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EFFECTS OF LONG-TERM EXPOSURE TO LOW LEVELS OF OZONE: A REVIEW

C. E. Melton

Civil Aeromedical Institute
Federal Aviation Administration
Oklahoma City, Oklahoma

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16. Abstract

Available literature regarding long-term effects of ozone on animals and humans is reviewed. Emphasis is placed on reports that have appeared since 1976, but some earlier reports are cited for completeness and perspective.

This review shows that ozone concentration is more important than is duration of exposure in determining the effectiveness of an ozone exposure (dose). This conclusion calls into question the validity of the Time-Weighted Average (TWA) as an index of severity of ozone exposure. The literature review further reveals that there is wide variation in susceptibility of different animal species to ozone, making it difficult to apply results of animal experiments to humans. It further appears that a dose of ozone that is acutely innocuous is also innocuous over the long term.

The effects of a symptom-producing dose of ozone are initially cumulative for the first two or three exposures, then an adaptive response may ensue that involves a plateau of response or even a reversal. These effects are shown by both animals and humans. The mechanisms are unknown.

Ozone probably causes damage by free radical formation. Free radical scavengers such as vitamins E and C may provide protection against ozone damage.

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EFFECTS OF LONG-TERM EXPOSURE TO LOW LEVELS OF OZONE: A REVIEW

The literature regarding the effects of ozone on living systems is mountainous. Most of this literature relates to the effects of urban/industrial air pollution (mixed gases plus particulates) on humans, animals, and vegetation. Much of the literature is concerned with industrial uses of ozone. All of this literature is, to some extent, pertinent to the subject of this review, but for obvious reasons it cannot all be cited. To do so would be to defeat the purpose of a review, which is to collect and clarify information about one segment of a larger question. This review, then, will be concerned with information related to long-term exposure of humans and animals to low levels of ozone.

Having set these limits, it then becomes incumbent on the reviewer to define "long term" and "low level." The definitions will be based on exposures that would be expected to occur in airline operations. Such exposures would be intermittent rather than continuous and the levels of ozone encountered in flight would ordinarily not be greater than about 2,000 $\mu g/m^3$ ($\sim$1.0 part per million by volume - ppmv at sea level) (33). The duration that qualifies for "long term" will be considered to be any exposure, continuous or intermittent, that goes on for days rather than hours. Not all the experiments cited in this review are directed at effects of low levels of ozone or effects of long-term exposure; however, there are aspects of each of these experiments that bear on the question of effects of chronic exposure to low levels of ozone.

A "dose" of ozone is defined as the product of concentration (C) of the gas and the duration of exposure (T). This concept has led to the formulation of the "Time-Weighted Average" (TWA) as an index of the severity of exposure. The TWA is calculated according to the following equation (1):

$$TWA = \frac{\Sigma C_i T_i}{\Sigma T_i}$$

Where: C_i = Concentration of the ith sample
T_i = Sampling time of the ith sample
i = Any one sample during the time to which the TWA applies.

The concept on which this formula is based assumes that sampling periods (e.g., 10 min) rather than continuous measurement will be used. The sum of the CxT values thus obtained is divided by the total sampling time to give the TWA. When the TWA is used in conjunction with a peak limit, harmful exposures should be prevented. The TWA alone only tells, at the end of exposure, what the average exposure was.

It is clear from the literature that concentration is more important than duration of exposure in causing ozone damage. Freeman et al. (12) exposed dogs to toxic levels of ozone for various periods of time after which the dogs were sacrificed and their lungs and respiratory passages examined histologically.

The number of macrophages in random microscopic fields along the terminal airways were related to ozone level. Dogs that were exposed to 1.0 ppmv for 16 h (16 ppmv-h) showed less than half the response (5 cells/field) shown by dogs exposed to 2.0 ppmv (13 cells/field) for 8 h (16 ppmv-h). Likewise, dogs exposed to 1.0 ppmv for 24 h (24 ppmv-h) showed about half the response of dogs exposed to 3.0 ppmv for 8 h (24 ppmv-h). This work shows that equal doses (CxT) did not produce equal responses; ozone level determined the extent of damage.

