400 $\mu g/m^3$ (0.8–1.7 ppm). Symptoms disappeared when ozone levels were reduced to 400 $\mu g/m^3$ (0.2 ppm). Young et al. (1963) failed to detect any significant changes in lung function tests (vital capacity, functional residual capacity, MMFR, $FEV_{0.75}$, or diffusing capacity) in 7 men engaged in argon-shielded electric arc welding when ozone concentrations were 400–600 $\mu g/m^3$ (0.2–0.3 ppm).

Kudrjavceva (1963) described a complex of symptoms that included headache, weakness, increased muscular excitability, and decreased memory among workers engaged in the manufacture of hydrogen peroxide and exposed to ozone concentrations ranging from 500–800 $\mu g/m^3$ (0.25–0.40 ppm) for 7–10 years. The investigator suggested that these reactions might be due to prolonged exposure to ozone under working conditions. Increased prevalence of bronchitis and emphysema, accompanied by decreased expiratory flow rate, was also reported in workers involved in the manufacture of hydrogen peroxide by Nevskaja & Diterihs (1957). Ozone concentrations of 80–1000 $\mu g/m^3$ (0.04–0.50 ppm) were measured; sulfuric acid aerosol was also present. Control groups were not included in either of these studies.

In several countries, occupational exposure limits for ozone have been set as maximum, time-weighted averages for an 8-h workday or 40-h week (ILO, 1977). In some countries, the exposure limit is 100 $\mu g/m^3$ (0.05 ppm), in others, 200 $\mu g/m^3$ (0.1 ppm). In addition, a short-term exposure limit of 600 $\mu g/m^3$ (0.3 ppm), for periods up to 15 min, has been tentatively proposed by the USA provided that there are not more than 4 such periods per day with at least 60 min between each period, and that the daily time-weighted average is not exceeded. The German Democratic Republic has set a limit of 200 $\mu g/m^3$ for exposure periods not exceeding 30 min and the USSR has proposed an 8-h mean value of 100 $\mu g/m^3$ (0.05 ppm).

6.3 Community Exposure

Epidemiological studies on the association between human health effects and exposure to photochemical oxidants have largely been carried out in the Los Angeles air basin. For most of these studies, investigators made use of measurements of photochemical oxidants obtained from a network of air monitoring stations operated by the Los Angeles County Air Pollution Control District. Oxidants were measured by the unbuffered potassium iodide method which yields oxidant values 15–25% lower than the values obtained with the 1 or 2% neutral-buffered potassuim iodide method more commonly used in regions outside the Los Angeles air basin.

As in all studies on urban populations, the observed health effects of photochemical oxidant exposure cannot be attributed only to oxidants. Photochemical smog typically consists of ozone, nitrogen dioxide, peroxyacylnitrates and other nitrate compounds, sulfates, other particulate aerosols, and reducing agents. In combination, these pollutants may have an independent, additive, or synergistic effect on human health. In general, however, ozone appears to be the most biologically active pollutant and the correlations between health effects and pollutant exposure were found when results concerning ozone, rather than other identified pollutants, were statistically analysed. Since controlled exposure studies in man and animals confirm the greater biological reactivity of ozone compared with other components of photochemical smog, it seems reasonable to conclude, for the purpose of developing health protection guidelines, that ozone is the principal agent responsible for the exposure-response relationships observed in epidemiological studies on photochemical oxidants.

6.3.1 Mortality

Studies conducted by the California State Department of Public Health (1955, 1956) over the periods July–November 1953 and 1954, and July–December 1955 revealed that, during these months, the daily mortality rate of Los Angeles residents, aged 65 or over, was strongly influenced by a heat wave but was not consistently altered either by variations in oxidant concentrations or by the occurrence of smog-alert days (ozone concentrations of 600 $\mu g/m^3$ (0.3 ppm) or more).

No significant correlations were found by Massey et al. (1961, unpublished data) between daily mortality rates and daily oxidant levels in an analysis of two areas of Los Angeles County, selected for similarities in temperature and for differences in air pollution levels (values not given). Hechter & Goldsmith (1961) also failed to find a significant correlation

between monthly mortality rates due to cardiorespiratory diseases and pollutant levels (monthly means of daily maxima that ranged from 80 to 400 μg/m³; 0.04–0.2 ppm) in Los Angeles County for the years 1956–58. The authors made Fourier curve analyses of the data in order to remove the major effect of season of year on mortality rate and pollutant levels. An association between respiratory and cardiac deaths and Los Angeles smog episodes with 1-h concentrations exceeding 400 μg/m³ (0.2 ppm) was observed by Mills (1957) but he failed to take into account seasonal fluctuations in deaths and pollutants. However, it is possible that the method of adjustment for seasonal effects might mask a real effect of pollutant concentrations on mortality. Therefore no conclusive statement can be made concerning the lack of an association between mortality rate and short-term variations in oxidant levels.

A model of daytime wind flow over Los Angeles was constructed by Mahoney (1971) to divide the city into 5 wind zones each about 10 kilometres in width and representing distance downwind along the path of air flow. The mortality rate of the white population, adjusted for age, sex, and income level, from noncancerous respiratory diseases during 1961 increased in successive downwind zones affected by the Los Angeles sea breeze. The adjusted mortality rate in the wind zone immediately adjacent to the Pacific Ocean was 53 per 100 000, while mortality in the afferent wind zone most remote from the ocean was 111 per 100 000. The author suggested that this geographical difference in mortality might be consistent with an effect of temperature, humidity, or oxidant air pollution.

6.3.2 Annoyance and irritation

In Los Angeles, 75% of the population complained that they were "bothered" by air pollution either at home or at work compared with 24% in San Francisco and 22% in the "rest of the State". Eye complaints, which were relatively high (33%) in Los Angeles County and in the "rest of the State", were low (14%) in the San Francisco Bay area. However, fewer cases of hayfever and sinus trouble were reported in Los Angeles County compared with other areas (Hausknecht, 1960).

The association of oxidant levels with eye irritation was investigated (Renzetti & Gobran, 1957) in a panel of office and factory workers and a second panel of scientists residing in the Los Angeles Basin. The data demonstrated increasing eye irritation with increasing maximum instantaneous oxidant concentrations over a range of values from 100 to 900 μg/m³ (0.05–0.45 ppm).

