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Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III of III

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Air Quality Criteria for Ozone and Related Photochemical Oxidants

Volume III of III

National Center for Environmental Assessment
Office of Research and Development
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Preface

In 1971, the U.S. Environmental Protection Agency (EPA) promulgated National Ambient Air Quality Standards (NAAQS) to protect the public health and welfare from adverse effects of photochemical oxidants. In 1979, the chemical designation of the standards was changed from photochemical oxidants to ozone (O_3). This document focuses primarily on the scientific air quality criteria for O_3 and, to a lesser extent, on those for other photochemical oxidants such as hydrogen peroxide and the peroxyacyl nitrates.

The EPA promulgates the NAAQS on the basis of scientific information contained in air quality criteria issued under Section 108 of the Clean Air Act. The previous O_3 criteria document, *Air Quality Criteria for Ozone and Other Photochemical Oxidants*, was released in August 1986 and a supplement, *Summary of Selected New Information on Effects of Ozone on Health and Vegetation*, was released in January 1992. These documents were the basis for a March 1993 decision by EPA that revision of the existing 1-h NAAQS for O_3 was not appropriate at that time. That decision, however, did not take into account some of the newer scientific data that became available after completion of the 1986 criteria document. The purpose of this revised air quality criteria document for O_3 and related photochemical oxidants is to critically evaluate and assess the latest scientific data associated with exposure to the concentrations of these pollutants found in ambient air. Emphasis is placed on the presentation of health and environmental effects data; however, other scientific data are presented and evaluated in order to provide a better understanding of the nature, sources, distribution, measurement, and concentrations of O_3 and related photochemical oxidants and their precursors in the environment. Although the document is not intended to be an exhaustive literature review, it is intended to cover all pertinent literature available through 1995.

This document was prepared and peer reviewed by experts from various state and Federal governmental offices, academia, and private industry and reviewed in several public meetings by the Clean Air Scientific Advisory Committee. The National Center for Environmental Assessment (formerly the Environmental Criteria and Assessment Office) of EPA's Office of Research and Development acknowledges with appreciation the contributions provided by these authors and reviewers as well as the diligence of its staff and contractors in the preparation of this document at the request of the Office of Air Quality Planning and Standards.

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6

Toxicological Effects of Ozone and Related Photochemical Oxidants

6.1 Introduction

A wide range of effects of ozone (O_3) has been demonstrated in laboratory animals (see reviews by U.S. Environmental Protection Agency [1986], Lippmann [1989, 1993], and Graham et al. [1991]). The major research findings are that environmentally relevant levels of O_3 cause lung inflammation; decreases in host defenses against infectious lung disease; acute changes in lung function, structure, and metabolism; chronic changes in lung structure and lung disease, some elements of which are irreversible; and systemic effects on target organs (e.g., liver, immune system) distant from the lung. The research also has served to expand understanding of the mechanisms of toxicity and relationships between concentration (C) and duration of exposure (time [T]). The framework for presenting the health studies of O_3 in animals begins with a discussion of respiratory tract effects and is followed by a presentation of systemic effects and interaction of O_3 with other common co-occurring pollutants. Respiratory tract effects are often interrelated; however, for purposes of presentation, effects on lung inflammation and permeability, host defenses, morphology, pulmonary function, biochemistry, and mutagenic/carcinogenic potential are discussed separately in the main text, drawing correlations where appropriate. This type of organization enables focus on specific effect categories. In the few cases where one study addresses several different categories of endpoints, cross references are made to the appropriate sections of the chapter. Each major section on a specific effect category is followed by a summary for that section. The summary and conclusions section for the entire chapter (Section 6.5) attempts to draw together findings on related endpoints and to highlight key issues, such as the relative importance of exposure concentrations and durations and the identification of potential risk factors.

A purpose of this criteria document is also to describe any key health effects of photochemical oxidants in addition to O_3. Nitrogen dioxide (NO_2) and nitric oxide are the other two primary photochemical oxidants; they have been evaluated recently in another criteria document (U.S. Environmental Protection Agency, 1993). Formaldehyde (HCHO), which is formed photochemically and can be toxic, also has been reviewed recently by the U.S. Environmental Protection Agency (EPA) (Grindstaff et al., 1991). Literature searches did not reveal any animal toxicology inhalation studies of peroxyacetyl nitrate (PAN) since the last O_3 document (U.S. Environmental Protection Agency, 1986). A myriad of other

individual photochemical oxidants are formed in ambient air (Chapter 3), but they have not been investigated by animal inhalation toxicology. The very few publications on the effects of exposures to a mixture of oxidants are summarized in Section 6.4, which discusses pollutant interactions. Therefore, other than in Section 6.4, this chapter does not address other photochemical oxidants. Even so, considering the limited literature within the aforementioned documents, the available evidence from animal toxicology studies shows that O_3 is the most potent of the oxidants for noncancer effects at environmentally relevant concentrations.

The animal toxicology database for O_3 is extremely large, making it necessary to adopt conventions for presenting succinctly the pertinent findings. Priority was placed on analysis of research published after closure of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986); however, for the purposes of broader interpretation, the older literature is very briefly summarized. Generally, only the highlights of the key recent studies and their interpretation are provided here. Confirmatory recent studies are mentioned and presented in the tables. Furthermore, studies having O_3 concentrations 1.0 ppm are highlighted with rare exception (e.g., genotoxicity studies). Genotoxicity studies at O_3 concentrations higher than 1.0 ppm were included to enable coverage of all the specific endpoints, some of which were tested only above 1.0 ppm. In most other cases, however, the 1-ppm cut point allows portrayal of the full array of the effects of O_3 that may occur from ambient air exposure and also avoids the potential for confounding mechanisms that can occur at very high, environmentally unrealistic concentrations. For example, very high levels of O_3 can cause severe pulmonary edema, resulting in types and magnitudes of pulmonary function changes that would not occur in ambient air. In summarizing the literature, changes from control are described if they were statistically significant at $p < 0.05$, rather than citing the probability values for each study. Where appropriate, critique of a statistical procedure is mentioned. A probability value is provided if it aids the understanding of trends observed in a study (e.g., $p < 0.1$).

As stated above, only literature published since the last O_3 criteria document is described in detail here. The earlier findings are summarized to facilitate cross-referencing. For example, in some cases, the older work is presented in overview in the beginning of each main section; in other cases, the overview is at the subsection level. Generally, the newer literature elucidates the influence of different exposure regimens and the mechanisms of several key effects, rather than portrays undiscovered categories of effects. The newer knowledge on molecular and biochemical interactions increases the understanding of mechanisms of effects. For example, it is unlikely that the O_3 molecule itself penetrates the lung and enters the circulation. As another example, the relationship between inflammation measured in tissue and lung lavage assists in the interpretation of lung lavage findings. Information on the immune system suggests that the cell-mediated limb may be more susceptible than the humoral limb. The ability of O_3 to decrease antibacterial host defenses has long been recognized, but only recently have viral defenses been analyzed. Much remains to be learned, but apparently antibacterial defenses are more at risk from O_3 exposure than antiviral defenses. Cellular and interstitial changes in the lungs of O_3-exposed animals were among the very early studies, with newer work adding to a detailed understanding of morphologic lesions in the pulmonary region identified through advanced morphometric procedures. Scientists just recently have begun to study the effects of O_3 on the nose and have discovered epithelial changes, identifying this tissue as a significant target site of O_3. One new body of information concerns the influence of exposure concentration, duration, and pattern. For several endpoints (e.g., increased lung

permeability), under acute exposure conditions, concentration has more impact than exposure duration. The importance of exposure duration is clearly illustrated by the newer chronic studies that show different patterns of effects (compared to acute exposures). The most sensitive indicators appear to be morphological changes (compared to pulmonary function changes) which is consistent with the concept that functional abnormalities follow morphological changes and may not become apparent until a given threshold is achieved. Studies using intermittent exposures (e.g., exposures every day versus every other week or month for equal times) indicate that interrupted exposures can produce equal or, in some cases, enhanced effects compared to uninterrupted exposures, suggesting a cumulative effect. Thus, seasonal "lows" in O_3 do not have benefit in these animal studies. Newer work also has mimicked and extended human clinical studies of repeated exposures. As with humans, the pulmonary function of rats was attenuated with several days of exposure. However, other changes (e.g., cellular) did not attenuate in the rat, illustrating the need for comprehensive evaluations. For the first time, a classical cancer bioassay has been performed with O_3. It helps put some of the earlier genotoxicity and carcinogenicity studies in perspective. This brief identification of the newer additions to the O_3 database is not meant to be a summary of effects; that is the last section of the chapter. Rather, it does show the importance of considering all the literature, not just the newer work in interpreting the effects of O_3. As mentioned, it was not feasible to repeat the 1986 O_3 criteria document herein; this makes it necessary to use both the current and the former document in evaluations.

Animal toxicological studies of O_3 are of major interest because they illustrate a fuller array of effects and exposure conditions than can be investigated in humans. Most experts accept a qualitative animal-to-human extrapolation (i.e., O_3 effects observed in several animal species can occur in humans if causative exposure concentrations, durations, and patterns also occur). However, there is less consensus on an approach to quantitative extrapolation (e.g., the exposures at which effects in animals actually occur in humans). Chapter 8, on extrapolation, provides more information on this topic.

6.2 Respiratory Tract Effects of Ozone

6.2.1 Biochemical Effects

6.2.1.1 Introduction

This section outlines studies designed to identify biochemical targets of O_3, as well as biochemical measurements of antioxidant and microsomal enzyme activities, lipids, and proteins. It should be noted that interpretation of biochemical changes resulting from whole lung measurements is complicated by the heterogeneity in cell type and function present in lung tissue and the changes in cell populations that result from O_3-induced inflammatory cell infiltration and epithelial cell and fibroblast proliferations. The ability to extrapolate from in vitro to in vivo studies and from high to low levels of O_3 is further complicated by an inability to detect biochemical changes in the whole lung when only a small proportion of the lung may be affected by O_3, especially at concentrations of O_3 less than 1 ppm. Interpretation of all biochemical measurements, therefore, needs to take into account the airway sites of O_3 interaction and concomitant changes in cell populations and numbers that take place at times other than the onset of exposure.

6.2.1.2 Cellular Targets of Ozone Interaction

In vitro experiments have indicated that O_3 has the potential to interact with a wide range of different cellular components that include polyunsaturated fatty acids (PUFAs); some protein amino acid residues (cysteine, histidine, methionine, and tryptophan); and some low-molecular-weight compounds that include glutathione (GSH), urate, vitamins C and E, and free amino acids (U.S. Environmental Protection Agency, 1986; Mustafa, 1990; Pryor, 1991, 1992). The mechanisms to explain the initial biochemical and physiological effects of O_3 exposure in vivo are therefore complex. Hypotheses have been developed on the direct action of O_3 with lung macromolecules, the reaction of secondary biochemical products that could result from the generation of free radical-precursor molecules, the release of endogenous mediators of physiological response, and the reactive oxygen intermediates and proteinases associated with the activities of inflammatory cells that subsequently infiltrate into O_3-damaged lungs (see Section 6.2.2). Based on some theoretical calculations, Pryor (1992) hypothesized that, because O_3 is so reactive, it most likely does not penetrate beyond the surface-lining fluids of the lung except in those terminal airway regions having minimal lining thickness where epithelial cells may well be relatively unprotected by either mucus or surfactant. In a review, Pryor (1991) proposed that O_3-induced cell damage more likely results from the reactions of more stable but less reactive ozonide, aldehyde, and hydroperoxide products of O_3 interaction with surface-lining fluid components than from direct interactions of O_3 with intracellular components. Although the alveolar lining fluid is relatively rich in saturated phospholipids, it does contain some lipids with unsaturated fatty acids, cholesterol, a protein A component, and small-molecular-weight compounds (e.g., GSH and uric acid) that have been shown to react with O_3 in both in vitro and in vivo studies (Effros et al., 1990; King and Clements, 1985; Shelley et al., 1984).

Polyunsaturated Fatty Acids

Hitherto, the major products of O_3-lipid interaction that account for cell membrane damage have been assumed to be lipid hydroperoxides. However, evidence for the production of hydrogen peroxide and aldehydes has been demonstrated. It has been proposed that, although Criegee ozonation (Figure 6-1) will ultimately lead to the production of ozonides in a lipophilic environment, in the aqueous environment of lung airways, the carbonyl oxide intermediate can form a hydroxyhydroperoxy compound, which on elimination of hydrogen peroxide yields another aldehyde or, in the presence of iron ions, can form an aldehyde and the very reactive hydroxyl radical (Teige et al., 1974; Pryor, 1991). Ozonation of aqueous emulsions of PUFAs, rat erythrocyte ghost membranes, and rat bronchoalveolar lavage (BAL) fluid has shown hydrogen peroxide and aldehyde generation with a much smaller proportion of ozonides and lipid hydroperoxides (Pryor et al., 1991). A mechanistic study by Santrock et al. (1992) of the ozonation of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine in unilamellar phospholipids confirmed the generation of the hydroxyperoxy compounds, which subsequently result in the generation of hydrogen peroxide and aldehydes with further oxidation to carboxylic acids (Figure 6-1). Similar studies conducted under nonaqueous conditions have demonstrated the production of secondary ozonides that, under physiological conditions, would be expected to decompose rapidly to reactive products (Lai et al., 1990). Madden et al. (1993) have demonstrated recently production of arachidonate-derived aldehydic substances and hydrogen peroxide from in vitro O_3 exposure (0.1 and 1.0 ppm for 1 h) of arachidonate in both a cell-free system and

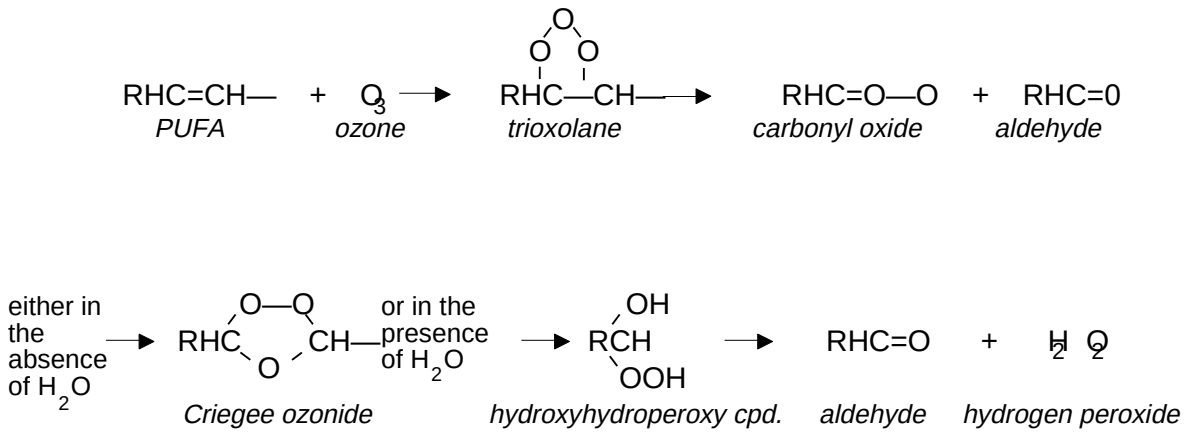

Figure 6-1. Major secondary products of ozone interaction with lung cells.

cultured human bronchial epithelial cells. Ozonides, aldehydes, hydrogen and lipid peroxides, and related reactive oxygen intermediates, together with the phospholipid from which the aldehyde has been removed, represent major products of O_3 interaction with lung cells that all have the potential to cause damage to membranes (see Figure 6-1).