Horvath and his associates (21) exposed healthy young men to various levels of ozone in an atmospheric chamber. The subjects underwent various levels of exercise while being exposed. The level of ventilation produced by exercise was not as important as was ozone concentration in predicting the degree of pulmonary function alteration. The effective dose was calculated as the product of ozone concentration, ventilation volume and time of exposure. With the exception of respiratory frequency, the majority of the variance was explained by ozone concentration rather than by ventilation during the exposure.

Silverman and others (36) exposed 28 young healthy adults (20 men, 8 women) to 0.37, 0.50, and 0.75 ppmv ozone for 2 h together with exercise calculated to increase pulmonary ventilation 2.5 times. Effective dose was calculated as the product of concentration, duration of exposure and ventilation. They found that exposure to a high concentration of ozone for a short period had more effect than did a lower concentration for a longer period, thus implying that there is not only a threshold effective dose, but also a threshold effective concentration.

Coffin, Gardner, Sidorenko, and Pinigin (7,14) exposed white mice and rats to benzene, SO_2 and NO_2 and demonstrated for NO_2 exposure only that brief peak levels were more toxic than was continuous exposure to lower levels, thus indicating that the prime importance of concentration of an irritant gas is a phenomenon not confined to ozone.

Intuitively, it can be seen that concentration of ozone should be more important in causing damage than would duration of exposure. In order to reach the cells lining the respiratory passages, ozone must diffuse through the surface mucus. The diffusion gradient will determine how rapid and how deep penetration will be; thus, the mass transfer of ozone into respiratory tissue will depend on the concentration at the mucous surface. An equation that defines the rate of movement of a gas from the lumen of the respiratory tract to the tissue is as follows (24):

$$\frac{dm}{dt} = -KgA\ (C_1 - C_2)$$

Kg is the mass transfer coefficient in centimeters per second, A is exposed airway surface, C_1 is the ozone concentration in the airway lumen and C_2 is the concentration of ozone in the airway wall. The expression $(C_1 - C_2)$ is the concentration gradient. Because ozone is rapidly destroyed in tissue, a steady-state diffusion profile will quickly develop and penetration of the gas into the airway wall will depend primarily on C_1. Implicit in this line of reasoning is the idea that ozone is evenly distributed and held at a constant concentration in the air of the bronchiole, alveolar duct or alveolus--probably by a valid

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assumption. Accordingly, no matter how long a small mass of ozone acts, beyond some undefined minimum time probably measurable in seconds or minutes, the response or damage would not be increased because depth of penetration into the tissue would not be increased. Stated another way, effect is primarily dependent on the partial pressure of ozone in the airway gas.

This principle was again demonstrated by Watanabe, Frank, and Yokoyama (39) on anesthetized, paralyzed, mechanically ventilated cats. Ozone was administered through a tracheal cannula in concentrations of 0.26, 0.50, and 1.0 ppm. The duration of exposure to ozone ranged from 2.6 to 6.5 h. A fourfold increase in concentration of ozone produced a fivefold increase in lung resistance at 1 h and 4 h.

The operational meaning of the predominant importance of ozone concentration over duration of exposure is that exposure to an ozone concentration that is acutely innocuous is also innocuous over the long term. As far as pulmonary effects and subjective symptoms in healthy humans are concerned, concentrations at or below 0.25 ppmv appear to lie below the threshold for such effects. In experiments on human subjects in this laboratory, 0.25 ppmv for 4 h with exercise had no effect; whereas, 0.3 ppmv for 3 h with exercise had effects on all subjects (20).

An extensive literature review on ozone effects was prepared by the American Society for Testing and Materials (ASTM) (1). The appendix to this document cites instances of human exposure and shows only one case of a biological effect of ozone, other than odor, below 0.30 ppmv. These observations emphasize that below ozone concentrations of about 0.30 ppmv, the concentration gradient from airway lumen to tissue is so shallow that the probability of ozone traversing the mucus covering the respiratory epithelium before it is destroyed is practically nonexistent. This concept has been mathematically modeled by Miller, Menzel, and Coffin (28).