Daily symptoms associated with eye and respiratory irritation were studied by Hammer et al. (1974) in a group of Los Angeles student nurses

in relation to daily oxidant levels. Headache, eye discomfort, cough, and chest discomfort were all found to be related to daily maximum hourly oxidant concentrations. On days when maximum hourly concentrations were 800–1000 μg/m³ (0.40–0.50 ppm), students reported 48% more cough and 100% more chest discomfort compared with days when oxidant levels were below the US air quality standard of 160 μg/m³ (0.80 ppm). Using "hockey stick" functions,[a] the threshold levels have been determined as maximum 1-h concentrations of 100 μg/m³ (0.05 ppm) for headache, 300 μg/m³ (0.15 ppm) for eye irritation, 530 μg/m³ (0.27 ppm) for cough, and 580 μg/m³ (0.29 ppm) for chest discomfort. Exposure-response relationships are shown in Table 11.

6.3.3 Athletic performance

The effect of oxidant concentrations on athletic performance was studied in Los Angeles by Wayne et al. (1967) in 21 competitive team events for high school cross-country runners from 1959–64. Since running times tended to improve throughout the season, team performance at a meeting was evaluated by determining the percentage of individual athletes who failed to improve when their running time was compared with that of their previous performance on the same course. Oxidant levels in the hour before the meeting, which ranged from below 100 μg/m³ (0.05 ppm) to 600 μg/m³ (0.3 ppm), were highly correlated ($r = 0.88$) with decreased performance, as shown in Fig. 9. Consistently high correlations were obtained when the observations were divided into the periods 1959–1961 ($r = 0.945$) and 1962–64 ($r = 0.945$). Correlations with other pollutants (carbon monoxide and particulates) and with meteorological variables (temperature, relative humidity, wind velocity, wind direction) were considerably lower and usually not significant. The authors speculated that the observed oxidant-athletic performance relationship could be due to a direct effect on oxygen use or to respiratory discomfort associated with exercise in an atmosphere containing a high concentration of oxidants. A statistical test for threshold values, based on segmental regression analysis ("hockey stick" functions) applied to these data (Barth et al., 1971; Hasselblad et al., 1976) gave a threshold estimate of 240 μg/m³ (0.12 ppm) (1-h value) with a 95% confidence interval of 134 to 326 μg/m³ (0.067–0.163 ppm).

[a] The term hockey stick function has been used by Hammer et al. (1974) and Hasselblad et al. (1976) to describe a function consisting of a curve with zero slope up to a certain point and increasing monotonically from that point.

Table 11. Relative increase in headache, eye discomfort, cough, and chest discomfort in relation to photochemical oxidant exposure of student nurses in Los Angeles[a]

Daily maximum 1-h oxidant level		Relative increase in symptom							
		Headache		Eye discomfort		Cough		Chest discomfort	
$\mu g/m^3$	(ppm)	Simple	Adjusted[b]	Simple	Adjusted[b]	Simple	Adjusted[b]	Simple	Adjusted[b]
≤160	(≤0.08)	1.00 (16.4)[c]	1.00 (10.6)	1.00 (8.9)	1.00 (5.2)	1.00 (13.0)	1.00 (9.5)	1.00 (3.5)	1.00 (1.8)
180	(0.09)	0.97	1.00	1.01	1.07	1.02	1.07	0.91	1.05
200–280	(0.10–0.14)	0.95	1.03	0.96	1.13	0.91	0.99	0.97	1.00
300–380	(0.15–0.19)	0.95	1.07	1.12	1.33	0.95	1.02	1.05	0.94
400–480	(0.20–0.24)	0.95	1.08	1.37	1.77	0.89	0.96	0.89	0.89
500–580	(0.25–0.29)	1.01	1.07	1.67	2.15	0.95	1.01	1.03	1.11
600–780	(0.30–0.39)	1.03	1.26	2.37	3.42	1.16	1.23	1.17	1.27
800–1000	(0.40–0.50)	1.02	1.41	3.93	6.11	1.48	1.77	2.00	3.22

[a] From: Hammer et al. (1974).

[b] Excludes all days when the symptom was reported in conjunction with either "feverish", "chilly", or "temperature".

[c] Bracketed figure gives baseline symptom rate as mean daily percentage of symptom reported.

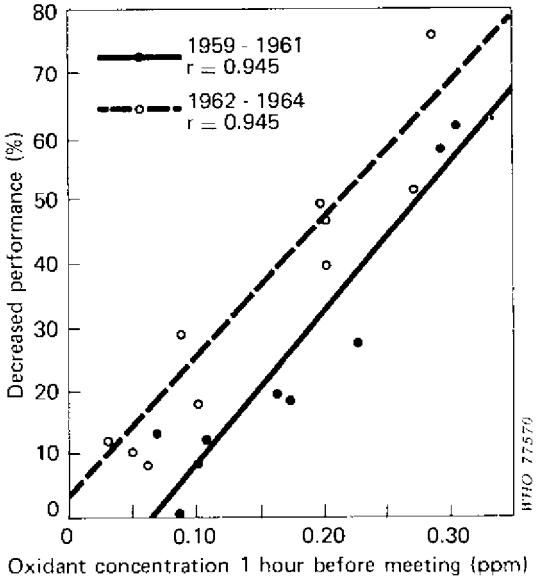

Fig. 9. Relation between oxidant concentration in the hour before the meeting and the percentage of team members with decreased performance. Oxidants, 1 ppm = 2000 μg/m³ (Adapted from: Wayne et al., 1967).

6.3.4 Effects on children

Ventilatory performance, measured twice a month for 11 months by the Wright Peak Flow Meter, in 78 elementary school children in two cities in the Los Angeles Basin was unaffected by differences in the daily mean oxidant levels which were 320 μg/m³ (0.16 ppm) and 220 μg/m³ (0.11 ppm) respectively (McMillan et al., 1969).