Evidence that interaction of O_3 with PUFAs takes place in vivo has not been so easily obtained. Goheen et al. (1986) investigated the effects of fat-free diets on rats exposed to air or to 0.96 ppm O_3 for 0, 1, 2, and 4 weeks and concluded that O_3 does not oxidize significant levels of the PUFAs linoleate (18:2) and arachidonate (20:4). However, cleavage of lung fatty acid double bonds has been demonstrated in an in vivo study reported by Rabinowitz and Bassett (1988) that involved rat exposures for 4 h to 2 ppm O_3. These authors, by using hydrogen peroxide treatment to convert ozonides and aldehydes to carboxylic acids, were able to demonstrate O_3-induced increases in glutaric and nonanoic acids that are the ozonolysis breakdown products of lung tissue arachidonic and oleic acids, respectively. More recent studies directed towards developing suitable biomarkers and dosimeters for O_3 exposure have analyzed rat BAL lipids after a 12-h exposure to 1.3 ppm and demonstrated the appearance of the aldehydes nonanal and heptanal (Cueto et al., 1992). Pryor et al. (1992) also have been able to identify cholesterol ozonation products extracted from whole lung tissue with the same exposure of rats to 1.3 ppm O_3 for 12 h.

Evidence of the role of hydrogen peroxide in O_3-induced lung damage has been described by Warren et al. (1988), who demonstrated diminished O_3-induced increased BAL protein in rats after 1 day of exposure to 0.64 ppm O_3, when treated with the hydrogen peroxide scavenger dimethylthiourea before exposure. Hitherto, the exhalation of ethane and pentane and tissue measurements of diene-conjugates and thiobarbituric acid reactive substances (TBARS) have been used as evidence for O_3-induced free radical autoxidation of lipids (U.S. Environmental Protection Agency, 1986). However, these measurements have been found to be relatively insensitive for use in inhalation experiments under conditions of low O_3 concentrations (<0.5 ppm). Ichinose and Sagai (1989) were unable to demonstrate any changes in lung TBARS as a result of exposing rats for 2 weeks to 0.4 ppm O_3. As noted by

Pryor (1991), malondialdehyde and other thiobarbituric-acid-reacting aldehydes can be produced by Criegee ozonation of olefinic fatty acids that include arachidonate, as well as by free radical peroxidative processes. In addition, malondialdehyde, being volatile as well as highly reactive, may be lost readily from the lung or during sample preparation. However, measurements of TBARS continue to be used for in vitro experiments designed to demonstrate possible mechanisms by which such agents as taurine (Banks et al., 1991) and uric acid (Meadows and Smith, 1987; Peden et al., 1993) may protect against O_3-induced lipid damage. Rietjens et al. (1987b), by preincubating rat alveolar macrophages (AMs) with either arachidonate (20:4) or phosphatidylcholine to alter PUFA content and membrane fluidity, respectively, demonstrated that PUFA content (not membrane fluidity) determined sensitivity to O_3 damage, measured as decreases in phagocytic activity.

Evidence for free-radical-mediated autoxidation comes indirectly from the demonstration that vitamin E depletion increases O_3 toxicity, as reported previously (U.S. Environmental Protection Agency, 1986) and more recently (Elsayed, 1987; Elsayed et al., 1988). More direct evidence for free radical generation has been obtained using electron spin-trapping technology that correlated increased radical signals in isolated lung lipids from rats exposed to increasing O_3 concentrations (0 to 1.5 ppm, effect beginning at about 0.5 ppm; 2 h) under conditions of carbon dioxide (CO_2)-stimulated respiration (Kennedy et al., 1992). However, the possible contribution of activated inflammatory cell generation of reactive oxygen intermediates to these observed free radical alterations to lung lipids needs to be considered.

Antioxidants

Although vitamin E directly reacts with O_3 at the same rate as PUFAs, vitamin C appears to react more effectively (Pryor, 1991), which, together with intracellular taurine (Banks et al., 1991) and BAL uric acid (Meadows and Smith, 1987; Peden et al., 1993) found in nasal and lung-lining fluids, may act as direct scavengers of O_3. Ozone-induced increases in lung polyamine metabolism in vitamin E-deficient rats suggests their possible role as antioxidants (Elsayed, 1987). Glutathione in its reduced form (GSH) represents another potential direct O_3 scavenger. In addition to being a major intracellular antioxidant, GSH is a component of airway-lining fluids found in BAL. Ozone would have to penetrate the cellular membrane without reaction if it is to directly interact with intracellular GSH, an event considered to be unlikely (Pryor, 1991, 1992). Previously observed oxidation of glutathione and, in some cases, its loss from the lung may more likely reflect its reaction with an O_3-derived oxidant, such as a hydroperoxide or an ozonide, mediated by glutathione peroxidase (GSHPx) and glutathione-S-transferases, respectively (Rietjens et al., 1987a), resulting in the formation of glutathione disulphide or mixed disulphides with sulphydryl (SH)-containing proteins. Although ozone-induced formation of glutathione sulfonate has been reported in vitro, such irreversible oxidation of GSH has not been observed in vivo (U.S. Environmental Protection Agency, 1986; Mustafa, 1990).

Proteins

Early studies reported that nonprotein sulfydryls (NPSHs) and the activities of various cytosolic, microsomal, and mitochondrial enzymes are decreased immediately following short-term exposures to relatively high levels (2 to 4 ppm) of O_3 (U.S. Environmental Protection Agency, 1986; Mustafa, 1990). However, although these early

biochemical effects could not be demonstrated after the first day of exposure to the lower O_3 concentration of 0.8 ppm, the methods employed may not have been sensitive enough to detect coenzyme and enzyme changes in the centriacinar region (CAR), which is a primary target of O_3. However, together with surfactant lipids, the surfactant protein A also has been examined as a potential target of O_3 interaction (Oosting et al., 1991c, 1992). In vitro studies by these authors have suggested that either hydrogen peroxide- or O_3-induced oxidation of methionine and tryptophan residues account for the observed changes in physicochemical properties of canine and human surfactant protein A, measured as an impairment of self-association and a decreased ability to aggregate phospholipid vesicles and to bind mannose (Oosting et al., 1991c). Similar responses were found in vivo; surfactant isolated from rats exposed for 12 h to 0.4 ppm O_3 was less able to stimulate AM superoxide anion generation than surfactant obtained from air-exposed control rats (Oosting et al., 1992). The previously reported presence of giant lamellar bodies in O_3-exposed rat lungs following exposure to 0.3 ppm for 3 h/day for 16 days is also consistent with the hypothesis that O_3 reacts with surfactant protein A (Shimura et al., 1984) and thereby interferes with its homeostatic role in surfactant release from alveolar Type 2 cell lamellar bodies and its subsequent reuptake by Type 2 cells and AMs.

6.2.1.3 Effects of Ozone Exposure on Lung Lipid Metabolism

Arachidonate Metabolites

Ozone-induced damage to airway epithelia (Leikauf et al., 1988) and AMs (Madden et al., 1991) in vitro has been associated with the production of arachidonic acid metabolites by both cyclooxygenase and lipoxygenase pathways. These metabolites have been implicated in a variety of different physiological processes that include changes in airway permeability, infiltration of polymorphonuclear leukocytes (PMNs) and eosinophils, and airway smooth-muscle reactivity, discussed elsewhere in this chapter (Sections 6.2.2 and 6.2.5). Leikauf et al. (1993) have examined the effects of fatty acid O_3-degradation products on human airway epithelial eicosanoid metabolism and concluded that the stimulating effects were increased with product chain length, with the 3-, 6-, 9-hydroxyhydroperoxides being more potent than their corresponding aldehydes. Madden et al. (1993) concluded that aldehydic degradation products of arachidonate, but not hydrogen peroxide, increased in vitro polarization of leukocytes, and decreased peripheral blood T-cell mitogenesis and natural killer (NK) cell cytotoxicity. In vivo experiments on rabbits, guinea pigs, mice, and rats of different ages exposed to 1.0 ppm O_3 have demonstrated increases in the products of arachidonic acid metabolism (see Section 6.2.2).

Surfactant

Although alveolar surfactant lipids purified from lavage fluids have been shown to be relatively enriched in saturated lipids, a varying percentage of the lipids do contain unsaturated fatty acids depending on the species studied (King and Clements, 1985; Shelley et al., 1984). These unsaturated lipids, together with the apoprotein as possible targets of O_3 interaction, may be expected to have an altered composition as a result of O_3 inhalation. However, surfactant-enriched material isolated by BAL from rats following an 8-h exposure to 0.8 ppm O_3 retained its ability to lower surface tension in spite of an increase in protein content (Nachtman et al., 1986). In long-term exposure studies, monkeys were exposed for 8 h/day to 0.15 and 0.3 ppm O_3 for 21 and 90 days (Rao et al., 1985a,b). In contrast to

measurements of total lung lipids that demonstrate a relative decrease in PUFAs after 21 days of exposure (Rao et al., 1985a), there was a relative increase in the proportion of PUFA in the percentage of BAL unsaturated fatty acids (increases from 34% in air controls to 41, 42, and 45% in BAL lipids recovered from monkeys exposed for 21 days to 0.15 ppm, 90 days to 0.15 ppm, and 90 days to 0.3 ppm O_3, respectively) (Rao et al., 1985b). The major increases were observed in linoleate (18:2) and arachidonate (20:4). Because these PUFAs are potential targets for O_3 interaction, their increase, rather than a decrease, in BAL fluid may best be explained by changes in surfactant lipid production associated with alveolar Type 2 epithelial proliferation (Section 6.2.4). Interestingly, a relative decrease in cholesterol ester with a concomitant increase in phosphatidylcholine was observed, which supports the hypothesis that cholesterol may represent a major target of O_3 interaction (Rao et al., 1985b; Pryor et al., 1992). The observed O_3-induced changes in BAL PUFA composition were consistent with those previously reported for rats by Roehm et al. (1972), but only for BAL lipids isolated from vitamin E-depleted rats following 6 weeks of exposure to 0.5 ppm O_3. Wright et al. (1990) were unable to detect changes in BAL lipid and fatty acids recovered from normally fed rats following 0.12-, 0.25-, and 0.5-ppm O_3 exposures for 20 h/day for 18 mo. Results from these studies are summarized in Table 6-1.

Tissue Lipids

In vivo pulse labeling with carbon-14-labeled acetate was used to estimate phospholipid biosynthesis (Wright et al., 1990). Although found to be diminished at certain time points (3 and 12 mo), no consistent trend could be demonstrated that would suggest that O_3 exposures of less than 0.5 ppm alter lung surfactant homeostasis. Bassett and Rabinowitz (1985), using isolated perfused lungs taken from rats after 3 days of continuous exposure to 0.6 ppm O_3, demonstrated an enhanced incorporation of glucose carbons into both fatty acid and glycerol-glyceride moieties of total lung lipids by 180 and 95%, respectively. The relative increase in carbon incorporation into free fatty acids, phosphatidic acid, phosphatidyl inositol, and sphingosine containing lipids was consistent with the needs of a dividing cell population for increased lipids synthesis associated with alveolar epithelial proliferative repair. It should be noted that, in a separate study, under the same exposure conditions of 0.6 ppm O_3 for 3 days, rat lungs demonstrated increased glycolytic activity and generation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) consistent with the energy and synthetic needs of a lung undergoing repair of O_3-induced damage (Bassett and Bowen-Kelly, 1986). Results from these studies are summarized in Table 6-1.

6.2.1.4 Effects of Ozone on Lung Antioxidant Systems

The O_3-induced increased levels of the antioxidant NPSHs, identified mainly as GSH in the lung, and the enzyme activities involved in GSH utilization, GSHPx and glutathione-*S*-transferase (GST), and for maintaining GSH in a reduced state, glutathione reductase (GR) and the NADPH-linked dehydrogenases of glucose-6-phosphate (G6PD) and 6-phosphogluconate (6PGD), typically have been attributed to concurrent morphological changes rather than to any specific biochemical response (U.S. Environmental Protection Agency, 1986). Numerous studies conducted in mice, rats, and monkeys show increases in many of these enzyme activities at exposures as low as 0.2 ppm O_3 for 1 week (rat) (U.S. Environmental Protection Agency, 1986). The earlier research also included studies of age-dependent responsiveness of rats (Tyson et al., 1982; Lunan et al., 1977; Elsayed et al.,

1982). Rats ranging in age from 5 to 90 days old were exposed to 0.8 or 0.9 ppm O_3 for 3 or 4 days or for about 20 days, depending on the experiment. Ozone altered activities of antioxidant enzymes in an age-dependent manner. Generally, prior to weaning, enzyme

Table 6-1. Effects of Ozone Exposure on Lung Lipids[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.25 0.5	235 490 980	20 h/day, 7 days/week for 18 mo	Rat, M (F344) 28 days old	Age-related increase in BAL and tissue phospholipids generally unaffected by O_3 exposure; at 0.5 ppm, total phospholipid increased at 6 and 12 mo.	Wright et al. (1990)
0.15 0.3	353 588	8 h/day for 90 days	Monkey (Bonnet)	Fraction of total lung lipid fatty acids that were PUFAs decreased from 22 to 9% and 6% following 0.15-ppm and 0.3-ppm exposures, respectively.	Rao et al. (1985a)
0.15 0.3	353 588	8 h/day for 21 and 90 days	Monkey (Bonnet)	BAL PUFAs (linoleate [18:2] and arachidonate [20:4]) increased, with a relative decrease in cholesterol esters.	Rao et al. (1985b)
0.5 0.6	980 1,176	Continuous for 0-4 weeks	Rat, M (S-D) 50 g	No change in lung fatty acid content; no acceleration of essential fatty acid deficiency in rats on fat-free diet.	Goheen et al. (1986)
0.5 1.0 1.5 2.0	980 1,960 2,940 3,920	2 h	Rat, M (CD) 65-85 days old	Extracted lung lipid EPR signal intensity proportional to O_3 concentration following pretreatment with spin-trapping agent and CO_2 stimulation of respiration in vivo.	Kennedy et al. (1992)
0.58	1,137	Continuous for 3 days	Rat, M (Wistar) 220-250 g	Increased lipid synthesis associated with increased glucose catabolism for ATP and NADPH generation.	Bassett and Bowen-Kelly (1986)
0.6	1,176	Continuous for 3 days	Rat, M (Wistar) 220-250 g	Increased synthesis of perfused lung glyceride-glycerol and fatty acid moieties of neutral lipids and phospholipids from glucose carbons, with a greater proportion of shingosine and inositol synthesis.	Bassett and Rabinowitz (1985)
0.8	1,568	18 h	Rat (F344) 260 g	Increase in BAL protein; no alteration in surface-tension-lowering ability of BAL.	Nachtman et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

activities decreased, and, at older ages, they increased. The reasons for these differences are not known, but may be due to differences in (1) dose of O_3 to the lung (due to differences in exposure concentrations in huddled neonates on bedding prior to weaning or to differences in doses delivered to lung target sites), (2) basal levels of antioxidants and antioxidant enzymes, or (3) cellular sensitivity. Increased lung enzyme activities can result from either increased activity within a particular cell population or increased numbers of cells with that activity. Age, nutritional, and species differences in O_3-mediated responses must therefore be interpreted with consideration of the underlying morphological changes (Section 6.2.4). Relevant studies are summarized in Table 6-2.