Animal Studies: Animals vary widely in their susceptibility to ozone, thus greatly complicating the choice of surrogates for humans. Such surrogates are important because studies on humans must be limited to exposures that are not damaging and evaluations must be noninvasive. With animals, exposure regimens can be widely variable and organ and tissue studies can be carried out on all subjects.

Mittler et al. (29,30) have shown that LD-50's for different animals vary greatly. They exposed mice, rats, guinea pigs, rabbits, and cats to levels of ozone ranging from about 12.0 to 60.0 ppmv. The 3-h LD-50's were 21.0 ppmv for mice, 21.8 for rats, 51.7 for guinea pigs, 36.0 for rabbits, and 34.5 for cats. The differences in susceptibility could not be accounted for on the basis of body weight or metabolic rate.

Birds exhibit relatively great resistance to ozone. Clamann (6) cites work showing that young turkeys were not killed by 417.0 ppmv ozone for 3 h; pigeons withstood 67.3 ppmv for 3 h; some parakeets died in 25.0 ppmv ozone while others did not; 54.0 ppmv ozone for 3 h was not lethal for canaries.

In a later study, Mittler et al. (30) exposed young rats to "low" concentrations of ozone (0.6-2.4 ppmv) 5 d/wk for 4 wk and compared their mean weight gain with that of control rats. Weight gain was significantly inhibited in the ozone-exposed animals by doses ranging from 0.6 ppmv for 10 d to 2.4 ppmv for 3 d. Exposure of mice to 2.4 ppmv continuously for 10 d caused the deaths of 21 of the 102 animals tested. All effects seen in rats and mice were confined to their lungs. Hemorrhage and pulmonary edema were common autopsy findings.

Stokinger et al. (37) exposed hamsters, rats, guinea pigs, and dogs to ozone levels that, at that time, were considered to be low (0.5-2.3 ppmv; av., 1.0 ppmv) for 6 h/d, 5 d/wk over a total period of 433 d. Rats and guinea pigs showed the highest mortality rate (33 and 30 percent, respectively) associated with ozone exposure. Ozone-exposed hamsters showed a lower mortality rate than did controls (11 percent as opposed to 50 percent) and no dogs, neither controls nor ozone-exposed, died. Mean weight gain was less in ozone-exposed rats than in controls, but the other species showed no significant effects. Examination of the dogs' eyes showed no effect of ozone after 1 year, either ophthalmologically while they were alive or histologically after sacrifice. Arterial blood oxygen saturation in guinea pigs was affected secondary to hypoxia brought on by pneumonia, pneumonitis or bronchiolitis.

There were no effects of ozone on arterial blood oxygen saturation in dogs. Lung pathology was qualitatively similar in all species examined except dogs, with guinea pigs showing the greatest damage. Terminal bronchiolar epithelium was hyperplastic, causing stenosis of tubes. Sloughing of the epithelium and leucocytic invasion were also common findings. Fibrosis extended from the bronchioles into the alveolar ducts. Emphysema was evident. Dogs showed only irritation of the trachea and large bronchi. Stokinger felt that the observed species differences could be accounted for by nasal scrubbing being better in some species than in others and by length of the respiratory tract. Unfortunately, these authors did not relate mortality rate to duration of exposure. Such data would be useful in assessing long-term effects. As it stands, it is not known whether or not most of the deaths occurred early in the experiment, thus qualifying as acute rather than chronic effects.

Later, in a review of ozone toxicology (38), Stokinger summarized the effects of chronic exposure to damaging levels of ozone as being (i) premature aging, evidenced in rabbits by premature calcification of the sternocostal cartilage, depletion of body fat, coarseness of the coat, dull cornea and sagging conjunctivae; (ii) lung-tumorigenesis (adenoma) in a lung tumor-susceptible strain of mice; and (iii) chronic pulmonary effects in guinea pigs, hamsters, rats, and mice. These effects consisted of emphysematous changes, chronic bronchitis, bronchiolitis, and fibrotic changes.