Kagawa & Toyama (1975b) and Kagawa et al. (1976) studied the weekly variations in lung function (airway conductance and ventilatory performance) of 20, normal, 11-year-old, school children in Tokyo in relation to variations in temperature and ambient concentrations of ozone, nitrogen dioxide, nitric oxide, sulfur dioxide, hydrocarbons, and particulate matter. Students were tested from June 1972 to October 1973. Ozone levels were determined by the ethylene-chemiluminescence method. Temperature was the factor most highly correlated with variations in specific airway conductance (negative correlation) and maximum expiratory flow rate ($\dot{V}_{max}$) at 25 and 50% FVC (positive correlation). Significant negative correlations between ozone and specific airway conductance, and between nitrogen dioxide, nitric oxide, sulfur dioxide, and particulate matter and $\dot{V}_{max}$ at 25 or 50% FVC were observed in some children. The

ranges of hourly pollutant levels, at the time of the lung function test (13h00), that were used for correlation during the study period were: 0–560 μg/m³ (0–0.28 ppm) for ozone, 40–550 μg/m³ (0.02–0.29 ppm) for nitrogen dioxide, 30–340 μg/m³ (0.01–0.13 ppm) for sulfur dioxide, and 50–490 μg/m³ for particulate matter (Toyama et al., 1977).

The effect of oxidant air pollution exposure on the incidence and duration of A_2 influenza in an epidemic that occurred in 1968–69 was studied retrospectively by Pearlman et al. (1971) in 3500 elementary school children from 5 southern California communities. These areas were selected to represent an exposure gradient for photochemical oxidants, although no difference in community oxidant exposure was present at the time (December 1968–January 1969) of the epidemic. Information from school absenteeism, questionnaires on illness, and haemagglutination-inhibition titres did not reveal any statistically significant morbidity differences corresponding to the pollution gradient that existed during the season of peak oxidant levels.

Several Japanese investigators have reported acute reactions among school children exposed to moderately elevated concentrations of oxidants of 200–400 μg/m³ (0.10–0.20 ppm), on smoggy days in a number of urban areas. Not only eye and respiratory irritation, but systemic symptoms such as paraesthesia, prostration, and convulsions were observed. In reviewing these studies the WHO Task Group was aided by several investigators and observers from Japan. In the opinion of the Task Group, eye and respiratory effects observed during these episodes may well have been caused by reported oxidant concentrations. However, systemic symptoms could not be explained only by the observed oxidant concentrations, but were more likely to be attributable to individual psychosomatic reactions among the students. This judgement is supported by the observation that acute systemic reactions were observed in only some of the students, most of whom were exercising at the time of peak oxidant levels. Thus, the students may well have experienced acute irritation of the pharynx and trachea, enhanced by their active status. However, the general population in the same neighbourhood, including young children in primary grades and school teachers, did not appear to have had these systemic symptoms. These comments are based on the following studies.

Fujii (1972) reported the acute effects of an episode of photochemical smog in Osaka, Japan, on 27 August 1971. The oxidant concentration measured by the NBKI method rose to an hourly value of 360 μg/m³ (0.18 ppm) by 14h00. At this time, the peak ozone concentration, determined by the chemiluminescent method was 480 μg/m³ (0.24 ppm) and the maximum sulfur dioxide concentration was 180 μg/m³ (0.07 ppm). A total of 249 school children reported symptoms including pain in the

throat, headache, coughing, breathing difficulties, eye irritation, and numbness in the limbs. On 14 September 1971, maximum hourly ozone concentrations reached 480 μg/m³ (0.24 ppm) and a total of 290 persons complained of similar symptoms. These data do not provide a basis for estimating symptom frequency in the general population, because the total population at risk was not reported.

In an evaluation of the clinical status of 15 high school children who were hospitalized after the onset of acute symptoms that began during photochemical smog episodes in Osaka, Japan, Adachi & Nakajima (1974) stated that "they were astonished by the seriousness of the conditions which surpassed by far the symptoms previously known in connection with the relation between photochemical oxidant concentration and its effect on man in the case of Los Angeles". Affected students were not only found to have irritation of the eyes and respiratory tract, but, systemic symptoms such as paraesthesia, muscle spasms as well as an increased leukocyte count and borderline elevation of serum alkaline phosphatase (3.1.3.1).

Based upon complaints found in schools that were registered with local health centres located in Japanese cities, the Japan Public Health Association (1976) reported a significant increase in subjective complaints, particularly eye irritation, when photochemical oxidant concentrations exceeded hourly values of 300 μg/m³ (0.15 ppm), as determined by the 10% NBKI method.

Mikami & Kudo (1973) described various local and systemic symptoms in 82 school children that were attributed to 3 photochemical air pollution episodes in Tokyo and Osaka in 1970, 1971, and 1972 with maximum 1-h concentrations ranging from 300 to 580 μg/m³ (0.15 to 0.29 ppm). A large proportion of cases were reported to have eye irritation, throat irritation, cough, breathlessness, headache, and chills. The authors pointed out that many of these symptoms had not been reported under Los Angeles smog conditions.

6.3.5 Effects on the incidence of acute respiratory and cardiovascular diseases

In studies on college students in the Los Angeles Basin, Durham (1974) showed that acute episodes of pharyngitis, bronchitis, and upper respiratory infections were associated with peak concentrations of oxidants and mean concentrations of sulfur dioxide and nitrogen dioxide. Oxidants, sulfur dioxide, and nitrogen dioxide (concentrations not given) were, in this order, consistently associated with the various episodes of illness. A comparison of selected high and low pollution days indicated

that photochemical air pollution might have been responsible for a 16.7% increase in acute respiratory symptoms seen in Los Angeles schools situated in areas of highest concentration compared with those in areas of lowest concentration. The author's method of data presentation did not permit the estimation of dose-response relationships or of threshold concentrations.

Brant & Hill (1964) and Brant (1965) did not find any correlation between admissions for cardiovascular conditions to Los Angeles County Hospital and oxidant levels (170 $\mu g/m^3$; 0.08 ppm as a mean value of daily 7-h measurements for the study periods) either on the day of admission or for 2 weeks prior to admission, for the period between 8 August and 25 December 1954. However, Sterling et al. (1966, 1967) found statistically significant but very low correlations between admissions to Los Angeles hospitals for respiratory episodes during the period 17 March–26 October 1961 and daily mean levels of oxidants (74 $\mu g/m^3$; 0.037 ppm), ozone (84 $\mu g/m^3$; 0.042 ppm), and carbon monoxide (11 mg/m^3; 9.5 ppm). Because of the limited time period and the extremely low correlation coefficients, it is difficult to conclude that a real relationship was demonstrated by these studies.