An increase in lung alveolar Type 2 cells and in infiltrating inflammatory cells adequately explained the observed increases in succinate oxidase, G6PD, and 6PGD activities observed after 3 days of continuous exposure of rats to 0.75 ppm O_3 when represented on a per-milligram-deoxyribonucleic-acid (DNA) basis (Bassett et al., 1988a). These cell types are enriched in mitochondria and in NADPH-generating capacity, needed for both lipid biosynthesis and GSH maintenance. Similarly, no significant changes in these enzyme activities could be detected after 3 days of exposure to the lower O_3 concentration of 0.35 ppm, further illustrating the need to take into account the concomitant changes in cell population and number when interpreting whole-lung enzyme measurements. Increases of 150 and 108%, respectively, were observed in the per-milligram DNA activities of the ornithine carboxylase and *S*-adenosyl-methionine decarboxylase enzymes involved in polyamine synthesis, which, together with enhanced tritiated thymidine incorporation into DNA, have been considered to be more sensitive measures of biochemical changes in lungs of rats exposed continuously for 3 days to 0.45 ppm O_3 (Elsayed et al., 1990).

The potential role of superoxide dismutase (SOD) and catalase in protecting the lung against O_3 toxicity is not clear. Bassett et al. (1989), using a pretreatment with a phenyl-urea compound (*N*[2·(2-oxo-1-imidazolindinyl)ethyl]-*N*′-phenylurea; EDU) that increased rat lung SOD and catalase activities, failed to demonstrate any protection against acute lung injury from a single 3-h exposure to 2.0 ppm O_3. However, Zidenberg-Cherr et al. (1991) have demonstrated that copper (Cu)- and manganese (Mn)-deprived mice may be more susceptible to continuous O_3 exposure of 1.2 ppm for 7 days. Rahman and Massaro (1992) have demonstrated protection against edemagenic exposures to ozone (2.5 ppm for 24 h) in rats pretreated with endotoxin. Endotoxin pretreatment is associated with increases in lung tissue mitochondrial Mn-SOD activity without any concomitant increases in catalase, GSHPx, and the cytosolic Cu,Zn-SOD enzymes. Although it is difficult to conclude that mitochondrial SOD might directly protect against O_3 interactions, these results do suggest a central role of mitochondrial SOD in the protection of the cell against oxidative stress (Rahman and Massaro, 1992).

Rahman et al. (1991) also have demonstrated that lungs from O_3-exposed rats had increased activities of Cu, Zn-SOD, Mn-SOD, catalase, and GSHPx after 5 days of exposure to 0.7 ppm O_3. These increases were attributed to enhanced gene expression, indicated by higher messenger ribonucleic acid (mRNA) concentrations, rather than to the infiltration of cells enriched with these enzyme activities. Chronic exposure of rats to an urban pattern of O_3 for 12 mo did not affect total SOD activity in rats, although GSHPx and GR activities per lung were increased (Grose et al., 1989). Use of microdissection techniques following 90 days and 20 mo of rat exposures to 0, 0.5, and 1.0 ppm O_3 have shown concentration-dependent

increases in SOD, GST, and GSHPx per milligram of DNA in the distal bronchioles. In contrast, decreases in GST and GSHPx activities in major bronchi and

Table 6-2. Effects of Ozone Exposure on Lung Antioxidants[a]

Ozone Concentration ppm	Ozone Concentration g/m^3	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, 0.25 spike	118 base, 490 spike	Base 13 h/day, 7 days/week; ramped spike 9 h/day, 5 days/week for 12 mo	Rat, M (F344)	Whole lung increase in GSHPx and GSH reductase activities. SOD activity and NPSH content not affected.	Grose et al. (1989)
0.12 0.2 0.64	235 392 1,254	Continuous for 7 days	Rat, M (S-D) 250-300 g	Pretreatment with the H_2O_2 scavenger dimethylurea decreased O_3-induced tissue DNA and protein and BAL protein, acid phosphatase, and *N*-acetyl-β-D-glucosaminidase. No effect of vitamin E or β-carotene.	Warren et al. (1988)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 90 days or 20 mo	Rat, M and F (F344)	Using microdissection techniques and representing data as units/mg DNA, GST, GSHPx, and SOD were increased in distal bronchioles after 90 days and 20 mo in a concentration-dependent fashion. After 90 days, SOD and GST were lower in major daughter bronchi. After 20 mo, SOD was increased in distal trachea; GSHPx was decreased in major bronchi but enhanced in minor bronchi; and GST decreased in major bronchi.	Plopper et al. (1994b)
0.35 0.75	686 1,470	Continuous for 3 days	Rat, M (Wistar) 200-250 g	0.75 ppm O_3-induced whole lung increases in GSHPx and GR not significant when corrected for increases in cell number. Increases in succinate oxidase, G6PD and 6PGD activities per mg DNA were consistent with increased Type 2 and inflammatory cell content. No increases per mg DNA at 0.35 ppm O_3.	Bassett et al. (1988a)
0.4	784	Continuous for 2 weeks	Rat, M (Wistar) 6 weeks old Guinea pig (Hartley) 6 weeks old	Small increases in whole rat lung levels of NPSH, vitamin C, GSHPx. Guinea pig GSHPx and GSH transferase activities decreased.	Ichinose and Sagai (1989)
0.41	800	12 h during day or night for 3 days or continuous for 72 h	Rat, M (Wistar) Guinea pig, M (Hartley) 9 weeks old	Rats: No effect of daytime exposure. Nighttime or continuous exposure increased activities of LDH, G6PD, GR, and GSHPx. Guinea pig: No daytime-only exposure. No effect on GR or GSHPx, G6PD increased after nighttime or continuous exposure; lactate dehydrogenase activity increased only after continuous exposure.	Van Bree et al. (1992)
0.45	882	Continuous for 2 days	Rat, M (S-D) 90 days old	Large increase in ornithine decarboxylase activity and DNA labeling reflecting polyamine metabolism and DNA synthesis and/or repair, respectively.	Elsayed et al. (1990)
0.5	980	Continuous for 5 days	Rat (Long-Evans) 10 weeks old	Ozone increased lung putrescine in both vitamin E-deficient or 1,000 IU/kg groups, but increases in spermidine content and decarboxylase activities of ornithine and *S*-adenosylmethionione only in vitamin E-deficient group.	Elsayed (1987)

Table 6-2 (cont'd). Effects of Ozone Exposure on Lung Antioxidants[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.5	980	Continuous for 5 days	Rat (Long-Evans) 10 weeks old	Ozone increased lung vitamin E level in supplemented rats and remained unchanged in all other tissues measured.	Elsayed et al. (1990)
0.5	980	2.25 h/day for 5 days	Rat, M (F344) 110 days old	Lung GSH initially enhanced, declining to control levels by Day 4. Lung ascorbate levels enhanced on Days 3 and 5 only.	Tepper et al. (1989)
0.64	1,254	Continuous for 7 days	Rat, M (S-D) 3-5 weeks old	The whole lung O_3-induced increase in ascorbate and GSH content unaffected by protein deficient diets.	Dubick et al. (1985)
0.64	1,254	Continuous for 7 days	Rat, M (S-D) 52 and 295 g	Whole adult lung contents of Cu,Zn-SOD and GSHPx increased by O_3 in all diet groups (ad libitum, 4-16% protein diets); GSHPx only increased in weanling rats fed 16% protein diet. Mn-SOD only increased in lungs from 4 and 16% protein-fed adult lungs.	Heng et al. (1987)
0.7	1,373	Continuous for 1-5 days	Rat, M (S-D) 45, 80, and 300 g	By 5 days, increased lung Cu,Zn-SOD, Mn-SOD, catalase, and GSHPx per DNA in all age groups. Adult lungs: Concomitant increases in mRNAs for Cu,Zn-SOD, catalase, and GSHPx without differences in mRNA stability.	Rahman et al. (1991)
0.8	1,568	8 h/day for 2 mo	Rat, M (S-D) 2 mo old	Absence of vitamin E exacerbates O_3-induced damage related to increases in whole lung levels of metabolic enzymes. No additional amelioration by diet supplementation above 50 IU vitamin E.	Elsayed et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

an increase in GSHPx were observed in minor bronchi after 20 mo of O_3 exposure (Plopper et al., 1994b). Rahman et al. (1991) concluded that changes in antioxidant enzyme activities in some cases could be associated with alterations in cellular pathology (see Section 6.2.4), whereas, in other cases, no correlation could be made even though the results were represented on a per-milligram-DNA basis. The observed changes in antioxidant enzyme activities appear to be site-specific and different at different airway locations. The response are concentration dependent and altered by the length of O_3 exposure (Plopper et al., 1994b).

Representing data on a per-gram, wet-lung basis, Ichinose and Sagai (1989) demonstrated increases in lung NPSH, vitamin C, and GSHPx but observed no effect on vitamin E levels, after continuous exposure of rats to 0.4 ppm O_3 for 2 weeks. In contrast, guinea pig lungs exhibited no changes in these antioxidant components when similarly exposed. However, although using the higher concentration of 0.64 ppm for 7 days of continuous O_3 exposure, Dubick et al. (1985) demonstrated that whole lung content of ascorbate and GSH was elevated, these changes were not significantly different when the data were represented on a per-100-g, wet-tissue basis. Rat BAL analysis following a 12-mo exposure to an urban pattern of O_3 demonstrated decreased vitamin E and enhanced ascorbate and protein levels (Grose et al., 1989). Because these antioxidants also have been shown to be targets of ozone interaction, any observed increases in their steady-state level suggest an increase in the ratio of production to degradation that could reflect either enhancement in cellular functions in response to continued O_3 exposure or alteration in the number of cells associated with their production.

In order to demonstrate that dietary vitamin E reduces the effects of O_3 exposure on lung biochemical parameters, comparisons between vitamin E-depleted and -supplemented diets have been used (U.S. Environmental Protection Agency, 1986) and reviewed by Pryor (1991). Elsayed et al. (1988) fed rats a test diet containing 0 or 50 International Units (IU) of vitamin E per kilogram for 2 mo prior to exposure to 0.8 ppm O_3 for 8 h/day for 7 days. Ozone exposure increased the whole-lung activities of mitochondrial, microsomal, and cytosolic enzymes. Vitamin E deficiency alone had no significant effect on these lung enzyme measurements, which were taken on a per-lung basis, but the addition of 50 IU vitamin E per kilogram to the diet prior to O_3 exposure diminished the observed O_3-induced increases in mitochondrial succinate cytochrome *c* reductase and GSHPx, microsomal NADPH cytochrome *c* reductase, and cytosolic GSHPx and SOD observed in vitamin E-deficient rats by up to 50%. Additional experiments using a relatively low range of vitamin E supplementation for short time periods demonstrated that, although absence of vitamin E in the diet exacerbates the effects of O_3 on lung injury, the magnitude of a protective effect does not increase proportionately with increased dietary vitamin E. These data support the conclusion that any supplementation beyond the normal recommended daily allowance for vitamin E may not necessarily provide humans with any additional protection against the effects of ambient O_3 exposure (Pryor, 1991). However, possible failure in these animal experiments to reach a steady-state tissue level of vitamin E may have obscured protective effects.

6.2.1.5 Effects of Ozone on Lung Protein Metabolism

Exposure of rodents to 0.45 ppm O_3 has been associated with increases in lung collagen, collagen synthesis, and prolyl hydroxylase activity associated with fibrogenesis (U.S. Environmental Protection Agency, 1986). These earlier studies showed an influence of exposure pattern on the responses. When rats were exposed to 0.8 ppm O_3 for 7 days, prolyl

hydroxylase activity continued to increase, but hydroxyproline content plateaued about Day 3 of exposure and remained elevated 28 days after exposure ceased (Hussain et al., 1976a,b). Last et al. (1984b) employed 90-day exposure regimens of rats to 0.96 ppm O_3 that included (1) a continuous 90-day exposure and (2) intermittent periods of 5 days (8 h/day) of O_3 and 9 days of air, repeated seven times with a total of 35 O_3 exposure days over a 90-day period. Both groups had equivalent increases in lung collagen content. When durations were decreased to 3 weeks, the continuous and intermittent (1 week O_3, then 2 weeks air) regimens resulted in equivalent increases in lung collagen. In nonhuman primates receiving 0.25 ppm O_3 daily or seasonally (every other month) for 18 mo, only the seasonal group had an increase in collagen (Section 6.2.4, Tyler et al., 1988). Results from studies of lung protein metabolism are summarized in Table 6-3.

More recently, Choi et al. (1994) examined the earliest time points from the onset of continuous O_3 exposure of rats to 1.0 ppm that caused alterations in extracellular matrix protein gene expression. These authors demonstrated an early increase in lung fibronectin mRNA at 2 days, which preceded an increase in Type I collagen mRNA observed at 4 days; however, increased collagen content indicated by lung hydroxyproline content was not significantly enhanced until after 7 days of exposure. Pickrell et al. (1987a) demonstrated concentration-dependent decreases in antiproteinase activities in serum and lung tissue of rats exposed to 0.5 and 1.0 ppm O_3 for 48 h. Exposure to 1.0 ppm was accompanied by a concomitant increase in inflammatory-cell-derived proteinases. A second study that examined lung collagen metabolism and proteinolysis in rat lungs exposed to 0.57 and 1.1 ppm O_3 for 19 h/day for 11 days suggested that collagen accumulation, in part, may result from decreased collagen degradation (Pickrell et al., 1987b).

