

Health risk evaluation and recommended guideline values for ozone

Summary and health risk evaluation

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

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

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

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

Metabolism

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

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

Animal studies

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

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

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

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

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

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

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

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

Controlled human studies

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

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

whether ozone potentiates the allergic reaction in humans.

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

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

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

Epidemiologic studies

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

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

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

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

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

Cancer

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

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

Recommended guideline values

Short-term exposure

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

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

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

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

Long-term exposure

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

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

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

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

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

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