6.3.6 Effects on the prevalence of chronic respiratory diseases and on pulmonary function

Deane et al. (1965) conducted a survey on the prevalence of chronic respiratory disease in outdoor male telephone workers in San Francisco and Los Angeles. Symptoms of persistent cough and phlegm were slightly less prevalent in Los Angeles than in San Francisco in the 40–49-year group but more prevalent in the 50–59-year group (31.4% compared with 16.3% in San Francisco). These data were adjusted for cigarette smoking. No differences in pulmonary function test results were found.

The prevalence of chronic respiratory symptoms and the results of pulmonary function tests were compared in 2 similar groups of nonsmoking, adult, male and female Seventh Day Adventists aged 45–64 years residing in Los Angeles and San Diego, respectively. Annual mean oxidant values (94 $\mu g/m^3$; 0.047 ppm and 76 $\mu g/m^3$; 0.038 ppm), were essentially the same in the 2 cities, but a mean of maximum daily concentrations in Los Angeles (290 $\mu g/m^3$; 0.144 ppm) was twice as high as that in San Diego (148 $\mu g/m^3$; 0.074 ppm). The survey was performed when oxidant levels were low and similar in both cities, to minimize effects attributable to acute oxidant exposures. No differences were found in symptom prevalence or in several measures of ventilatory function.

Prevalence rates for chronic respiratory disease were uniformly low (less than 4%) in both groups (Cohen et al., 1972).

6.3.7 Effects on patients with pre-existing diseases

6.3.7.1 *Asthma*

The association between reported asthma attacks in 137 patients from Pasadena, California, and oxidant levels during the period 3 September–9 December 1956 was studied by Schoettlin & Landau (1961). Daily records of the time of onset of asthma attacks were kept by each patient and collected weekly. The daily number of patients afflicted with asthma was moderately well correlated ($r = 0.37$) with concurrent maximum hourly oxidant readings. Asthma attacks were more weakly correlated with temperature, relative humidity, and water vapour pressure than with oxidants. The number of patients having attacks on days when maximum 1-h oxidant levels were higher than 500 $\mu g/m^3$ (0.25 ppm) was significantly greater ($p = 0.05$) than the number of those having attacks on days with lower oxidant levels. Unfortunately, the analysis failed to isolate the pronounced seasonal variation of asthma. In the northern hemisphere, asthma attack rates tend to reach a peak in October and November (Booth et al., 1965), and decline sharply in late November and early December. This pattern corresponds closely to seasonal declines in peak oxidant levels. Hence, it is possible that observed asthma-oxidant correlations have been secondary to simultaneous seasonal changes in asthma frequency and oxidant levels.

6.3.7.2 *Chronic respiratory diseases*

Studies over 18 months on 25 patients with severe, chronic, obstructive lung disease at a chronic disease centre in Los Angeles County showed that the prevailing levels of oxidants, oxidant precursors, temperature, and relative humidity did not produce any effects on lung function (Rokaw & Massey, 1962).

In studies on the effects of ambient air pollution exposure on armed forces veterans with chronic respiratory disease living in the Domiciliary Unit and Chronic Disease Annex of the Los Angeles Veterans Administration Center, subjects were evaluated once a week by pulmonary function tests and respiratory symptom questionnaires (Schoettlin, 1962). Analysis of variance did not show any statistically significant effects of air pollution (concentrations not reported) on respiratory symptoms or function, although maximum concentrations of oxidant and oxidant precursors consistently accounted for more of the variation in frequency

of symptoms and clinical signs of disease than maximum temperature, relative humidity, or pollen counts.

6.3.8 Cancer

A 5-year prospective study of lung cancer from 1958 to 1963 was conducted by Buell et al. (1967) among 69 160 members of the California American Legion. Long-term residents of Los Angeles County had slightly lower age-smoking adjusted lung cancer rates (95.4 per 100 000) than residents of the San Francisco Bay area counties and San Diego County (102 per 100 000). These urban groups, in turn, had higher rates than all other California counties (75.5 per 100 000). Nonsmokers followed the same geographical pattern: 28.1 per 100 000 for Los Angeles, 43.9 per 100 000 for San Francisco and San Diego, 11.2 per 100 000 for all others. Smokers of more than one packet of 20 cigarettes a day in Los Angeles had the highest lung cancer rates, 241.3 per 100 000, compared with San Francisco–San Diego, 226 per 100 000, and with other counties, 137.5 per 100 000. The duration of exposure necessary to induce lung cancer may have been longer than the actual ozone exposures in this population study. Thus, it would appear worthwhile to extend these observations to subsequent years.

6.3.9 Motor vehicle accidents

The association between automobile accidents and days of elevated oxidant levels (120–480 $\mu g/m^3$; 0.06–0.24 ppm) in Los Angeles was studied from August to October in 1963 and 1965. Applying a sign-test and nonparametric correlation analysis to the data, Ury (1968) found a statistically significant relationship between oxidant levels and automobile accidents. Concentrations of carbon monoxide, oxides of nitrogen, and other pollutants would also be relatively elevated when oxidants were high and may have contributed to the results reported. Furthermore, the association of accidents with oxidant levels may be confounded by the fact that traffic jams produce both more accidents and a greater output of oxidant precursors.

6.4. Summary Tables

Studies on the health effects of controlled, industrial, and community exposures that provide quantitative information useful for establishing guidelines for the protection of public health with respect to photochemical oxidants are summarized in Tables 12, 13 and 14.