Chronic exposures of monkeys to 0.61 ppm 8 h/day for 1 year demonstrated increased lung collagen content, even 6 mo postexposure (Last et al., 1984b). Further analysis also has demonstrated that the collagen isolated from these O_3-exposed lungs exhibited abnormalities, as indicated by increased levels of the difunctional cross-link dehydrodihydroxylysinonorleucine (DHLNL) and of the ratio of DHLNL to hydroxylysinonorleucine (HLNL) (Reiser et al., 1987). Although collagen content remained elevated, difunctional DHLNL and HLNL cross-link levels returned to normal by 6 mo postexposure, whereas trifunctional mature cross-links (hydroxypyridinium) remained elevated. These data suggest that structurally abnormal collagen is actively synthesized during O_3 exposure and that it becomes irreversibly deposited in the lungs.

Because O_3-induced lung effects are multifocal by nature, it is reasonable that changes in collagen content within the lung may not be easily detectable by measuring alterations in whole lung hydroxyproline at earlier time points or in those experiments that have used lower O_3 concentrations. For example, Wright et al. (1988) calculated values for the extent of lung collagen deposition using measured synthesis rates and concluded that 18 mo of exposure (20 h/day) of rats to concentrations up to 0.5 ppm O_3 did not change either synthesis or accumulation of lung collagen. On the other hand, Chang et al. (1992) demonstrated sustained thickening of rat lung extracellular matrix on long-term exposure to a simulated urban pattern of O_3 exposure (baseline of 0.06 ppm, 7 days/week, with a slow rising peak for 9 h/day, 5 days/week to 0.25 ppm) of up to 38 weeks. More recently, Last et al. (1993a, 1994) observed excess stainable collagen in the lung CAR of rats exposed to 0.5 and 1.0 ppm O_3 for 6 h/day, 5 days a week for 20 mo. Biochemical analyses demonstrated slight

but significant increases in collagen with relatively more hydroxylysine-derived cross-links in female but not male rats, when compared with age-matched,

Table 6-3. Effects of Ozone Exposure on Lung Proteins[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.25 0.5	235 490 980	20 h/day for 18 mo	Rat, M (F344) 28 days old	Age-related increases in hydroxyproline content as a measure of collagen were unaffected by O_3 exposure.	Wright et al. (1988)
0.125 0.25 0.5	245 490 980	1 year	Rat (F344) 6 weeks old	No changes in collagen content, increased turnover at 0.25 ppm after 3 or more mo of exposure.	Filipowicz and McCauley (1986b)
0.12 0.5 1.0	235 980 1,960	6 h/day 5 days/week 20 mo	Rat, M and F (F344) 4-5 weeks old	Excess stainable collagen in the CAR at 0.5 ppm. Biochemical analysis demonstrated slight but significant increases in collagen in female but not male rats exposed to 0.5 ppm with increased hydroxylysine-derived cross-links.	Last et al. (1993a, 1994)
0.25	490	8h/day 7 days/week, "daily" for 18 mo or "seasonal" O_3 odd months for 18-mo period (9 mo of O_3)	*Macaca fascicularis* 6 mo old	Increased collagen content in seasonal group only.	Tyler et al. (1988)
0.4	784	12 h	Rat, M (Wistar) 8 weeks old	Surfactant less able to stimulate AM superoxide anion generation, confirming in vitro results suggesting damage to surfactant protein A.	Oosting et al. (1992)
0.5	980	4 h/day for 2 days and 6 weeks	Sheep, F 23-41 kg	At 2 days, increased sulfated glycoproteins secretion; at 6 weeks, diminished tracheal mucosal gland hyperplasia secretion.	Phipps et al. (1986)
0.5 1.0 1.5	980 1,960 2,940	Continuous for 48 h	Rat, F (F344) 12-14 weeks old	Concentration-dependent decrease in antiproteinase activity at 0.5 and 1.0 ppm. Increases in acid proteinase activity 1.0 and 1.5 ppm correlated with increased inflammatory cell content.	Pickrell et al. (1987a)
0.57 1.1	1,117 2,156	19 h/day for 11 days	Rat, F (F344) 120-180 g	After 11 days of 1.1 ppm O_3, inflammatory cell infiltrate and Type 2 cell and fibroblast proliferation, increased cathepsin D and AM elastase activity, decreased rate of intracellular collagen degradation, and increased extracellular matrix collagen turnover (indicated by enhanced BAL hydroxyproline). These changes preceded increased collagen content observed 50 days PE.	Pickrell et al. (1987b)
0.61	1,196	8 h/day for 1 year	Monkey (Cynomolgus) 6-7 mo old	Increased lung collagen content associated with elevated abnormal cross-links that were irreversibly deposited.	Reiser et al. (1987)

Table 6-3 (cont'd). Effects of Ozone Exposure on Lung Proteins[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.8	1,568	Continuous for 3 days	Rat (S-D) 24-365 days old	Increased lung dry weight, protein, and collagen synthesis greatest after 60 days of age.	
0.8	1,568	6 h/night for up to 90 days	Rat, M (S-D) 10-12 weeks old	Increased lung content of collagen. No change in hydroxypyridinium or elastin.	Last et al. (1993b)
1.0	1,960	Continuous for 14 days	Rat, M (Wistar) 200-250 g	Lung mRNAs for c-myc proto-oncogene and fibronectin enhanced on Day 2 and Type I collagen mRNA not increased until Day 4, preceding increases in collagen hydroxyproline observed on Day 7.	Choi et al. (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

air-exposed control animals. It should be noted that no excess of mRNA for Type I procollagen was observed by in situ hybridization in lungs of rats exposed to 1.0 ppm for 20 mo, although increases after a 2-mo exposure under similar conditions did indicate some increase expression of this mRNA in alveolar interstitial cells (Last et al., 1993a).

Ozone exposure also affects airway secretion of mucous glycoproteins. After 2 days of exposure of sheep to 0.5 ppm O_3, with subsequent evaluation of tracheal sulfated glycoprotein and ion fluxes in vitro, there was an increase in basal secretion that was associated with a moderate hypertrophy of lower tracheal submucosal glands (Phipps et al., 1986). Although 7 days of exposure resulted in hypertrophy of upper and lower tracheal submucosal glands, glycoprotein secretion was reduced, but chloride secretion was increased, which can be explained by a relative decrease in gland mucous content.

6.2.1.6 Effects of Ozone Exposure on Lung Xenobiotic Metabolism

Previous studies have demonstrated that exposure to 0.75 to 1.0 ppm O_3 for a few hours diminishes microsomal cytochrome P-450 content and decreases the activities of benzo[*a*]pyrene hydroxylase and benzphetamine *N*-demethylase of lungs isolated from several different experimental animal species (U.S. Environmental Protection Agency, 1986). Because bronchiolar Clara cells and alveolar Type 2 cells are considered to be relatively enriched with microsomal cytochrome P-450 enzyme systems, it is reasonable that damage and subsequent proliferative repair of these cell types would be expected to change the lung's capacity to conduct xenobiotic metabolism. In a series of rat studies, Takahashi et al. (1985) and Takahashi and Miura (1985, 1987, 1989, 1990) have demonstrated that, although intermittent exposure of 0.4 ppm O_3 for 7 h/day for 14 days did not affect microsomal metabolism, increasing the concentration to 0.8 ppm (Takahashi et al., 1985) or exposing the rats continually to 0.2 and 0.4 ppm for 14 days (Takahashi and Miura, 1985) increased cytochrome P-450 content and the activities of cytochrome P-450 reductase, benzo[*a*]pyrene hydroxylase, and 7-ethoxycoumarin *O*-deethylase (see Table 6-4). These increased microsomal activities were sustained in rats exposed continuously for up to 12 weeks to 0.1 to 0.4 ppm O_3, with a greater response being observed in the activity of benzphetamine *N*-demethylase, suggesting preferential increase in the associated P-450 cytochrome isozyme (Takahashi and Miura, 1987). Ozone-induced increases in cytochrome P-450 also have been shown not to result in concomitant increases in microsomal xenobiotic metabolism (Rietjens et al., 1988). Rat lung microsomal benzo[*a*]pyrene oxidation and benzphetamine demethylation were found to be enhanced after a 6-mo continuous exposure to 0.5 ppm O_3 (Filipowicz and McCauley, 1986a). More recent studies have explored O_3-induced changes in cytochrome P-450 isozyme patterns and correlated changes in lung xenobiotic metabolism with Clara cell enlargement and increased numbers during a 14-day exposure of rats to 0.4 ppm O_3 (Takahashi and Miura, 1990; Suzuki et al., 1992). These authors also demonstrated, by immuno-electron microscopy, the presence of cytochrome P-450b (IIB1) in the Clara cell endoplasmic reticulum.

Changes in the extent and pattern of formation of benzo[*a*]pyrene products were investigated by Bassett et al. (1988c) in lungs from rats undergoing epithelial proliferative repair resulting from 3 days of continuous exposure to 0.6 ppm O_3. Although metabolism to all benzo[*a*]pyrene metabolites was enhanced 4.7-fold, the relative proportion of metabolism involving quinone formation was enhanced from 10 to 25%. The toxicity of other inhaled pollutants that undergo lung xenobiotic metabolism may therefore be dependent not only on

Table 6-4. Effects of Ozone Exposure on Lung Xenobiotic Metabolism[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.2 0.4	196 392 784	Continuous for 4-12 weeks	Rat, M (Wistar) 19-22 weeks old	Concentration-dependent increases in NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content during 4-12 weeks exposure to 0.2 and 0.4 ppm O_3, reaching a maximum at 12 weeks with concomitant increases in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities. NADH-cytochrome b_5 reductase activity unaffected. Four weeks at 0.1 and 0.2 ppm demonstrated a preferential increase in benzphetamine *N*-demethylase activity, with no alterations in coumarin hydroxylase activity.	Takahashi and Miura (1987)
0.2	392	Continuous for 2 weeks	Rat, M (Wistar) 19-22 weeks old	Increases in cytochrome P-450 isozymes ascribed to constitutive types rather than induction of other types.	Takahashi and Miura (1990)
0.2 0.4	392 784	Continuous for 7 and 14 days	Rat, M (Wistar) 22-24 weeks old	By 14 days, NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content enhanced with concomitant increases in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities by Day 7; no change in NADH-cytochrome b_5.	Takahashi and Miura (1985)
0.4 0.8	784 1,568	7 h/day for 14 days	Rat, M (Wistar)	No effect at 0.4 ppm. 0.8 ppm increased NADPH-cytochrome P-450 reductase activity and cytochrome P-450 content, with concomitant increases on Day 7 in benzo[*a*]pyrene hydroxylase and 7-ethoxycoumarin *O*-deethylase activities that further increased by Day 14.	Takahashi et al. (1985)
0.4	784	Continuous for 6 h, 1-14 days	Rat, M (Wistar) 5 weeks old	By 24 h, Clara cell number decreased, but by 14 days had increased. Increase in cytochrome P-450b (IIB1) on Days 7 and 14.	Suzuki et al. (1992)
0.5	980	Continuous for 1 year	Rat, M (F344)	Microsomal benzo[*a*]pyrene oxidation and benzphetamine demethylase activities enhanced after 6 mo and 1 year of exposure.	Filipowicz and McCauley (1986a)
0.6	1,176	Continuous for 3 days	Rat, M (Wistar) 200-220 g	In isolated perfused lung, increase in overall benzo[*a*]pyrene metabolism but with a greater proportion being metabolized to quinones.	Bassett et al. (1988c)
0.8	1,600	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Cytochrome P-450, cytochrome b_5, and NADPH-cytochrome P-450 reductase enhanced per lung and per gram lung but not per milligram microsomal protein. No concomitant increases in all cytochrome P-450-dependent reactions, suggesting alterations in isozyme patterns.	Rietjens et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

O_3-induced changes in airway protective barrier function and clearance mechanisms, but also on O_3-induced changes in epithelial cell activation and detoxification reactions.

6.2.1.7 Summary

In vitro studies have provided an indication of a wide range of initial biochemical targets of ozone interaction that include lipid PUFAs, SH-containing proteins, and small-molecular-weight electron donors such as GSH and vitamins E and C. Demonstration that these interactions occur in vivo and are responsible for subsequent cytotoxicity has been more difficult to characterize and mainly has required the use of relatively high (>1 ppm) concentrations of O_3. However, because of the high reactivity of ozone and the relatively high abundance of PUFAs in both cell membranes and epithelial lining fluids, PUFAs are considered to be the most likely initial target of interaction of O_3 with the lung. Current understanding of this interaction is that, in the relatively aqueous environment of the lung airways, hydroxyhydroperoxy intermediates are formed that break down to form aldehydes and hydrogen peroxide. Alternatively, it has been proposed that, in more hydrophobic environments (e.g., within a cell membrane), O_3 interaction with PUFAs yields ozonides and their free radical products. Ozonides, aldehydes, hydrogen peroxide, and other lipid break-down products and oxygen intermediates are therefore considered to be secondary products of the initial O_3 interaction with PUFAs that would account for the observed alterations of cell lipids, SH-containing enzyme proteins, and antioxidants associated with O_3-induced cell damage. As a result of these observations, it has been hypothesized that O_3 most likely does not reach far beyond the surface lining fluids of the upper and lower airways, which are rich in mucopolysaccharides and surfactant lipids, respectively. However, at points where coverage is either discontinuous or thin, epithelial cell components might be expected to directly interact with inhaled O_3.

A wide array of lung biochemical measurements have been made at different times from the onset of O_3-exposure. These measurements have included lung lipids, antioxidants, and enzyme and structural proteins that, in some cases, can be attributed to particular cell populations. However, many of these biochemical determinations cannot be interpreted fully without consideration of the changes in cell population that occur as a result of O_3 exposure. In addition, the sensitivity of some of these measurements has been limited by the relatively small percentage of the whole lung affected by O_3 exposure. The more recent biochemical determinations being made on airway samples isolated by regional microdissection should help overcome some of these limitations.