Problems of exposure of spacecraft crews to on-board air pollutants prompted an investigation into the effect of reduced ambient gas pressure on ozone toxicity (23,25). These workers exposed dogs, rats, mice, guinea pigs, and rhesus monkeys for 90 d to 190 $\mu g/m^3$ ($\sim$0.10 ppmv) ozone mixed with either air or 100 percent oxygen at ambient and reduced pressures. Reduced pressure accounted for a lower mortality rate in ozone-exposed animals. However, as with Stokinger's chronic-exposure experiments, these workers did not state when in the 90-d period the animals died. One dog was sacrificed when it was believed to be dying of ozone

exposure. Its lungs were hemorrhagic and edematous. Other animals that died were described as showing signs consistent with ozone poisoning. Monkeys were entirely unaffected by the experiment. In another series of studies involving acute exposure to ozone for 2 weeks, these workers showed that 800 $\mu g/m^3$ (~0.40 ppmv) in air at ambient pressure of 700 mm Hg caused the deaths of 5 out of 5 dogs, 2 out of 4 monkeys, 50 out of 50 rats, 34 out of 40 mice, and 8 out of 8 guinea pigs. Again, the authors did not provide the distribution of deaths over the 2-week period.

Freeman et al. (12) exposed dogs to various levels of ozone for 18 months. Dogs were exposed to 1.0 ppmv for 8, 16, or 24 h/d and others to 2.0 or 3.0 ppmv for 8 h/d. The animals' lungs were examined by light and electron microscopy. The response was dependent on the concentration of ozone; the earliest response (1.0 ppmv for 8 h/d) consisted of macrophages in and around the bronchiolar-ductal region and in adjacent alveoli. Macrophages increased with higher concentrations of ozone and fibrosis appeared. At higher concentrations, peribronchiolar collars of fibroblasts were apparent, reducing the caliber of the small airways.

Bartlett et al. (2) exposed 3- to 4-week-old rats to 0.20 ppmv ozone for 1 month. Ozone did not apparently affect health or lung growth in exposed rats. The ozone-exposed rats were morphometrically indistinguishable from the air-exposed controls. Lung volume, however, was 16 percent greater in the exposed group than in the control group. This overdistension was reflected in increased chord length and in alveolar surface area. The authors speculate that ozone adversely affected characteristics of lung collagen.

Penha and Werthamer (32) exposed tumor-resistant mice to 2.5 ppmv ozone for 120 d. The exposure regimen consisted of 2 h exposures of mice in groups of 10 to ozone-containing air or to air only each day of the study. Histological study of the lungs showed proliferative changes in the tracheal epithelium of ozone-treated mice around the 45th day of exposure. Cellular proliferation in the bronchioles formed papillary projections around the 80th day, becoming more pronounced by the 120th day. Ozone-exposed mice were also more susceptible to mycoplasma infection than were controls. In 120 days postexposure, most lung pathology reversed. The authors concluded that ozone should be added to the list of pulmonary carcinogenic compounds.

The respiratory system of monkeys most nearly resembles that of man; therefore, studies on primates are of special value. Unfortunately, because of acquisition costs and difficulty and expense of housing, few institutions are equipped to carry out studies on primates. Dungworth et al. (10) studied effects of ozone levels representative of those found in the California South Coast Basin on rhesus and bonnet monkeys. Monkeys were exposed to concentrations of ozone ranging from 0.20 to 0.80 ppmv, 8 h/d for 7 d. Companion controls consisted of identically treated monkeys, except that they were exposed to air only. At the end of the exposure period, all animals were killed, their lungs removed and examined histologically. The authors state, "The most obvious and consistent pulmonary lesion in all exposed monkeys occurred in respiratory bronchioles. The extent and severity of damage, but not its nature, varied with the level of

ozone exposure. There was a progressive decrease in amount of damage from proximal to distal orders of bronchioles and the farthest point reached depended on dosage. With 0.2 ppmv, the lesion was largely limited to the proximal generation of respiratory bronchioles whereas with 0.8 ppmv the damage extended to involve, minimally, proximal portions of alveolar ducts." The authors also state that the threshold for detectable biochemical and morphological effect of the same chronic regimen in rats was 0.1 ppmv. They conclude, because the threshold levels in bonnet monkeys was less than 0.2 ppmv, that similar effects would be produced in sensitive people by a level of ozone in the neighborhood of 0.2 ppmv. In rats exposed to 0.2 ppmv ozone for 30 d, the effect reached a peak by the 4th day and leveled off thereafter (35).