Table 12. Controlled human studies

I. Sensory effects

Ozone concentration		Length of exposure		Effects	Response[a]	Subjects	Reference
µg/m³	(ppm)	days	h/day				
400 700 1000	(0.2) (0.35) (0.5)	1 1 1	3 and 6 3 and 6 3 and 6	Diminution in various measures of visual perception; noneffect dose not determined; no mention of dose-response relationship.	Not applicable	22 healthy males, 6 healthy females	Lagerwerff (1963)
⩾200	(⩾0.1)	1	working hours (study repeated for 123 days)	Increasing eye irritation with increase in oxidant exposures; no apparent effects below 200 µg/m³ (0.1 ppm).	Not applicable	20 women office workers	Richardson & Middleton (1957, 1958)
40–100	(0.02–0.05)			Immediate odour perception after beginning of exposure; odour perception disappeared in ½–12 min; odour considerably stronger at higher dose.	9/10 at 40 µg/m³ 13/14 at 100 µg/m³	10–14 healthy males	Henschler et al. (1960)
15–40	(0.008–0.02)			Odour perception immediately after beginning of exposure; the most sensitive person perceived odour at the level of 15 µg/m³ (0.008 ppm).	Not available	20 healthy subjects	Eglite (1968)

II. Effects on respiratory function

A. *Exposure to ozone*

Ozone concentration		Length of exposure		Effects	Subjects	Reference
µg/m³	(ppm)	days	h/day			
2000	(1.0)	1	1	Consistent increase in airway resistance; exposures at 200, 800, and 1200 µg/m³ (0.1, 0.4, 0.6 ppm) caused effect in some subjects, but no dose-response pattern.	4 healthy males	Goldsmith & Nadel (1969)

[a] Response = $\frac{\text{number of subjects showing effect described}}{\text{total number of subjects}}$

Table 12. Controlled human studies—*continued*

II. Effects on respiratory function
A. *Exposure to ozone* (contd)

Ozone concentration		Length of exposure		Effects	Subjects	Reference
µg/m³	(ppm)	days	h/day			
1800	(0.9)	1	5 min	Highly significant decrease of airway conductance after inhalation with exercise.	4 healthy males	Kagawa & Toyama (1975a)
1200–1600	(0.6–0.8)	1	2	Significant reduction in diffusing capacity of lung and in $FEV_{0.75}$[b]; substernal soreness present in all subjects.	10 healthy men and one healthy woman	Young et al. (1964)
1000	(0.5)	6/wk × 12 wks	3	Significant decrease in $FEV_{1.0}$[c]; no effect at 400 µg/m³ (0.2 ppm).	12 healthy males	Bennet (1962)
1000	(0.5)	1	6	Significant change in airway conductance and pulmonary resistance (dry cough and chest discomfort).	20 healthy subjects (19 men and 1 woman)	Kerr et al. (1975)
1000	(0.5)	1	2	Decrease in pulmonary function measurements.	7 healthy males	Hackney et al. (1975a)
800	(0.4)	1	2 and 4	After 2-h exposure: R_{aw}[d] increase, FVC[e] decrease MMFR[f] decrease; after 4-h exposure: R_{aw}[d] increase, FVC[e] decrease, MMFR[f] decrease and additionally $FEV_{1.0}$[c] decrease; RV[g], FRC[h], and TCL[i] did not change.	22 healthy male subjects	Rummo et al. (1975, unpublished data)
740 & 1500	(0.37 & 0.75)	1	2	Significant decrease in ventilatory function and in closing volume under intermittent exercise; effect more pronounced at higher dose.	6–10 healthy males	Bates et al. (1972), Hazucha et al. (1973)
740, 1000 & 1500	(0.37, 0.50 & 0.75)	1	2	Dose dependent change of ventilatory pattern; significant reduction in MEFR[j] at 50% of vital capacity at 740 µg/m³ (0.37 ppm) or more, under exercise.	28 healthy subjects (20 males and 8 females)	Folinsbee et al. (1975)
740	(0.37)	1	2	Significant increase in total respiratory resistance under intermittent light exercise	2 healthy and 3 sensitive[l] males.	Hackney et al. (1975a)
500	(0.25)	1	2	No consistent changes in lung function.	3 healthy and 3 sensitive[l] males	Hackney et al. (1975a)
200–500	(0.1–0.25)	1	30 min	Tendency towards increase in breathing frequency and volume under exercise.	4 healthy male subjects	Ohmori (1974)
200	(0.1)	1	2	Increase of R_{aw}[d] and $AaDO_2$[k] in 7 of 11 test subjects under intermittent light exercise.	11 healthy male subjects	von Neiding et al. (1977)

B. *Exposure to mixtures of ozone and other air pollutants*

740 + 960 sulfur dioxide	(0.37 + 0.37 sulfur dioxide)	1	2	More pronounced decrease in ventilatory function and in closing volume than in single exposure to ozone at 740 µg/m.^{3} (0.37 ppm).	4 healthy males	Bates & Hazucha (1973)
200 + 9400 nitrogen dioxide	(0.1 + 5 nitrogen dioxide	1	2	Increases in R_{aw}[d] and $AaDO_2$[k] similar to those observed with ozone alone at 200 µg/m^3 (0.1 ppm).	11 healthy male subjects	von Nieding et al. (1977)
200 + 9400 nitrogen dioxide + 13 000 sulfur dioxide	(0.1 + 5 nitrogen dioxide + 5 sulfur dioxide)	1	2	Increases in R_{aw}[d] and $AaDO_2$[k] similar to those observed with ozone at 200 µg/m^3 (0.1 ppm) + nitrogen dioxide at 9400 µg/m^3 (5 ppm); recovery time delayed.	11 healthy male subjects	von Nieding et al. (1977)
50 + 100 nitrogen dioxide + 260 sulfur dioxide	(0.025 + 0.05 nitrogen dioxide + 0.1 sulfur dioxide)	1	2	No effect on R_{aw}[d] and $AaDO_2$[k]; sensitivity of the respiratory tract to acetylcholine increased.	11 healthy male subjects	von Nieding et al. (1977)

C. *Exposure to peroxyacetylnitrate*

1500	(0.3)	1	5 min	Increase in oxygen uptake with exercise; no effect at rest; significant decrease in MEFR[i] during the recovery phase following exercise.	32 college males	Smith (1965)
1350	(0.27)	1	42 min	Minor changes in cardiorespiratory and temperature regulation parameters.	20 healthy male subjects	Raven et al. (1974)

[a] Response = $\frac{\text{number of subjects showing effect described}}{\text{total number of subjects}}$