In vivo experiments have demonstrated cleavage of total lung lipid PUFA double bonds, with arachidonate being a major target of O_3 interaction, the breakdown of cholesterol, and the production of aldehydes and hydrogen peroxide (results that are consistent with ozonation of cell membrane and epithelial lining lipids). The protein A component of the alveolar surfactant system also has been identified as a possible primary target of O_3 interaction. Changes observed in lung lipid biosynthesis during the first few days from the onset of O_3 exposure can be accounted for by concomitant alveolar epithelial proliferative repair. However, lavage-recovered lipids from monkeys following O_3 exposures of 0.12 ppm for 90 days have demonstrated a relative increase in PUFAs and decrease in cholesterol-esters, suggesting some long-term alteration in surfactant lipid composition. However, age-related changes in lavage-recovered lipids and total lung lipid biosynthesis have been shown to be relatively unaffected in rats exposed to 0.5 ppm O_3 for periods of up to 18 mo.

Many studies have utilized whole-lung measurements of antioxidant enzyme changes as indicators of biochemical responses to O_3 exposure. The increased levels of the cytosolic enzymes G6PD, 6PGD, GR, and GSHPx and mitochondrial succinate dehydrogenase observed during the first week from the onset of exposure to O_3 levels of 0.5 to 1.0 ppm are most likely a result of the epithelial proliferation and infiltration of inflammatory cells taking place during this period. Failure to observe similar biochemical changes at lower O_3 concentrations most likely reflects an inability to detect focal changes of altered pathology when using whole-lung tissue samples. Longer-term exposure of rats to an urban pattern of O_3 with daily peaks of 0.25 ppm has demonstrated increases in tissue GSHPx and GR but not SOD. These enzyme changes could reflect changes in either cellular antioxidant capacity in response to chronic O_3 exposure or the steady-state cell population.

Although no long-term changes in collagen content have been observed in rats exposed to <0.5 ppm O_3 for 18 mo, extracellular matrix thickening has been observed in rats exposed to an urban pattern of O_3 with daily peaks of 0.25 ppm for 38 weeks. Exposure of female but not male rats for 20 mo to concentrations of 0.5 and 1.0 ppm O_3 for 6 h/day has demonstrated increased centriacinar stainable collagen and collagen and difunctional cross-links. Similar results were obtained in lungs from monkeys exposed to 0.61 ppm O_3 for 1 year, providing a sensitive indicator that long-term O_3 exposure does cause some fibrogenic alterations to the lung extracellular matrix.

Ozone-induced changes in the extent and pattern of lung microsomal metabolism of xenobiotics have provided consistent results, which may, in part, reflect changes in the numbers and function of bronchiolar epithelial Clara cell and alveolar epithelial Type 2 cells at different durations of O_3 exposure. These cell types are relatively enriched with cytochrome P-450-dependent enzyme systems. Changes in both lung activation and detoxification reactions represent important effects when considering whether or not low-level O_3 exposures alter the ability of the lung to deal adequately with the co-exposure to inhaled xenobiotics found in urban air.

6.2.2 Lung Inflammation and Permeability Changes

6.2.2.1 Introduction

The barrier functions of the airway epithelia have been investigated by isotope tracer techniques for detecting mucosal permeability and by analysis of the BAL for total protein and albumin concentrations. Under normal conditions, the airway epithelia restrict the penetration of exogenous particles and macromolecules from airway lumen into airway interstitium and blood. The integrity of the zonula occludens (tight junctions) is regarded as a major factor in providing barrier properties to the airway epithelia so that only a small amount of intratracheally introduced tracers finds its way across the airway epithelia into the blood. However, disruption of the epithelial barrier creates a leak across the airway mucosa, resulting in increased permeability of serum proteins into the air spaces and of intraluminal exogenous tracers into the blood. Therefore, permeability is generally detected by either the tracer transport from airway spaces to blood or measurement of total protein and albumin in the BAL. Both of these measures are, therefore, taken into account in discussing permeability changes in this section. Although BAL protein measurement offers a good marker for detecting permeability changes, it is important to note that the proteins in the BAL can result from tissue injury and secretory activity, in addition to leakage of the serum proteins across the airway mucosa (Hatch et al., 1989; Hatch, 1992).

Inflammatory cells in the lung constitute an important component of the pulmonary defense system. In their unstimulated state, the inflammatory cells present no danger to other cells or tissues, but, on activation, they are capable of generating proteolytic enzymes such as elastase and reactive oxygen species such as superoxide, hydrogen peroxide (H_2O_2), and the hydroxyl radical. These oxidants can cause substantial injury to cell membranes and intracellular components by their effects on membrane lipids and proteins (biochemical effects of O_3 were described in Section 6.1). Ozone exposure also can cause the epithelial or activated inflammatory cells to liberate arachidonic acid, which is free to enter enzymatic lipoxygenase or cyclooxygenase pathways that lead to the production of leukotrienes (LTs) and prostaglandins (PGs), respectively. Although some of the studies indicate a lack of change in the production and release of cellular mediators following O_3 exposure, other studies demonstrate an elevation in the levels of arachidonic acid and its metabolites in the bronchial washings of rats, as well as humans (see Chapter 7) exposed to O_3 under controlled conditions. The changes in the lung levels of arachidonic acid metabolites generally were observed in animals exposed to O_3 concentrations higher than 0.5 ppm. These cellular mediators can cause a wide range of pathophysiological changes. For example, LTB_4 can cause PMN aggregation and degranulation in vitro and margination of circulating PMNs to capillary endothelium in vivo, whereas LTC_4 and LTD_4 can cause contraction of vascular smooth muscle, PGE_1 has bronchodilator activity, and LTD_4 and $PGF_{2\alpha}$ are regarded as bronchoconstrictors. Because of the toxic potential of the products released by PMNs, AMs, mast cells, and other inflammatory cells, it has been suggested that the recruitment of these cells into the pulmonary interstitium is associated with lung injury and associated edema. An inflammatory response in the lung and an elevation of transmucosal permeability are observed after O_3 exposure, but the interdependence of these two events is a topic of debate. Although AMs are involved in cellular changes during the course of inflammation, AMs are discussed only in terms of their primary function in the section on host defense (Section 6.2.3.4).

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) discussed studies available at that time on the inflammatory and permeability effects of O_3. These studies recognized the increased thickness of the alveolar septa, presumably due to increased cellularity after acute exposure to O_3 and excess collagen after chronic exposure to O_3. The inflammatory cell response was reported in rats and monkeys receiving single or repeated exposures to O_3 concentrations ranging from 0.2 to 0.8 ppm (Castleman et al., 1980; Brummer et al., 1977; Moore and Schwartz, 1981; Crapo et al., 1984). Exposures to O_3 also resulted in increased mucosal permeability, as detected by the nonspecific diffusion of phenol red from the lung into circulation (Williams et al., 1980) or the appearance of serum proteins in the air spaces. Increased BAL levels of total protein, albumin, and immunoglobulin (Ig) G were detected in rats, dogs, and guinea pigs exposed acutely to O_3 concentrations ranging from 0.1 to 2.5 ppm (Alpert et al., 1971; Reasor et al., 1979; Hu et al., 1982). For example, Hu et al. (1982) found that a 72-h exposure of guinea pigs to $\geq$0.26 ppm O_3 increased BAL protein immediately after exposure and that, when the exposure duration was decreased to 3 h, protein increased 10 to 15 h postexposure (not immediately after exposure ceased).

6.2.2.2 Permeability Changes

A number of studies have demonstrated an increase in airway mucosal permeability following inhalation exposure to O_3 concentrations of $\geq$1.0 ppm (Table 6-5).

Table 6-5. Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration μg/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 1.2	196 2,352	2 h/day for 1, 2, 6, and 13 days	Rabbit, M (NZW) 2-4 mo old	Increase in AM number at 7 days following single exposure to 0.1 ppm and increase in number of AMs and PMNs on 1-day after cessation of 6 or 13 days of exposure. Increase in number of PMNs at 24 h after single exposure to 1.2 ppm.	Driscoll et al. (1987)
0.1 0.3 1.0	196 588 1,960	2 h	Rabbit, M (NZW) 15-16 weeks old	Increase in levels of PGE_2 and $PGF_{2\alpha}$ in BAL immediately after exposure to 1.0 ppm O_3 only. No significant effects were observed on the levels of 6-keto-$PGF_{1\alpha}$, TXB_2 or LTB_4	Schlesinger et al. (1990)
0.1 0.3 1.2	196 588 2,352	2 h In vitro and in vivo	Rabbit, M (NZW) 2-4 mo old	In vitro: Increase in PGE_2 after 0.3 ppm and increase in $PGF_{2\alpha}$ after 1.2 ppm by AMs. In vivo: Increase in the release of PGE_2 and $PGF_{2\alpha}$ by AMs after 1.2 ppm, but no effect of 0.1 ppm.	Driscoll et al. (1988)
0.1 0.3 1.0	196 588 1,960	2 h In vitro	Rat (S-D) 12-18 weeks old	Increased production of arachidonic acid metabolites by AMs at 1.0 ppm only.	Madden et al. (1991)
0.1 to 10	196 to 19,600	2 h In vitro	Cow	Increased production of PGE_2 and $PGF_{2\alpha}$ by tracheal epithelial cells after exposure to 0.1 and 0.3 ppm. Increased production of other arachidonic acid metabolites at > 1.0 ppm.	Leikauf et al. (1988)
0.1 0.2 0.4 0.8	196 392 784 1,568	2, 4, and 8 h	Rat (F344) 90 days old Guinea pig (Hartley) 60 days old	C × T exposure design; BAL 25 h after exposure started. PMNs measured in rats only; no C and T interaction; effect dependent on C. Exponential and polynomial response surface model used. Similar protein responses at low C × T products; generally, the influence of T increased as C increased. Exponential model explained 86% of the data.	Highfill et al. (1992)
0.1 0.3 1.2	196 588 2,352	2 h In vitro	Rabbit, M (NZW) 2-4 mo old	Exposure of AMs to ≥0.3 ppm O_3 resulted in increased secretion of factors capable of stimulating migration of inflammatory cells.	Driscoll and Schlesinger (1988)
0.1 0.2 1.2 4.0	196 392 2,352 7,840	Continuous for 1 to 12 weeks	Rat, M (Wistar) 16 weeks old for 1 week exposure; 21 weeks old for longer exposures	Number of AMs in BAL increased after exposure for 11 weeks to 0.2 ppm. Infiltration of PMNs did not occur.	Mochitate et al. (1992)
0.12 0.3	235 588	24 h 48 h and 72 h	Mice, M (C57BL/6J[B6]); (C3H/HeJ[C3]) 6-8 weeks old	BAL immediately PE. Comparable increases in BAL protein, AMs, PMNs, and lymphocytes in the two strains after exposure to 0.12 ppm, but greater number of inflammatory cells and protein concentration in B6 than in C3 mice after exposure to 0.3 ppm.	Kleeberger et al. (1993a)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, F (F344/N) 12-18 weeks old	Increased number of PMNs in nasal lavage, but not in BAL at 18 h after 0.12 ppm; increased number of PMNs in BAL, but not in nose after 1.5 ppm; number of PMNs decreased with time in nose, with a concomitant increase in BAL PMNs after 0.8 ppm.	Hotchkiss et al. (1989a)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344/N) 12-18 weeks old	AMs and PMNs increased in number in BAL at various times PE at 0.8 ppm.	Hotchkiss et al. (1989b)
0.12 to 0.96	255 to 1,882	6 h, 24 h, or 2 days	Rat, M (S-D) 250-300 g	Total protein in BAL increased after exposure to 0.4 ppm for 6 h and 0.12 ppm for 1 or 2 days. Transport of radiolabeled albumin from blood to the airways increased after 6- or 24-h exposure to 0.4 ppm and after 2 days exposure to 0.2 ppm.	Guth et al. (1986)
0.2 0.5 1.0 2.0	392 980 1,960 3,920	4 h	Mouse (Swiss Albino) 19-25 g Guinea pig (Hartley) 314-522 g Rat (S-D) 280-350 g Rabbit (NZW) 1.7-2.5 kg Hamster (Golden Syrian) 94-107 g	Species differences in responsiveness. At 18-20 h PE, total protein in BAL increased in guinea pigs exposed to 0.2 ppm, whereas mice, hamsters, and rats responded to 1.0 ppm, and rabbits responded only to 2.0 ppm.	Hatch et al. (1986)
0.2 0.4 0.6 0.8	392 784 1,178 1,568	6, 8, 12, and 24 h/day for 3 days	Rat (S-D) 10-12 weeks old	C and T matched such that all C × T = 14.4 ppm · h. BAL immediately after exposure ceased. Increase in PMNs equivalent in all O_3 groups. Increase in protein equivalent for 6-, 8-, and 12-h exposure groups, all of which are greater than protein in 24-h groups. Equivalent results for BAL PMNs.	Gelzleichter et al. (1992b)
0.2 0.4 0.6 0.8	392 784 1,176 1,568	7 h/day for 1, 2, or 4 days	Rat, M (PVG) 12-16 weeks old	BAL approximately 17 h PE. The proportion of AMs in the BAL decreased, with a concomitant increase in the proportion of PMNs after 1 or 2 days exposure to 0.6 ppm O_3. No significant effect on total number of lavageable cells or on the ability of neutrophils to injure epithelial cells.	Donaldson et al. (1991, 1993)
0.25 peak over a bkg of 0.06	490 118	13 h bkg, rose to peak and returned to bkg over 9 h	Rat, M (F344) 60 days old	Interstitial AMs increased in number in proximal alveolar region and TBs at one week of exposure, but the effects had subsided by 3 weeks of exposure.	Chang et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.3 2.0	588 3,920	24, 48, or 72 h for 0.30 ppm and 3 h for 2.0 ppm	Mice (C57BL/6J[B6]); (C3H/HeJ[C3]) DBA/2J (D2), hybrids, and recombinant inbred strains (R1): BXD and BXH 6-8 weeks old	Inflammatory response was greater in B6 than in C3 or D2 mice. F1 progeny was categorized as resistant; F2 generation segregated into 45:16 for resistant vs. susceptible phenotypes. Among BXD R1 strains, 4 of 10 responded discordantly to the two exposures (0.3 and 2.0 ppm). Among BXD R1, 4 of 16 were discordant.	Kleeberger et al. (1990, 1993b)
0.35 0.5 1.0	686 980 1,960	2.25 h/day for 5 days	Rat, M (F344) 3-4 mo old	Persistent increase in BAL protein and progressive inflammation at 0.5 ppm.	Tepper et al. (1989)
0.35 0.5 0.65 0.8	686 980 1,274 1,568	2, 4, and 7 h	Rat, M (F344) 13 weeks old	C × T exposure design. All exposures included 45 min of CO_2 for 1 h to increase ventilation. BAL after pulmonary function tests completed. The quadratic model explained 92% of the variance. The models suggest that C may have a more dominant influence than T.	Tepper et al. (1994, in draft)
0.38 0.76 1.28 2.04	750 1,500 2,500 4,000	1, 2, 4, and 8 h daytime	Rat (Wistar) 7 weeks old	C × T exposure design. BAL protein measured at various times PE. Daytime exposures: At 0.76 ppm, maximal increase 22 h after exposure started; after 4 and 8 h of exposure, protein still elevated at 54 h from start of exposure.	Rombout et al. (1989)
0.13 0.26 0.38	250 500 750	4, 8, and 12 h nighttime		Nighttime exposures: Temporal increase and decrease of protein more gradual, with maximal response at 36 h after exposure started. Protein still elevated 72 h after start of 8- or 12-h exposure to 0.26 or 0.38 ppm. Smallest tested C × T effect was with 0.13 ppm × 4 h. Both: Multivariate regression analysis. Polynomial function shows that T has progressive influence as C increases.	
0.4 0.6	784 1,176	8 h/days for 90 days	Monkey, M (Bonnet) 5.2-8 years old	Inflammatory response in RBs at 0.64 ppm.	Moffatt et al. (1987)
0.4	800	12 h during day or night	Rat, M (Wistar) Guinea pig, M (Hartley) 9 weeks old	Nighttime exposure of rats resulted in greater increase in BAL protein, albumin, and PMNs than the daytime exposure. A similar difference was not observed in guinea pigs.	Van Bree et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.5	980	2 h	Dog, M (Mongrel) 15 ± 0.9 kg, Baboon, M 25-40 kg	No effect on levels of 6-keto $PGF_{1\alpha}$, PGE_2, TXA_2, TXB_2, or $PGF_{2\alpha}$ in BAL.	Fouke et al. (1990, 1991)
0.5	980	Continuous exposure for 1-14 days	Mouse, F (Swiss) 20-25 g	PGE and total protein levels in BAL increased after the exposure, peaked at 3 days, then declined with time, but remained higher than the controls at 7 days; protein still increased at 14 days. Total cells in BAL decreased on Days 1 to 3 after exposure.	Canning et al. (1991)
0.5 1.0	980 1,960	4 h	Guinea pig, M (Hartley) 300-400 g	Depleting lungs of ascorbic acid enhanced effects of 0.5 but not 1.0 ppm on BAL protein. Depletion of lung nonprotein sulfhydryl had no effect.	Slade et al. (1989)
0.75	1,410	Continuous exposure for 3 days	Rat, M (Wistar) 200-250 g	Increased number of PMNs and AMs and elevated levels of albumin in the BAL. At 4 days PE, no PMNs were detected, but AM numbers and albumin levels were elevated.	Bassett et al. (1988a)
0.8	1,568	2 h	Rat, M (S-D) 300 g	Transient increase in tracheal and bronchoalveolar permeability, as revealed by tracer transport from airways to blood and tracer localization in intercellular spaces.	Bhalla et al. (1986) Bhalla and Crocker (1986)
0.6 0.8	1,176 1,568	2 h exposures during rest or exercise	Rat, M (S-D) 47-52 days old	Airway permeability increased after exposure of resting animals; trends of greater and more persistent effects in exercising group.	Bhalla et al. (1987)
0.8	1,568	2 h	Rat, M (S-D) 50-60 days old	Increased transport of radiolabeled tracers from blood to the air spaces following exposure.	Bhalla and Crocker (1987)
0.8	1,568	3 h	Rat, M (S-D) 250-300 g	The number of PMNs in lung parenchyma increased immediately after exposure, peaked at 8 h PE, and returned to baseline by 16 h PE. Total protein and albumin levels in BAL increased immediately after exposure, peaked at 8 h PE, and then declined with time, but the albumin levels were higher than the controls at 24 h PE.	Bhalla and Young (1992)
0.8	1,568	3 h	Rat, M (S-D) 250-300 g	Time-related changes in tracheal permeability, detected by tracer transport, and PMN influx in tracheal wall following exposure. Increase in permeability prior to increase in PMNs.	Young and Bhalla (1992)
0.8	1,568	2 h	Rat, M (F344) 11-12 weeks old	Increased DTPA transport across the tracheal mucosa and elevated levels of protein and albumin in BAL. Effects attenuated in leukopenic rats or rats pretreated with indomethacin or FPL 55712.	Bhalla et al. (1992)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.8	1,568	2 h	Rat, M (F344) 250-275 g	PMNs isolated from blood of O_3-exposed rats displayed deformation of shape, indicative of motility and greater cell adhesion than the PMNs from air-exposed rats.	Bhalla et al. (1993)
0.8	1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Increase in PGE_2 in BAL in 5-week-old mice only; effect blunted by indomethacin pretreatment.	Gilmour et al. (1993b)
0.96	1,882	8 h	Monkey, M (Rhesus) 2-8.5 years old	Number of labeled PMNs into lung tissue and BAL increased immediately after exposure, peaked at 12 h PE, and returned to baseline by 24 h PE. Total labeled and unlabeled PMNs in BAL remained elevated at 24 h, but returned to control levels by 72 h PE. Total protein in BAL was elevated only at 24 h PE.	Hyde et al. (1992)
1.0	1,960	4 to 24 h	Rat (S-D) 63-70 days old	Total protein and PMNs in BAL and PMNs in the CAR of the lung increased with exposure duration, but the number of AMs in BAL decreased. Treatment with anti-rat-PMN serum resulted in elimination of PMNs in BAL, but it did not affect the O_3-induced increase in BAL protein.	Pino et al. (1992a,b)
1.0	1,960	5 min O_3 delivered to a localized area of lung via a Teflon catheter fitted to bronchoscope	Dog, M (Mongrel) 21.2 ± 0.5 kg	Number of PMNs in the subepithelial tissue increased at 1-3 h PE. Number of BAL PMNs increased at 24, but not at 1-3 h PE.	Kleeberger et al. (1989)
1.0	1,960	6 h	Rat, F (S-D) 8-9 and 13-17 weeks old	BAL 16 h PE. Enhanced responsiveness to O_3-induced inflammation and elevated protein levels in BAL developed during pregnancy, was maintained during lactation, and disappeared following lactation.	Gunnison et al. (1992b)
1.0	1,960	2, 4, or 6 h	Rat, M (S-D) 13, 18 days and 8 and 16 weeks old	BAL immediately after exposure. PGE_2 concentrations in BAL greatest in 13-day-old rats after 2 h of exposure, but in older rats the response was seen after 6 h of exposure. In 13-day-old rats, 50% of leukocytes in BAL were dead after 6 h of exposure; no such effect on 16-week-old adults. No age dependence for BAL protein increase or PMN increase.	Gunnison et al. (1992a)
1.0	1,960	2 h	Rat (S-D) 18 days or 14 weeks old Rabbit (NZW) 6, 11, 16, or 30 weeks old	BAL immediately after exposure. In youngest animals, greater amounts of PGE_2 and $PGF_{2\alpha}$. In youngest rabbits, 6-keto $PGF_{1\alpha}$ and TXB_2 increased. No effect on LTB_4. No age dependent effects on BAL protein or cell number.	Gunnison et al. (1990)