Fukase et al. (13) exposed mice for 3 h/d for 4 d to 0.2, 0.5, or 1.0 ppmv ozone and examined their lungs for reduced glutathione (GSH) levels. Experimental and control mice were exercised in a motor-driven rotating cage. Body weight, as percent of control, decreased in the ozone-exposed group as a function of ozone level. Lung weight and GSH level increased with ozone dosage. Exercise increased all three effects of ozone by about three times.

Miller and coworkers (27) exposed mice to aerosols of Streptococcus pyogenes concurrently with and at various times after exposure to ozone (0.08-1.0 ppm). At all levels of ozone, death rates caused by lung infection was enhanced and such enhancement was maximal when the infectious agent was given concurrently with ozone. Nine hours postexposure, the difference mortality (between controls and ozone-treated mice) had fallen to about one-fifth of the mortality at zero time after exposure. While this work does not pertain to long-term exposure to ozone, it does tell something about the persistent effect of ozone postexposure.

In summary, animal studies have shown that: (i) Common laboratory small mammals are more sensitive to ozone than are dogs and monkeys. The reason offered in explanation of this variability includes references to differences in nasal scrubbing, different lengths of the respiratory tubes, different respiratory rates and biochemical differences. (ii) Ozone damage is confined to the pulmonary tree; no primary extrapulmonary effects were described at dosages below 1.0 ppmv. (iii) Ozone adversely affects susceptibility to lung infection and the effect persists for hours after exposure. (iv) Exercise exacerbates the effects of ozone. (v) There is a scarcity of data relating ozone level and duration of exposure to effects of ozone.

Human Effects: Bennett in 1962 (3) was impelled to investigate ozone contamination of jetliner cabins because of complaints about deterioration of latex foam in passenger oxygen masks. He pointed out that little information was available about chronic exposure of people to low concentrations of ozone. He figured that crews flying about 600 h/yr would be in flight conditions likely to involve ozone exposure for about 370 h or about 1 h/d, on the average. Bennett experimentally exposed two groups of men 3 h/d, 6 d/wk for 12 weeks in an attempt to produce conditions similar to those encountered in flight. The men worked together in two offices into which ozone was introduced from ultraviolet generators. The air in one office was 0.2 ppmv (Group A) with respect to ozone and the other was 0.5 ppmv (Group B). Group A subjects complained of no symptoms and experienced 0.66 upper respiratory infection (URI)/subject during the exposure period compared with 0.95 URI/person in a control group. There

were no clinical changes attributable to ozone nor were there any significant changes in forced vital capacity (FVC) or in forced expiratory volume (FEV). Group B experienced no subjective symptoms and had 0.80 URI/person during the experimental period. FVC trended downward, but without statistical significance. FEV was significantly depressed. All parameters returned to normal in 6 weeks.

The experiments of Hackney et al. (17) do not qualify as "chronic" exposure but do yield valuable information regarding dose-response relationships. A group of normal adult men were exposed for 2 h on each of 2 successive days to 0.37 or 0.50 ppmv ozone. Significant effects were found on forced expiratory parameters by 0.50 ppmv. There were no significant effects of exposure to 0.37 ppmv. The interesting comment was made, but not elaborated upon, that "The group changes in physiological measurements did not achieve statistical significance until the second exposure day in many cases, and in most cases the decrements in function were markedly worse on the second day." Hackney offers the opinion that the "zero effect threshold" lies between 0.25 and 0.50 ppmv. Oxidant-induced alterations in blood parameters (several sulfhydryl enzymes and red blood cell (RBC) fragility) took place after 2.75 h of exposure to 0.50 ppmv ozone.

Chaney and coworkers (4) exposed healthy young nonsmoking men to 0.40 ppmv ozone (784 $\mu g/m^3$) for 4 h on each of 4 successive days with and without exercise. Nine blood biochemical parameters were measured including serum glutamic-pyruvic acid transaminase (SGPT), alkaline phosphatase, lactic acid dehydrogenase, RBC cholinesterase, RBC glucose-6-phosphate dehydrogenase (G6PDH), RBC and serum glutathione reductase, serum vitamin E and several serum proteins. No effects of ozone alone or in combination with exercise were found on most of the parameters. Positive effects were found on serum vitamin E, G6PDH and complement C_3 by 0.4 ppmv ozone in combination with exercise.