[b] $FEV_{0.75}$ = 0.75 second forced expiratory volume
[c] $FEV_{1.0}$ = one second forced expiratory volume
[d] R_{aw} = airway resistance
[e] FVC = forced vital capacity
[f] MMFR = mid-maximal expiratory flow rate
[g] RV = residual volume
[h] FRC = functional residual capacity
[i] TLC = total lung capacity
[j] MEFR = maximum expiratory flow rate
[k] $AaDO_2$ = alveolar to arterial oxygen pressure difference
[l] sensitive (subjects) = those with a prestudy history of cough, chest discomfort, or wheezing associated with allergy or air pollution exposure, but with normal base-line pulmonary function studies

Table 12. Controlled human studies—*continued*

II. Effects on respiratory function

D. *Exposure to irradiated automobile exhaust*

Ozone concentration		Length of exposure		Effects	Subjects	Reference
$\mu g/m^3$	(ppm)	days	h/day			
Oxidants 440–540	(0.22–0.27)		Short term (no details given)	No significant changes in reaction time, vital capacity, and submaximum work performance on the bicycle ergometer.	14 healthy subjects	Holland et al. (1968)
Carbon monoxide 17–33 mg/m^3	(15–29)					
Carbon dioxide 1520–2660 mg/m^3	(800–1400)					
Nitric oxide 470–710	(0.38–0.58)					
Nitrogen dioxide 1300–1900	(0.7–1.0)					
Hydrocarbons traces						
Aldehydes	(0.2–0.7)					
Formaldehyde 250–300	(0.2–0.24)					

E. *Exposure to ambient air with an elevated concentration of oxidants*

400–1400	(0.2–0.7)		2–90 h	Improvement in lung function upon residence in clean filtered room for 40 h or longer; threshold concentration not determined.	46 patients with chronic lung disease	Motley et al. (1959)
100–460	(0.05–0.23)	21	24	Decrease in airway resistance and increase in arterial partial oxygen pressure during week of residence in clean filtered air; threshold concentration not determined.	15 patients with moderately severe chronic lung disease	Balchum (1973)

III. Changes in the electroencephalogram

Ozone concentration		Length of exposure		Effects	Response[a]	Subjects	Reference
μg/m³	(ppm)	day	h/day				
10	(0.005)	1	3 min	Decrease in alpha rhythm.	1/3	3 healthy subjects	Eglite (1968)
15	(0.008)	1	3 min	Decrease in alpha rhythm.	2/3	3 healthy subjects	Eglite (1968)
20	(0.01)	1	3 min	Decrease in alpha rhythm.	3/3	3 healthy subjects	Eglite (1968)

[a] Response = $\frac{\text{number of subjects showing effect described}}{\text{total number of subjects}}$

Table 13. Studies on the effects of industrial exposures

Ozone concentration		Averaging time	Effects	Reference
$\mu g/m^3$	(ppm)			
1600–3400	(0.8–1.7)	1 h	11 of 14 welders complained of respiratory symptoms; symptoms disappeared when ozone levels were reduced to 400 $\mu g/m^3$ (0.2 ppm); nitrogen dioxide probably present.	Challen et al. (1958)
600–1600	(0.3–0.8)	1 h	Increased frequency of chest tightness and throat irritation among welders; no complaints at 500 $\mu g/m^3$ (0.25 ppm); welders also exposed to nitrogen dioxide and particulates but concentration levels not given.	Kleinfeld et al. (1957)
500–800	(0.25–0.40)	long-term	Increased frequency of headache, weakness, change in neuromuscular sensitivity, and decrease in memory among workers manufacturing hydrogen peroxide and exposed to ozone for 7–10 years; threshold concentration not determined.	Kudrjavceva (1963)
400–600	(0.2–0.3)	1 h	No evidence for changes in vital capacity or functional residual capacity in welders; nitrogen dioxide probably present.	Young et al. (1963)
80–1000	(0.04–0.50)	long-term	Increased prevalence of bronchitis and emphysema in workers engaged in manufacture of hydrogen peroxide for many years; sulfuric acid aerosols also present; threshold concentration not determined.	Nevskaja & Diterihs (1957)

Table 14. Studies on the effects of community exposures

Hourly oxidant concentration		Effects	Subjects	Reference
$\mu g/m^3$	(ppm)			
500 and above	(0.25 and above)	Increased frequency of asthma; possible confounding of asthma-oxidant association with seasonal effects; other pollutants present but not reported.	137 patients with asthma	Schoettlin & Landau (1961)
~ 1000	(~ 0.50)	Increased symptoms began at hourly concentrations of (headache) 100 $\mu g/m^3$ (0.05 ppm), (eye irritation) 300 $\mu g/m^3$ (0.15 ppm), (cough) 530 $\mu g/m^3$ (0.265 ppm), (chest discomfort) 580 $\mu g/m^3$ (0.29 ppm); threshold levels determined by "hockey stick" functions.	102 student nurses	Hammer et al. (1974)
240 and above	(0.12 and above)	Impaired performance determined by failure to improve running times; threshold determined by "hockey stick" functions.	116 high school cross-country runners	Wayne et al. (1967)
> 300	(0.15)	Increased frequency of complaints, particularly eye irritation.	7440 school children	Japan Public Health Association (1976)
0–560	(0–0.28) (range at time of lung function test)	Specific airway conductance of sensitive subjects was significantly decreased with increasing hourly oxidant concentrations; temperature, nitric oxide, nitrogen dioxide, sulfur dioxide, and particulate matter also showed significant correlations with various respiratory function tests of highly reactive children; threshold concentration not determined.	20 healthy 11-year-old school children	Kagawa et al. (1976); Toyama et al. (1977)

7. EVALUATION OF HEALTH RISKS FROM EXPOSURE TO PHOTOCHEMICAL OXIDANTS

There appears to be sufficient information from experimental and epidemiological studies to justify an attempt to establish guidelines on the exposure limits for ozone and to review those for "oxidants" (as measured by the neutral-buffered potassium iodide method (NBKI)), proposed by a WHO Expert Committee in 1972. The Task Group appreciated the fact that photochemical air pollution contains other substances besides ozone, such as nitrogen dioxide, peroxyacetylnitrate, and possibly many other gaseous and particulate products of atmospheric photochemical reactions. However, present knowledge about the composition of photochemical pollution, the concentrations of individual components, and their possible impact on human health is so limited that no attempt can be made to estimate exposure limits for any single compound other than ozone. The Task Group was, of course, aware that some sensory effects of photochemical air pollution (such as eye irritation) might be due to a large extent, to these poorly defined components of the photochemical oxidant mixture. As nitrogen dioxide is an important air pollutant in its own right, it has been discussed in a separate criteria document (World Health Organization, 1977).