Table 6-5 (cont'd). Lung Inflammation and Permeability Changes Associated with Ozone Exposure[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
1.0	1,960	1 h	Guinea pig, M (Hartley) 300-400 g	Appearance of horseradish peroxidase in plasma, following its intratracheal administration, was accelerated at 2 and 8 h PE, but not at 24 h PE.	Miller et al. (1986)
1.0	1,960	1 h	Guinea pig, M (Hartley) 250-300 g	The concentrations of PGE_1, 6-keto $PGF_{1\alpha}$, and TXB_2 in BAL increased at various times following exposure.	Miller et al. (1987)
1.0	1,960	3 h isolated perfused lung	Rat (S-D) 350 ± 42 g	No effect on BAL protein.	Joad et al. (1993)
1.8	3,528	2 or 4 h	Rat, M (Wistar) 200-250 g	A decrease in number of AMs in BAL immediately after exposure. PMNs and albumin content of BAL increased at 1 day PE. Increased albumin levels, but not PMNs, persisted on Day 3 PE.	Bassett et al. (1988b)
2.0	3,920	4 h	Guinea pig, M (Hartley) 300-350 g	Interstitial PMNs increased in number immediately after exposure, but declined by 24 h PE. BAL PMNs were maximal by 3-6 h and remained elevated by 3 days PE.	Schultheis and Bassett (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

In rats exposed for 2 h to 0.8 ppm O_3, labeled tracers, such as diethylenetriaminepentaacetate (DTPA) and bovine serum albumin, introduced into the airway lumen were transferred to blood to a greater extent than in the air-exposed rats (Bhalla et al., 1986; Bhalla and Crocker, 1986; Crocker and Bhalla, 1986). The rapidly rising concentration of the tracers in the blood during the initial period of instillation of the tracers into the airways reflected both the accumulation, due to slow instillation over a 5-min period, of the tracers in the airway lumen and subsequent transfer across the respiratory epithelium. The changes in permeability observed in this study were transient in nature, returning to the baseline value by 24 h postexposure in the trachea and by 48 h in the distal airways. Reversible increases in airway epithelial permeability also were observed in guinea pigs acutely exposed to 1 ppm O_3 (Miller et al., 1986). The rate of appearance of intratracheally administered horseradish peroxidase increased in blood at 2 and 8 h after O_3 exposure, as compared to rats at 24 h postexposure to O_3 and air-exposed controls. When rats were exercised at a level that increased the minute ventilation ($\dot{V}_E$) twofold, the effect of 0.8 ppm O_3 was not only greater than in rats exposed at rest, but the increased permeability persisted longer (Bhalla et al., 1987).

Guth et al. (1986) analyzed the permeability effects of O_3 by injecting radiolabeled albumin into the blood and measuring it in the BAL, as well as by measuring the total protein concentration in the BAL. This study revealed a concentration-dependent increase in permeability following a 6-h exposure of rats to 0.4 ppm or following 1 or 2 days of exposure to 0.12 ppm O_3. For example, after a 2-day exposure to 0.12 ppm, there was a 71% increase in BAL protein. Tracer transport also was increased in rats exposed for 2 h to 0.8 or 2.0 ppm O_3 (Crocker and Bhalla, 1986; Bhalla and Crocker, 1987).

The relative influence of concentration and duration of O_3 exposure was evaluated by three laboratories using BAL protein as an indicator of effects. In the first study, Rombout et al. (1989), exposed rats for 1, 2, 4, or 8 h to 0.38, 0.76, 1.28, or 2.04 ppm O_3 during the daytime (16 C × T products). A similar nighttime exposure study was conducted using 0.13 to 0.38 ppm O_3 and 4, 8, or 12 h of exposure (nine C × T products). The smallest C × T product causing an increase in protein was 0.52 ppm · h (0.13 ppm × 4 h). A multivariate regression analysis accounted for 88.6% of the variance in the daytime data and 73.2% in the nighttime data. Animals exposed during the night were more responsive. A quadratic polynomial function showed that the influence of T increased with increasing C and that the influence of T was still important at the lowest O_3 concentration tested (0.13 ppm). The second study employed rats and guinea pigs, each having 12 C × T products (0.1, 0.2, 0.4, and 0.8 ppm O_3; 2, 4, and 8 h) (Highfill et al., 1992). Using additional modeling approaches, they obtained similar results to those of Rombout et al. (1989) . For example, the exponential response surface model explained 86% of the variance in the data and showed that the influence of T increased as C increased. However, at low C × T products, similar BAL protein increases were observed. Further modeling of these data (Highfill and Costa, 1995) again showed that C and T had interdependent influences. Tepper et al. (1994) performed a similar C × T study with 12 C × T products (0.35 to 0.8 ppm O_3, 2 to 7 h). However, rats were exposed to 8% CO_2 for 45 min of each hour to increase ventilation, and BAL was conducted on lungs that had been measured for pulmonary function. The response surface predicted by the modeling again indicated that the influence of T increased as C increased. Histopathological observations in the rats support the findings that C had more influence than T. Tepper et al. (1994) compared their analysis of BAL protein to that of Highfill et al. (1992) and found very good agreement, even though there were experimental differences. However,

in Tepper et al. (1994), there were larger constants for C terms, indicating that C had a greater influence than in the Highfill et al. (1992) study, probably because Tepper and co-workers increased ventilation (and hence O_3 dose) by using concurrent CO_2 exposures.

Gelzleichter et al. (1992b) exposed rats to a single O_3 C × T (14.4 ppm · h) composed of 16 products (0.2 to 0.8 ppm O_3, 6 to 24 h/day for 3 days). They found that the 24 h/day exposure groups had significantly fewer responses than the other groups, which were all equivalent. Thus, in this study, C and T had equivalent influences on the response, except when T was 24 h/day. This study was well conducted, but had some basic differences from the Rombout et al. (1989) and Highfill et al. (1992) studies in that the longer exposure durations (i.e., 24 h/day) involved a mixture of daytime and nighttime exposure that likely altered the dose-rate of O_3. Also, Gelzleichter et al. (1992b) used one C × T product, whereas the other studies used several C × T products.

6.2.2.3 Concomitant Changes in Permeability and Inflammatory Cell Populations in the Lung

Polymorphonuclear leukocyte infiltration in the lung following O_3 exposure has been investigated in a number of studies (Table 6-1), either by analyzing the cellular content of the BAL or by counting PMNs in lung sections. Bassett et al. (1988a) found an increase in the number of inflammatory cells in the BAL of rats continuously exposed for 3 days to 0.75 ppm O_3. The inflammatory response was accompanied by elevated levels of albumin and lactate dehydrogenase, suggesting increased permeability and cellular injury. Comparable changes were also observed in rats acutely exposed to a higher O_3 concentration (Bassett et al., 1988b). In another study, a random count of PMNs in the lung sections at 4-h intervals, following a 3-h exposure of rats to 0.8 ppm O_3, revealed a gradual increase in the number of PMNs, with a peak at 8 h postexposure and a return to the baseline value by 16 h postexposure (Bhalla and Young, 1992). The total protein and albumin concentrations in the BAL also increased after the exposure, peaking at 8 h postexposure. Although the protein concentrations returned to baseline by 16 h postexposure, the albumin levels remained above the controls after 24 h. Alveolar changes, consisting of thickened septa, parenchymal cellularity, and increased numbers of free cells, began to increase between 12 and 16 h postexposure and were still increasing at 24 h postexposure.