Effects by day of exposure were inconsistent. For RBC G6PDH the ozone by day by pre-post effect was significant and indicated that heavy exercise was more effective than was light exercise in causing G6PDH levels to vary. Complement C_3 showed a significant ozone by exercise effect; for the heavy exercise group this measurement increased linearly with time.

Folinsbee, Bedi, and Horvath (11) addressed the question of cumulative effects of low levels of ozone on healthy adult men. They used three groups of subjects, each exposed to a different level of ozone 2 h/d for 3 d. Group 1 (10 subjects) was exposed to 0.20 ppmv, Group 2 (10 subjects) to 0.35 ppmv and Group 3 (8 subjects) to 0.50 ppmv. Additionally, two subjects were exposed 2 h/d on 4 consecutive d to 0.50 ppmv ozone. On the days preceding and following ozone exposure, subjects underwent an identical protocol while being exposed to ozone-free air. In this closely controlled experiment these workers showed that there was an increased effect of ozone on all forced expiratory parameters the second day of exposure followed by a decreased effect on the third day of exposure. This pattern of cumulative effect and adaptation was also apparent in the two men exposed for 4 d to 0.50 ppmv. These effects were apparent only for 0.35 and 0.50 ppmv; 0.20 ppmv ozone had no measurable effect either subjectively or objectively, thus reemphasizing that there is a threshold level of ozone in the range of 0.20-0.30 ppmv as well as a threshold dose. These authors also pointed out that lung function was affected by the end of the first hour of

exposure. These observations on human subjects point up some significant facts. (i) The threshold ozone level for healthy people is probably between 0.25 and 0.30 ppmv. (ii) Once the threshold level is exceeded, impediment to airflow through terminal bronchioles ensues quickly—probably in a matter of minutes. (iii) The effect over time is cumulative for at least the first 4 h of exposure. (iv) Adaptation (as discussed in the next section) follows the cumulative effect. (v) The magnitude of the response is proportional to the level of ozone as is the cumulative effect.

Adaptation: It has been known for many years that the magnitude of the response to ozone is not linear with respect to time, but that a response plateau develops after the first few exposures. This phenomenon has been variously called "adaptation," "adjustment," "tolerance," or "resistance." It occurs in animals as well as humans.

Hackney et al. (18) have studied the phenomenon of adaptation in four residents of Southern California and four residents of Toronto and Hamilton, Ontario, Canada, thus providing a comparison of susceptibility in smog-exposed and nonexposed people. Subjects were exposed to purified air and, on another occasion, to 0.37 ppmv ozone in an experimental chamber. Exposure lasted 2 h during which they exercised half the time at a level that produced a doubling of ventilation. Forced expiratory measurements were made every 30 min during exposure and other measurements were made preexposure and postexposure. Two of the Canadian subjects developed clinical illness during exposure with cough, substernal discomfort and upper airway irritation. The other two Canadians showed upper airway irritation; one of these also complained of chest discomfort. Two of the Californians reported upper airway irritation; the other two reported no symptoms. Group means for forced expiratory parameters were not significantly different. Two of the Canadians showed losses in FEV_1, FVC, and total lung capacity (TLC). One Californian showed a small decrease in FEV_1, and the other three showed no changes. Canadians also showed increased RBC fragility as a result of ozone exposure, whereas Californians did not. These experiments indicate a difference in susceptibility to exposure to a low level of ozone between (presumably) ozone-adapted Californians and nonadapted Canadians.

Hackney and others (19) exposed six men to 0.50 ppmv ozone experimentally 2 h/d for 4 d. Five of the subjects showed cumulative effects of ozone on symptoms and forced expiratory parameters for the first 3 d, then showed a reversal on day 4 to values near control values. One subject showed no significant changes from control throughout the experiment.