7.1 Exposure Conditions

Exposure of man to ozone must have occurred for millions of years, as ozone, present naturally in higher tropospheric layers, is also found regularly in the lower atmosphere even in completely uninhabited regions like the Antarctic. These natural concentrations have been reported to have values ranging from 10 to 100 $\mu g/m^3$ (0.005–0.05 ppm). It is difficult to determine the proportions of natural to man-made oxidants (including ozone) that occur in rural areas in most countries. In general, ozone concentrations greater than 120 $\mu g/m^3$ (0.06 ppm) are considered to be related to man-made activities. Ozone may be transported over hundreds of kilometres and rural populations may be exposed to the pollutant, which earlier was considered to exist only in urban areas. A characteristic of such exposures is that the precursors have vanished and ozone can therefore persist for days in succession, since it does not come into contact with other pollutants that act as its scavengers.

In large urban areas with strong sunshine and dense traffic or other sources of precursors, photochemical air pollution is a daylight phenomenon with maximum 1-h ozone concentrations sometimes as high as 300-

800 μg/m³ (0.15 0.4 ppm), occurring around noon or somewhat later. Such peak concentrations are preceded by nitrogen dioxide peaks and accompanied by concurrent rises in peroxyacetylnitrate concentrations. In contrast to oxides of sulfur and smoke, ozone exposures are always intermittent, the peak concentrations rarely lasting for more than 2–3 h. In the low temperature season, photochemical reactions are much less likely to occur at rates sufficient to produce large quantities of ozone.

Unless certain industrial technological processes (e.g., welding) are in operation, ozone concentrations indoors tend to be considerably lower than those outdoors due to the presence of reactive surfaces, air conditioning, and indoor smoking.

7.2 Exposure-effect Relationships

Information presented in sections 5 and 6 is sufficient to evaluate the relationship between exposure and the associated effects, at least for some biological changes observed in man and experimental animals.

7.2.1 Animal data

There is a considerable amount of evidence that short-term, prolonged, or repeated exposure to ozone concentrations ranging from 200 to 400 μg/m³ (0.1–0.2 ppm) can cause a variety of biological changes in several animal species and that these effects become more pronounced with higher concentrations and increased exposure time (see Table 10). These effects are, of course, also influenced by other factors such as the animal species, the length of the interval between exposures, the presence of other pollutants, low temperature, and physical activity.

The host's pulmonary defence mechanisms against infectious microorganisms are affected in several animal species by exposure to ozone (section 5.2.6). This may result in rapid multiplication of infectious microorganisms *in situ*, causing disease and eventually death. An increase in mortality, resulting from the joint action of infectious microorganisms and ozone, has been demonstrated in artificially infected mice after a 3-h exposure to ozone at 160 μg/m³ (0.08 ppm). These effects were dose-related.

Pathomorphological changes in the respiratory tract of various animal species, such as the rat, cat, rabbit, and mouse, have been observed at ozone concentrations of about 400 μg/m³ (0.2 ppm) and higher (section 5.2.1). Short-term exposures (up to 24 h) produce oedema, degeneration and destruction of type I alveolar cells, loss of ciliated epithelium, and

breakdown of capillary endothelium. When the exposure is repeated or its length extended, the biological changes become more severe and include emphysema, atelectasis, vascular lesions, bronchopneumonia, and fibrosis.

Various functional changes in the respiratory tract begin at levels of about 520 $\mu g/m^3$ (0.26 ppm) (section 5.2.2). The activities of several enzymes in the lung tissue are also influenced at exposure levels of about 500 $\mu g/m^3$ (0.25 ppm) (section 5.2.3).

After pre-exposure to ozone, animals appear to become tolerant to ozone concentrations that would otherwise cause pulmonary oedema (section 5.2.5). The development of tolerance in small rodents is related to pre-exposure levels of at least 600 $\mu g/m^3$ (0.3 ppm). This tolerance does not protect animals from such effects of ozone as inflammation, alterations in alveolar macrophage functions, and impairment of respiratory functions, that can occur at concentrations lower than those that produce oedema.

Although the primary target for ozone is the respiratory system, a number of studies have indicated that exposure to ozone may also result in some extrapulmonary effects, but the mechanisms of such action are not clear (section 5.3). For example, ozone exposure at 400–500 $\mu g/m^3$ (0.2–0.25 ppm) for less than 2 h produced changes in the circulating lymphocytes (increasing the number of binucleated cells) and increased the number of spherocytes (section 5.3.2.1). It has also been shown that exposure of pregnant mice to ozone at 200–400 $\mu g/m^3$ (0.1–0.2 ppm) for 7 h per day, 5 days per week, for 3 weeks significantly increased neonatal mortality (section 5.3.3).

The available information concerning the carcinogenicity and mutagenicity of ozone is inadequate for the definite evaluation of such effects (sections 5.2.4 and 5.4).

Biological effects produced by the exposure of experimental animals to a combination of ozone and nitrogen dioxide or by exposure to complex pollutant mixtures containing oxidants, such as irradiated automobile exhaust, are generally similar to those produced by exposure to pure ozone. However, one study in which mice were exposed to a mixture of ozone and nitrogen dioxide indicated that the effect (reduction in resistance to respiratory infection) of a single exposure to this mixture was additive, and that repeated exposure to this mixture might result in a synergistic action.

7.2.2 Controlled human exposures

Although limited in number, human volunteer studies with short-term controlled exposure to oxidants have proved useful for establishing

exposure-effect relationships for ozone at levels ranging from about 200–700 μg/m³ (section 6.1). Some of these studies provide evidence of changes in the respiratory function of healthy subjects that are related to exposure. Physical exercise tends to enhance the respiratory effects of ozone (section 6.1.3).

Three investigators found a significant increase in airway resistance with exposure to an ozone level of 740 μg/m³ (0.37 ppm) for 2 h. One of the investigators did not find any effect with a 2-h exposure to a level of 500 μg/m³ (0.25 ppm). However, this particular study was conducted on subjects from southern California who were later found to be less sensitive to ozone. Another investigator, using similar test conditions, found a significant increase in airway resistance in 7 out of 11 subjects at an ozone level of 200 μg/m³ (0.1 ppm) for 2 h (section 6.1.3.1).