In trachea of rats exposed for 3 h to 0.8 ppm O_3, a peak of PMN infiltration at 12 h postexposure was preceded by a decline in the number of PMNs in pulmonary capillaries, suggesting exit of PMNs from the blood vessels and their migration across the endothelial cells into the tracheal wall (Young and Bhalla, 1992). Although a significant change in the tracheal population of PMNs did not occur until 12 h after the end of exposure, tracheal permeability, as detected by DTPA transport, increased immediately following O_3 exposure. The results of this study suggest that the initial changes in tracheal permeability may be independent of an inflammatory response, but the recruited PMNs may serve to sustain the increased permeability and to amplify O_3 effects at later stages. This conclusion was based on the observed shift of PMNs from the vascular compartment into the tracheal wall and a concurrent peak of increased permeability. In comparable studies, Pino et al. (1992a) exposed rats to 1.0 ppm O_3 for periods ranging from 4 to 24 h. Total protein and the number of PMNs in the BAL increased with time, with the maximum increase at the end of 24 h of continuous exposure. The number of AMs was lower in the exposed animals than in the controls. By morphometry, the peak PMN response in the terminal bronchioles (TBs) and alveolar ducts

(ADs) occurred at 4 h after an 8-h exposure. In dogs, local exposure of peripheral airways to 1 ppm O_3 for a short period (5 min) produced a recognizable inflammatory response (Kleeberger et al., 1989). An increase in the number of PMNs was detected in the subepithelial tissue within 3 h after 5 min of exposure of the dogs, but the response had subsided 24 h later. In BAL, on the other hand, an increase in the number of PMNs was not observed at 3 h postexposure; the number of PMNs increased at 24 h.

Hotchkiss et al. (1989a,b) have investigated the effects on AMs and PMNs of a 6-h O_3 exposure of rats to 0.12, 0.8, or 1.5 ppm O_3 and compared the inflammatory responses by nasal lavage and BAL, as well as by morphometry in the nose and the CAR of the lung, a site at which abnormal cellular changes generally occur following O_3 exposure. Animals were examined 3, 18, 42, or 66 h after exposure ceased. From lavage data, 0.12 ppm O_3 had no effect. At 0.8 ppm, there was an increase in the number of nasal PMNs lavaged immediately after exposure, which tapered off (no significant change at 42 h postexposure). In contrast, BAL PMNs increased later, beginning at 18 h postexposure and peaking at 42 h postexposure. From morphometric data, 0.12 ppm O_3 caused an increase in nasal PMNs 66 h postexposure. At 0.8 ppm, nasal PMNs increased to their greatest extent immediately after exposure and still were increased at later time periods. However, PMNs in the lung increased only at 18 and 66 h postexposure. The interpretation of these results was based on the presence of potential competing mechanisms in the nose and lungs. Therefore, the attenuation of the nasal effects are matched by simultaneous enhancement of the inflammatory response in the lung. Whether such a balance between nasal and alveolar PMNs represents a specialization restricted to rats or is a more general phenomenon remains to be investigated. A similar balance was not observed in humans exposed to O_3 (see Chapter 7). Subtle differences in species, O_3 concentrations, and exposure durations, however, need to be considered when making interspecies comparisons.

Hyde et al. (1992) investigated the inflammatory response in monkeys exposed to 0.96 ppm O_3 for 8 h. Polymorphonuclear leukocytes were isolated from peripheral blood, labeled with indium-111-labeled tropolonate and infused into the cephalic vein of monkeys 4 h before necropsy. Labeled PMNs in the lung tissue and the BAL peaked at 12 h and returned to control values by 24 h postexposure. The total number of labeled and unlabeled PMNs in the BAL, however, remained elevated at 24 h postexposure, but returned to baseline by 72 h. Furthermore, the PMN peak at 24 h postexposure coincided with the maximum increase in BAL protein at this time point. These studies suggest a strong correlation between BAL protein concentration, epithelial necrosis, and inflammatory cells (especially eosinophils) in bronchi, but not in the trachea or bronchioles. This observation may represent a species-specific response. In rats, the inflammatory response in the terminal airways involved an increase in the number of migratory cells, including PMNs, but not eosinophils (Pino et al., 1992a). The available literature suggests that the precise time point at which the maximum change in the number of inflammatory cells occurs is variable and may be dependent on several factors, including animal species, concentration, duration of exposure, and mode of analysis (i.e., BAL versus morphometry of lung parenchyma). Because the PMNs sampled by BAL represent only a small fraction of the cells shown to be present in the air spaces by morphometry (Downey et al., 1993), the inflammatory response detected by analyzing BAL may or may not match the response obtained by microscopic analysis of tissue sections. Even when the PMN response detected by the BAL analysis accurately reflects the tissue PMNs (Hotchkiss et al., 1989a), the times at which the PMN response peaks do not necessarily coincide when the analyses are made by the two procedures. Therefore, the mode of analysis

(BAL versus morphometry) and the time at which this analysis is made need to be taken into account when analyzing the inflammatory response. This recommendation is consistent with the conclusion of Schultheis and Bassett (1991) that BAL does not necessarily reflect cellular changes in the lung interstitium.

Another approach to studying the inflammatory impact of O_3 and its effects on airway permeability is based on exposure of rats to drugs that destroy leukocytes or block the activity of chemical mediators released by these cells. To determine whether the PMNs play a role in O_3-induced increased permeability, Pino et al. (1992b) studied O_3 effects in PMN-depleted rats. Although ip injection of anti-PMN serum resulted in a nearly complete depletion of PMNs in rats, it did not affect the increase in BAL protein following an 8-h exposure to 1.0 ppm O_3. In comparable studies, rats were rendered leukopenic by ip injection of cyclophosphamide (Bhalla et al., 1992). A 2-h exposure of untreated rats to 0.8 ppm O_3 caused a significant increase in the tracheal mucosal permeability, as measured by enhanced trachea-to-blood transport of ^{99m}Tc-radiolabeled DTPA immediately postexposure and accumulation of protein and albumin in BAL at 12 h postexposure. Pretreatment with cyclophosphamide, a potent immunosuppressive agent, which can be toxic to the lung, did not change baseline values but did eliminate the O_3 response. The reasons for the discrepancy between these results and the results of the anti-PMN serum treatment study of Pino et al. (1992b) are not entirely clear, but it is likely that the O_3 effects are dependent, in part at least, on an interaction between different inflammatory cell types. Therefore, it is not unreasonable to assume that, in the absence of PMNs, their role is taken up by another cell type. The attenuation of O_3 effects also was observed in rats pretreated with indomethacin, an inhibitor of cyclooxygenase products, and FPL55712, which blocks LTD_4 activity by preventing its binding to the receptors (Bhalla et al., 1992). Based on these results, it was proposed that, although O_3 is capable of producing direct injury to cells, inflammatory cells and their products may contribute to the injury process (Bhalla et al., 1992). This conclusion is supported by the recent studies of Joad et al. (1993). In the isolated perfused rat lung, PMNs (but not O_3) increased BAL protein concentration. However, PMNs acted synergistically with O_3 in the induction of epithelial injury in the bronchioles. The recent demonstration of the effects of O_3 (0.8 ppm, 2 h) on some of the cellular activities of vascular PMNs (Bhalla et al., 1993) further suggests potential mechanisms involved in the stimulation of PMNs and the induction of inflammatory response. Polymorphonuclear leukocytes isolated from the blood of rats exposed to O_3 displayed shape changes, indicative of cell motility, and greater adhesion to epithelial cells in culture than did the PMNs from rats exposed to purified air.

6.2.2.4 Sensitive Populations

In addition to investigating the inflammatory response and permeability changes in healthy adult animals, studies in recent years have analyzed the effects of O_3 on lung inflammation and airway permeability in different animal species, in potentially susceptible subpopulations, and under special conditions (Table 6-1). Hatch et al. (1986) performed an interspecies comparison to determine their relative responsiveness to O_3. Although the baseline BAL protein concentration of all the species was nearly the same, there were noticeable differences in changes in BAL protein concentration among different species following their exposure to O_3. Significant changes were observed in guinea pigs exposed to 0.2 ppm O_3. Mice, hamsters, and rats responded at O_3 concentrations of 1 ppm and above, but rabbits responded only to 2 ppm O_3. In the case of rats, no differences were observed in the

sensitivity between males and females. When Slade et al. (1989) depleted guinea pigs of lung ascorbic acid, they were more susceptible to an O_3-induced increase in BAL protein when the 4-h exposure was to 0.5 but not 1.0 ppm. Depletion of lung nonprotein sulfhydryls did not enhance susceptibility. Although Hu et al. (1982) report no elevation in BAL protein in O_3-exposed, vitamin C-deficient guinea pigs, when their data were statistically reanalyzed by Slade et al. (1989), vitamin C deficiency enhanced the effects of 0.5 but not 1.0 ppm O_3.

Kleeberger et al. (1990) found interstrain differences in inbred mice with regards to inflammation and permeability changes following high-concentration (2-ppm) O_3 exposure for 3 h that led the investigators to propose that the PMN response to O_3 may be controlled by a single autosomal recessive gene at a chromosomal location designated as "Inf" (inflammation) locus. In the follow-up studies, Kleeberger et al. (1993a) exposed the "susceptible" (C57BL/6J) and the "resistant" (C3H/HeJ) strains of mice to lower concentrations of O_3. Although changes in inflammatory response and BAL protein were observed after exposure for 24 to 72 h to 0.12 as well as 0.3 ppm O_3, the elevation in response in the susceptible strain over that in the resistant strain was observed only at 0.3 ppm O_3. Further studies with recombinant inbred strains of mice suggested that genes at different loci may be responsible for responses to 24-h (Inf locus) and 48-h (Inf-2 locus) O_3 exposures (Kleeberger et al., 1993b).

Gunnison et al. (1992a) exposed rats aged 13 and 18 days and 8 and 16 weeks old to 1 ppm O_3 for 2, 4, or 6 h. In the experiments to be discussed here, BAL was performed immediately after exposure. Ozone exposure resulted in an increase in protein in the BAL and a decrease in the number of leukocytes, but this decrease was not specific for a certain age group. A weak relationship was observed between age and the number of lavageable PMNs; a slightly greater influx of PMNs was observed in the younger rats. A strong inverse relationship was, however, observed between age and leukocyte viability. Approximately 50% of the total leukocytes recovered in the BAL from 13-day-old rats exposed for 6 h were dead, as compared to about 10% dead in the 16-week-old rats. Furthermore, 13-day-old animals were more responsive to a 2-h O_3 exposure than the other age groups of rats in terms of PGE_2 levels in BAL; PGE levels were enhanced more in older animals with the longer exposure durations. The authors attribute the increase in PGE_2 to an increased release of arachidonic acid, rather than an effect on metabolism or formation on PGE_2. Gunnison et al. (1990) also have shown that levels of several eicosanoids in rabbits show a similar pattern of age-responsiveness.

Factors such as physical activity and pregnancy, in addition to age, can modify the airway sensitivity of rats to O_3. Van Bree et al. (1992) have reported circadian variation in response to O_3. In rats exposed to 0.4 ppm O_3 for 12 h, about 70% more PMNs were recovered in the BAL after nighttime exposure than after daytime exposure. This increase was attributed to greater physical activity and increased ventilation in the nocturnal animals. In guinea pigs, a similar difference between daytime and nighttime exposures was not observed; instead, the variations appeared to be related to random physical activity. The nighttime exposures also caused a greater increase in BAL protein and albumin in rats but not in guinea pigs. Gunnison et al. (1992b) have found that pregnant rats are more responsive to O_3 (1 ppm for 6 h) than virgin females, as measured by an enhanced inflammatory response and as detected by the analysis of protein, PMNs, leukocytes, and enzyme activities in BAL at 18 h postexposure. When O_3 exposure occurred on Day 17 of pregnancy or Days 3, 13, and 20 of lactation, the magnitude of the increase in BAL protein and number of PMNs was greater than the magnitude of increase in virgin rats. No such increased responsiveness was observed in

rats at Day 10 to 12 of pregnancy or 14 days after lactation ceased. Enzyme changes followed a similar pattern.

6.2.2.5 Repeated Exposures

The magnitudes of some of the effects of O_3 observed after an acute exposure were smaller following repeated exposure. This phenomenon has been referred to as tolerance, adaptation, or attenuation. Most of the older literature is on tolerance, which classically is defined as the phenomenon in which a previous exposure to a nonlethal concentration of O_3 provides protection against an otherwise lethal level. These studies and others at high O_3 levels are discussed in the last O_3 criteria document (U.S. Environmental Protection Agency, 1986).

Tepper et al. (1989) observed attenuation of pulmonary function changes in rats exposed for 2.25 h/day for 5 days, but a corresponding attenuation of lung inflammation did not occur. Histologic examination of the lung sections revealed substantially more inflammatory cells in alveoli after 5 days of exposure to 0.5 ppm O_3 than after a single exposure to the same O_3 concentration. Increased protein concentration in the BAL observed after a single exposure also persisted after 5 days of repeated exposures. The morphologic studies of Moffatt et al. (1987) identified an inflammatory response in the respiratory bronchioles (RBs) of bonnet monkeys exposed for 8 h/day for 90 days to 0.64 ppm O_3. Significantly greater numbers of AMs, mast cells, and PMNs reflected persistence of inflammation following repeated exposures. Chang et al. (1992) exposed rats to an ambient pattern of O_3. In this morphometric study, the responses (epithelial inflammation in the proximal alveolar region and the TBs, interstitial edema, and infiltration of AMs) to 1 week of O_3 exposure had subsided after 3 and 13 weeks of exposure. Donaldson et al. (1993) did not find a change in the total number of cells in BAL of rats exposed for 7 h/day for 4 days to O_3 concentrations ranging from 0.2 to 0.8 ppm. The number of PMNs, however, increased after exposure to 0.6 and 0.8 ppm O_3. This increase was greatest after the first day of exposure, but it was resolved by Day 4. In the studies of Mochitate et al. (1992), the number of BAL AMs of rats exposed to 0.2 ppm O_3 for 11 weeks was about 60% greater than in the air-exposed controls. The preferential increase in the number of small AMs was not dependent on an enhancement of DNA synthesis. It was concluded that AMs adapt to long-term exposures as a result of recruitment of immature AMs from an influx of monocytes. No increase in the number of PMNs was observed in the BAL of exposed rats.

When analyzing the PMN data from different studies like the ones discussed above, it is important to make a distinction between the PMN response in the lung interstitium versus that observed in the BAL. It is possible that, although the inflammatory response may persist after repeated exposures, the PMNs do not necessarily continue to migrate from pulmonary interstitium into the air spaces. As a result, the inflammatory response is detected in the histological sections of the lung but not in the BAL.