Douglas, Curry, and Geffkin (8) examined the question of tolerance by exposing mice to a "low" concentration of ozone for 3 h (5.4 ppmv) and then exposing them together with controls to a lethal concentration of ozone (36.9 ppmv). All animals showed effects of the high dose of ozone; all the nonpreconditioned controls died while only 30 percent of the preconditioned mice succumbed. These authors hypothesized that induction of superoxide dismutase by pretreatment with a nonlethal dose of ozone might afford protection against the subsequent dose of ozone, the toxicity of which was allegedly due to the superoxide radical. No such relationship was actually found.

The experiments cited in this section support and extend the idea that exposure to ozone causes some sort of adaptation to occur. The mechanism and full significance of this phenomenon remain obscure.

Protection: Concepts regarding the mechanisms of ozone toxicity are adequately summarized in the National Research Council's publication, "Ozone and Other Photochemical Oxidants" (31). Suffice it to say that the oxidant property of ozone derives from the formation of an oxygen singlet when ozone breaks down. This singlet has an unsatisfied electron orbit and, thus, acts as an avid electron acceptor. In this manner free radicals are produced that themselves act as electron acceptors, causing a free radical chain reaction. Carbon to carbon double bonds in unsaturated lipids are easily oxidized by ozone. Because such lipids are universal components of cell membranes, such oxidations are believed to be one of the prime mechanisms of cellular injury by ozone. Ionizing radiation acts by a similar mechanism; indeed, the toxicities of ozone and ionizing radiation are additive.

Vitamins E and C are free radical scavengers, leading to the idea that these and similar compounds may protect against ozone toxicity. Goldstein et al. (16) claimed that p-aminobenzoic acid (PABA), p-aminohippuric acid, and anthranillic acid protected against ozone toxicity in vitro and in vivo. It was reported that these organic acids protected RBC acetylcholinesterase from damage by 40 ppmv ozone. Also, rats injected with PABA survived almost twice as long as untreated rats when exposed to 15 ppmv ozone. Because these acids are nontoxic and are present in nature, the authors suggested that they may have use in protecting susceptible people from ozone.

Roehm, Hadley, and Menzel (34) theorized that ozone toxicity represented an acceleration of the molecular events seen in vitamin E deficiency. These workers exposed rats continuously to 1.0 ppmv ozone and determined the time for 50 percent survival of the experimental population. Vitamin E supplement in the diet extended survival time to 18.5 d for the experimental group as compared with 8.2 d for controls.

Menzel and coworkers (26) showed that dietary supplementation with 100 or 200 mg of vitamin E prevented the formation by methyl oleate ozonide of Heinz body inclusions in human RBC's in vitro. Methyl oleate ozonide has been proposed as an intermediary in ozone toxicity. Heinz bodies are believed to be polymers of methemoglobin (Fe^{3+} hemoglobin) attached to the inner surface of the RBC membrane.

Giri et al. (15) point out that release of histamine, serotonin, bradykinin, and prostaglandins have been suggested as possible mediators of ozone-induced inflammatory reactions in the pulmonary airways. Rats were exposed for 4 h to 4.0 ppmv ozone. Exposure began 60 to 90 min after treatment with aspirin, indomethacin, hydrocortisone, or heparin. Control rats were treated with equivalent volumes of phosphate buffer. At the end of the exposure period the rats were killed and their lungs and tracheas were removed, weighed wet, dried, and reweighed. The difference in wet and dry weights was taken as a measure of pulmonary edema. Aspirin exacerbated the edematogenic effect of ozone. Aspirin alone (without ozone exposure) had no effect. Hydrocortisone did not affect ozone-induced pulmonary edema. Indomethacin and heparin produced significant reductions in pulmonary edema in ozone-treated rats. The authors interpret their findings to mean that one or more of the prostaglandins are involved in ozone toxicity and that some anti-inflammatory drugs ameliorate the effects of ozone.

Evolution of ethane and pentane when unsaturated fatty acids undergo peroxidation is a fairly common reaction. It takes place, for example, when fats become rancid and it also occurs during the peroxidation of lipids by ozone in vivo. Dumelin, Dillard, and Tappel (9) measured ethane and pentane in expired air of rats on vitamin E-deficient diets and on vitamin E-enriched diets after the rats were exposed to 1.0 ppmv ozone for 1 h. Vitamin E supplementation was associated with significantly decreased amounts of ethane and pentane in the rats' expired air, indicating protection against lipid peroxidation by ozone.