In two studies, an improvement in lung function was noted when patients with chronic pulmonary disease breathed filtered air for 40 h or more, as compared with unfiltered ambient air. The ambient air concentrations of oxidant in the two studies ranged from 400–1400 μg/m³ (0.2–0.7 ppm) and up to 400 μg/m³ (0.2 ppm) respectively (section 6.1.3.5).

Result of a 7-month study in which female employees were exposed to unfiltered ambient air during office hours suggested that the lowest 1-h oxidant level that could be associated with eye irritation was about 200 μg/m³ (section 6.1.2).

Although *in vitro* studies using human cells have shown some evidence of a joint action of ozone and nitrogen dioxide, this has not been clearly demonstrated in *in vivo* studies. For example, whereas a potentiated increase in airway resistance was observed with exposure to a combination of ozone at 740 μg/m³ (0.37 ppm) and sulfur dioxide at 960 μg/m³ (0.37 ppm) for a period of 2 h, it was not observed with exposure to a combination of ozone at 500 μg/m³ (0.25 ppm) and nitrogen dioxide at 560 μg/m³ (0.30 ppm). Exposure to ozone at 50 μg/m³ (0.025 ppm) combined with nitrogen dioxide at 100 μg/m³ (0.05 ppm) and sulfur dioxide at 260 μg/m³ (0.1 ppm) did not have any effect on airway resistance; however, this combined exposure resulted in enhancement of the bronchoconstrictor effect of acetylcholine (section 6.1.3.2).

7.2.3 Industrial exposure

Acute symptoms of chest tightness, irritation of the throat, and coughing have been documented in welders exposed to 1-h ozone levels of 600–1600 μg/m³ (0.3–0.8 ppm). These symptoms disappeared when ozone concentrations fell to 500 μg/m³ (0.25 ppm) or less. Possible chronic effects of repeated occupational exposure to ozone are not well documented,

although one investigator reported that workers exposed to ozone levels in the range of 500–800 μg/m³ (0.25–0.40 ppm) for 7–10 years had an increased frequency of headaches and weakness, increased muscle excitability, and impaired memory (section 6.2).

7.2.4 Community Exposure

Several studies have shown more frequent eye irritation, reduced athletic performance, changes in lung function of children, and increased frequency of asthma attacks, all of which have been associated with changes in hourly oxidant levels (sections 6.3.2, 6.3.3, 6.3.4, 6.3.7). As in all studies of the effects of community exposures, it is difficult to determine precisely the lowest level at which adverse effects become manifest. However, most of these effects were observed when 1-h oxidant levels were in the range of about 200–500 μg/m³ (0.1–0.25 ppm). Although other pollutants such as nitrogen dioxide, particulate matter, and sulfur dioxide were simultaneously present, the strongest correlation of the observed effects was with hourly levels of photochemical oxidants.

On the other hand, there is no evidence, so far, that long-term exposure to photochemical oxidants at levels currently present in urban air is associated with increased mortality (section 6.3.1), and there is no evidence that chronic respiratory diseases such as bronchitis, emphysema, and lung cancer are more prevalent in communities with high oxidant exposures (section 6.3.6). However, it should be pointed out that the number of epidemiological studies concerned with such associations is small.

7.3 Guidelines on Exposure Limits

The exposure-effect relationships discussed in section 7.2 make it possible to draw the following conclusions concerning the exposures to oxidants and ozone at which the effects in man begin to appear:

(*a*) There is presumptive evidence from one controlled exposure study that some effects on the lung function of healthy human subjects might occur with exposure to an ozone level of 200 μg/m³ (0.1 ppm) for 2 h.

(*b*) There is also evidence from general population studies that suggests that 1-h ambient oxidant levels in the range of about 200–500 μg/m³ (0.1–0.25 ppm) may affect lung function in children, increase the frequency of asthma attacks, cause more frequent eye irritation, and reduce athletic performance.

(*c*) There is limited evidence from controlled exposure studies that living in an environment with 1-h oxidant levels within the range of 400–1400 $\mu g/m^3$ (0.2–0.7 ppm) may exert additional stress on patients with chronic pulmonary disease.

(*d*) There is convincing evidence from controlled human exposure studies that airway resistance may be increased in healthy human subjects following exposure to ozone levels of 700–800 $\mu g/m^3$ (0.35–0.40 ppm) for 2 h.

Animal data generally support the results of human studies. However, some effects have been observed in animals at an ozone level of about 200 $\mu g/m^3$ (0.1 ppm) or even less, which have not yet been demonstrated in man. For example, in animals, short-term exposures to such concentrations appear to reduce resistance to pulmonary infections.

The role of ozone and other photochemical oxidants in the etiology of cancer is not clear. The only available epidemiological study did not indicate any association between exposure to oxidants and the risk of lung cancer, and experimental studies on the carcinogenicity and mutagenicity of ozone in animals are not adequate for evaluation. Nevertheless, the Task Group felt that there may be reason for concern about the possible carcinogenicity of ozone (based primarily on some biochemical considerations regarding the mechanism of the biological effects of ozone). This aspect of its toxicity should be kept under continual surveillance.

On the basis of all these considerations, the Task Group agreed that 1-h levels of ozone of 100–200 $\mu g/m^3$ (0.05–0.1 ppm) (measured by the chemiluminescence method) could be used as a guideline for the protection of public health. The relatively high natural concentrations of ozone precluded the use of any safety factor.

The Task Group also agreed that a 1-h maximum level of 120 $\mu g/m^3$ (0.06 ppm), which is approximately the highest natural background concentration of oxidants, would be the best single value estimate of the exposure limit for oxidants in the ambient air. This level is in agreement with the long-term goal for photochemical oxidants (as measured by the NBKI method) proposed by a WHO Expert Committee (World Health Organization, 1972).

The issue was raised as to whether the proposed guideline was realistic in view of natural exposure levels and the long-distance transport of ozone. In response to this question, the Group expressed the view that every effort should, nevertheless, be made to develop control strategies for achieving the proposed guideline or at least, for not exceeding it more than once a month.

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