Chow and Kaneko (5) studied the effect of dietary vitamin E on rats exposed continuously for 7 d to 0.8 ppmv ozone. Control rats were kept on a vitamin E-deficient diet for 3 or 4 months; experimental rats were fed the same basic diet but with vitamin E supplementation. Ozone caused a significant increase in activity of glutathione (GSH) peroxidase, pyruvate kinase and lactic dehydrogenase, and a decrease in GSH level in RBC's of vitamin E-deficient rats but not in those that received vitamin E supplementation.

The free radical mechanism of ozone toxicity appears to be emerging as the most acceptable theory. Such toxicity is apparently ameliorated by free radical scavengers, the most common of which is vitamin E.

Conclusions:

The National Research Council has sponsored an excellent review of all aspects of ozone pollution (31) that covers the literature through 1976. The present review has, therefore, been focused primarily on literature that has appeared since 1976, though some papers of an earlier date have been included for perspective and completeness. Not many papers have appeared in the last 4 years that are directly pertinent to long-term effects of ozone, though some of them certainly are pertinent to the problem of ozone contamination of airliner cabins. This literature review does allow certain conclusions to be drawn and points to areas where our knowledge is deficient.

The evidence is clear that there is a threshold level for toxic manifestations of ozone exposure as well as a threshold dose. Dose is defined as the product of ozone concentration and duration of exposure. Of these two determinants, ozone concentration is by far the most important.

Operationally, this may mean that peak concentrations are more important than the duration of exposure in assessing ozone-induced symptoms. For example, brief exposures to 1.0 ppmv may be more effective in causing "ozone sickness" than would be sustained exposure to 0.20 ppmv. Furthermore, the effects of exposures to 1.0 ppmv would not be reversed by subsequent flight segments when there was no ozone present; thus, a time-weighted average may not give an accurate index of exposure without due consideration being given to peak exposures. Federal Aviation Regulations, Part 121.220, limits peak ozone levels to 0.25 ppmv, sea level equivalent. From the available literature one would conclude that this level is innocuous to healthy humans for indefinite exposure. The literature does not adequately address the effect such a level would have on people with various diseases, particularly pulmonary diseases. Asthmatics have been exposed to 0.20 ppmv ozone for 2 h experimentally (22). This dose of ozone caused no measurable changes in pulmonary function of asthmatic subjects, but some blood biochemical changes were ascribed to ozone. No other experiments on humans with other pulmonary diseases are known.

Animal experiments point to increased susceptibility to streptococcal infection during ozone exposure. Thus, one might expect to find a higher rate of illness in people chronically exposed to ozone, but Bennett's experiment (3) on human subjects actually points to the contrary conclusion--that ozone protects against upper respiratory infection.

It is difficult, if not impossible, to draw meaningful conclusions from animal experiments about hazards to humans of long-term exposure to ozone. Laboratory animals differ both quantitatively and qualitatively from humans in their responses to ozone.

Repeated daily exposure to ozone causes a peculiar biphasic response. For the first day or so there is a so-called cumulative effect, a worsening of the response. Then a stabilization period, or even a reversal, occurs when the individual "adapts" to ozone. The mechanism involved in these responses is completely unknown. It could be that the rate of repair increases, that the induction of some unidentified enzyme occurs. or that damage merely attains a steady state with repair. These phenomena show considerable variability and occur in animals as well as in humans. Apparently, the adaptation is permanent and would tend to lessen the hazard of chronic ozone exposure to aircrewmembers.

The consensus favors the free radical mechanism of ozone toxicity. Investigations into the effectiveness of free radical scavengers in mitigating ozone toxicity have not been as extensive as one could wish; however, it seems clear from animal experiments that vitamin E is effective in reducing mortality from high doses of ozone. It seems to be a safe assumption that vitamin E would likewise reduce the toxicity of low doses of ozone and help prevent cumulative effects. Some of the individual variability in susceptibility to ozone may be based on dietary content of free radical scavengers.

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