6.2.2.6 Mediators of Inflammation and Permeability

Although the presence of PMNs in the lung in large numbers is regarded as evidence of a morphological response to O_3, the release of chemical mediators by inflammatory cells indicates a state of activation and represents the functional modification as a consequence of O_3 exposure. Mediators with biological and chemotactic properties have been shown to be released as a result of stimulation or injury of AMs, epithelial cells, and PMNs. Arachidonic

acid metabolites play an important role in a variety of processes, including inflammatory response and permeability changes. Driscoll and Schlesinger (1988) found that, although AMs isolated from rabbit lungs continually released chemoattractant factors for monocytes and PMNs, an in vitro exposure of AMs to 0.3 and 1.3 ppm O_3 resulted in the increased secretion of factors that stimulated the migration of PMNs. Driscoll et al. (1988) also found increased eicosanoid biosynthesis following O_3 exposure. In the latter studies, elevated levels of PGE_2 and $PGF_{2\alpha}$ were detected in the supernatant following in vitro exposure of rabbit AMs to 0.3 and 1.2 ppm O_3 for 2 h. In a parallel in vivo study, an effect was seen only at the higher O_3 concentration. In vitro exposure in a roller-bottle system of rat AMs to 1 ppm (but not 0.1 ppm) O_3 causes stimulation of both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism, as shown by substantial increases in the levels of 6-keto-$PGF_{1\alpha}$, thromboxane B_2 (TXB_2), PGE_2, LTB_4, LTD_4, and 15-hydroxy-eicosatetraenoic acid (15-HETE) in the supernatant of the AM culture (Madden et al., 1991). The authors attribute these effects to both an increase in the availability of arachidonic acid and a stimulation of cycloxygenase and lipoxygenase activities. Another in vitro study also demonstrated effects on arachidonic acid metabolism (Leikauf et al., 1988) . Interpretation of these in vitro studies is difficult. When Gunnison et al. (1990) compared the effect of in vitro and in vivo exposures of AM to O_3 on eicosanoid metabolism of AMs in culture, a disparity was found. Cultured AMs from O_3-exposed rabbits had a decrease in the elaboration of $PGF_{2\alpha}$; in vitro exposure caused an increase.

Changes in the levels of eicosanoids also have been observed in vivo studies. Schlesinger et al. (1990) found elevation of PGE_2 and $PGF_{1\alpha}$ in BAL of rabbits immediately following a 2-h exposure to 1 ppm O_3. Age may play a role. Five-week-old (but not 9-week-old) mice had increased levels of PGE_2 in BAL (Gilmour et al., 1993b). Lower O_3 concentrations did not affect the levels of BAL eicosanoids. Hyde et al. (1992) found an increase in BAL concentrations of $PGF_{2\alpha}$, PGD_2, and PGE_2 following an 8-h exposure of monkeys to 0.96 ppm O_3. Prostaglandin concentrations in the BAL, detected using an antibody that did not distinguish PGE_1 from PGE_2, also increased with time following a continuous exposure of mice to 0.5 ppm O_3 (Canning et al., 1991). The peak levels of PGE at 3 days were followed by a decline with time, but the levels remained higher than the controls after 14 days of exposure. The time course of changes in the PGE levels was matched by the time sequence of changes in BAL protein following exposure to 0.5 ppm O_3. Plasma concentrations of 6-keto-$PGF_{1\alpha}$ and PGE_1 also were elevated in guinea pigs exposed for 1 h to 1 ppm O_3 (Miller et al., 1987). Kleeberger et al. (1989) delivered 1 ppm O_3 for 5 min to a lobar bronchus of dogs using a wedged bronchoscope. An analysis of the lavage fluid collected at 1 min postexposure revealed significant increases in the concentrations of PGD_2 and histamine. Although, in this study, the concentration of TXB_2 did not change after O_3 exposure, significant increases in concentrations of TXB_2 in the plasma and BAL were observed following acute exposure of guinea pigs to 1 ppm O_3 (Miller et al., 1987) and humans to lower levels of O_3 (see Chapter 7). In addition, increased plasma concentrations of $PGF_{1\alpha}$ and PGE_1 were observed in the guinea pigs exposed to O_3. Fouke et al. (1990, 1991) were unable to detect changes in the BAL concentrations of 6-keto-$PGF_{1\alpha}$, PGE_2, TXB_2, and $PGF_{2\alpha}$ in baboons and mongrel dogs exposed to 0.5 ppm O_3 for 2 h. The reasons for the lack of this response in the baboon are not entirely clear, but the lower O_3 concentration used in this study, as compared to the exposure concentrations in dogs and guinea pigs, and species differences offer possible explanations for the discrepancy. Prostaglandin E_1 and E_2 have been shown to

influence the inflammatory processes in the lung. Intrabronchial or iv administration of PGE_2 was accompanied by increased accumulation of the inflammatory cells in the lung and elevation of BAL protein (Downey et al., 1988). A possible mechanism involved in the proinflammatory effects of the PGEs included arteriolar vasodilation without venodilation, resulting in increased transfer of proteins and cells from blood into the lung by hydrostatic pressure.

6.2.2.7 Summary

The airway epithelial lining serves as an efficient barrier against penetration of exogenous particles and macromolecules into the lung tissue and circulation and against entry of endogenous fluids, cells, and mediators into the air spaces. Disruption of this barrier following O_3 exposure represents a state of compromised epithelial defenses, leading to increased transepithelial permeability. Inflammatory cells represent another important component of pulmonary defenses. The recruitment of these cells into the lung following O_3 exposure could result in the release of mediators capable of damaging other cells in the lung.

Toxicological studies from several laboratories demonstrate alterations in epithelial permeability and inflammatory responses in animals exposed to O_3 concentrations of 1.0 ppm and below. In these studies, an inflammatory response, as detected by an increase in the number of PMNs in the BAL or in lung parenchyma, was accompanied by either an increased tracer transport across the airway mucosa or an elevation in the levels of total protein or albumin in the BAL. These changes were observed in animals exposed to O_3 concentrations as low as 0.1 ppm in rabbits (2 h/day for 6 days of exposure [Driscoll et al., 1987]; 0.12 ppm in mice (24 h-exposure [Kleeberger et al., 1993a]) and rats (6-h exposure [Hotchkiss et al., 1989a] and 24-h exposure [Guth et al., 1986]); and 0.2 ppm in guinea pigs (4-h exposure [Hatch et al., 1986]). Although monkeys also exhibit inflammatory responses, concentrations this low have not been tested in this species. The magnitude of response and the time at which it peaked appeared to vary with O_3 concentration, exposure duration, and the mode of analysis. Investigations of $C \times T$ relationships for BAL protein in both rats and guinea pigs showed that T had increasing influence as C increased (Rombout et al., 1989; Highfill et al., 1992; Highfill and Costa, 1995; Tepper et al., 1994). However, at low $C \times T$ products, similar increases were observed (Highfill et al., 1992). The responsiveness to O_3 also depended on the animal species tested (Hatch et al., 1986) and increased under certain conditions, such as physical activity (Van Bree et al., 1992) and pregnancy and lactation (Gunnison et al., 1992b).

To determine the impact of inflammatory cells on O_3-induced airway permeability, rats were exposed to drugs that either destroyed the inflammatory cells or blocked the activity of their products. Treatment of rats with anti-PMN serum resulted in the depletion of PMNs but did not affect the increase in BAL protein produced by O_3 exposure (Pino et al., 1992b). Depletion of all the leukocytes by cyclophosphamide or treatment of rats with PG and LT antagonists resulted in an attenuation of the O_3 effects on permeability (Bhalla et al., 1992).

Inflammatory cells, when activated, are capable of releasing mediators with pathophysiologic and a variety of modulating activities. An increase in the release of arachidonic acid metabolites following O_3 exposure has been shown after both in vitro (Driscoll and Schlesinger, 1988; Driscoll et al., 1988; Madden et al., 1991; Leikauf et al., 1988) and in vivo exposures (Schlesinger et al., 1990; Miller et al., 1987; Canning et al., 1991).

Some of the effects seen after an acute exposure to O_3 are modified on repeated exposure. The responses following repeated exposures included persistence or an increase in the number of PMNs or AMs on exposure of rats to 0.5 ppm (2.25 h/day) for 5 days (Tepper et al., 1989) or exposure of monkeys to 0.64 ppm for 90 days (Moffatt et al., 1987). Other studies report a reduced inflammatory response following repeated exposures (Chang et al., 1992; Donaldson et al., 1993). The section on morphometry (Section 6.2.4) has an extended discussion of microscopically evaluated inflammatory responses.

In brief, these studies show that acute exposures to O_3, at concentrations of 0.12 ppm and above, are capable of producing inflammatory and permeability changes in laboratory animals. It is clear that an assessment of the effects of O_3 and interpretation of the results requires that several factors be taken into consideration; these include O_3 concentration, duration of exposure, exposure conditions (e.g., repeated versus continuous exposure, daytime versus nighttime exposure, rest versus exercise during exposure), animal species, method of evaluation, sensitive populations, and time of analysis postexposure.

6.2.3 Effects on Host Defense Mechanisms

6.2.3.1 Introduction

The mammalian respiratory tract has a number of closely integrated defense mechanisms that, when functioning normally, provide protection from the adverse effects of a wide variety of inhaled particles and microbes (Green et al., 1977; Kelley, 1990; Schlesinger, 1989; Sibille and Reynolds, 1990). For simplicity, these interrelated defenses can be divided into two major parts: (1) nonspecific (transport and phagocytosis) and (2) specific (immunologic) defense mechanisms. A variety of sensitive and reliable methods have been used to assess the effects of O_3 on these components of the lung's defense system to provide a better understanding of the health effects associated with the inhalation of this pollutant.

The previous *Air Quality Criteria for Ozone and Other Photochemical Oxidants* (U.S. Environmental Protection Agency, 1986) provided a review and evaluation of the scientific literature published up to 1986 regarding the effects of O_3 on host defenses. This section briefly summarizes the existing database through 1986; describes the data generated since 1986; and, where appropriate, provides interpretations of the data. This section also discusses the various components of host defenses, such as the mucociliary escalator, the phagocytic and regulatory role of the AMs, the immune system, and integrated mechanisms that are studied by investigating the host's response to experimental pulmonary infections.

6.2.3.2 Mucociliary Clearance

This nonspecific defense mechanism removes particles deposited on the mucous layer of the conducting airways by ciliary action. Ciliary movement directs particles trapped on the overlying mucous layer toward the pharynx, where it is swallowed or expectorated. The effectiveness of the mucociliary transport system can be measured by the rate of transport of deposited particles, the frequency of ciliary beating, and the structural integrity of the cells that line the conducting airways. Impaired mucociliary clearance can result in an unwanted accumulation of cellular secretions, increased infections, chronic bronchitis, and complications associated with chronic obstructive pulmonary disease.

Studies cited in the previous criteria document (U.S. Environmental Protection Agency, 1986) provided evidence on the effect of O_3 on the morphologic integrity of the mucociliary escalator and its ability to transport deposited particles from the respiratory tract.

For example, a number of studies with various animal species reported morphologic damage to the cells of the tracheobronchial tree from exposures to O_3 (see Section 6.2.4). The cilia had become noticeably shorter or were completely absent. Based on such morphologic observations, related effects such as ciliostasis, increased mucous secretion, and a slowing of mucociliary transport rates might be expected. Functional studies on mucociliary transport of deposited particles from the respiratory tract have, in general, observed a delay in particle clearance in early time periods following acute exposure. For example, a 4-h exposure of rats to 0.8 ppm O_3 slowed early clearance of inhaled latex spheres (Phalen et al., 1980).

Since the publication of the previous criteria document (U.S. Environmental Protection Agency, 1986), several studies have been performed on the effects of acute O_3 exposure on the mucociliary transport apparatus (Table 6-6). Retarded mucociliary particle clearance was observed following a 2-h exposure of rabbits to 0.6 ppm O_3; extended exposures (up to 14 days) caused no effects (Schlesinger and Driscoll, 1987). Acute exposure of adult sheep for 4 h/day for 2 days to 0.5 ppm O_3 increased basal secretion of glycoproteins in sheep trachea, whereas a longer exposure (4 h/day, 5 days/week for 6 weeks) to 0.5 ppm O_3 reduced tracheal glycoprotein secretions (Phipps et al., 1986). In a similar manner, continuous exposure of ferrets to 1.0 ppm O_3 for 3 days increased tracheal gland secretion of glycoproteins, which remained elevated following 7 days of exposure (McBride et al., 1991). Because the integrity of the periciliary space is vital for efficient mucociliary action, O_3-induced hyper- or hyposecretion by the mucous glands along the conducting airways can alter the effectiveness of the mucociliary escalator.

Mariassy et al. (1990) exposed sheep during the first week of life to 1.0 ppm O_3 for 4 h/day for 5 days and observed retardation of normal morphologic development of the tracheal epithelium and a decrease in the tracheal mucous velocity. In a similar manner, exposure of sheep during the first week of life for 4 h/day for 5 days to 1.0 ppm O_3 decreased epithelial mucosa density and retarded the developmental decrease of tracheal mucous cells and their carbohydrate composition (Mariassy et al., 1989). Finally, exposure of adult sheep for either 2 h or for 5 h/day for 4 days to 1.0 ppm O_3 decreased tracheal mucous velocity (Allegra et al., 1991).

6.2.3.3 Alveolobronchiolar Transport Mechanism

In addition to the transporting of particles deposited on the mucous surface layer of the conducting airways, particles deposited in the deep lung may be removed either up the respiratory tract or through interstitial pathways to the lymphatic system (Green, 1973). The pivotal mechanism of alveolobronchiolar transport involves the movement of AMs with phagocytized particles to the bottom of the mucociliary escalator. Failure of the AMs to phagocytize and sequester the deposited particles from the vulnerable respiratory membrane can lead to particle entry into the interstitial spaces. Once lodged in the interstitium, particle removal is more difficult and, depending on the toxic or infectious nature of the particle, its interstitial location may allow the particle to set up a focus for pathologic processes. Although Phalen et al. (1980) and Kenoyer et al. (1981) observed decreases in early (tracheobronchial) clearance after acute O_3 exposure of rats; late (alveolar) clearance was accelerated.

Table 6-6. Effects of Ozone on Host Defense Mechanisms: Physical Clearance[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, spike rising to 0.25	118 base, spike rising to 490	13 h/day, 7 days/week base; ramped spike 9 h/day, 5 days/week; 6 weeks	Rat, M (F344) 35 days old	Increased retention of asbestos fibers in the lung parenchyma.	Pinkerton et al. (1989)
0.1 0.6 1.2	196 1,176 2,352	2 h/day for 1 or 13 days	Rabbit, M (NZW) 2.5-3.0 kg	Acceleration of early alveolar particle clearance at 0.1 and 0.6 ppm for 13 days. After single exposure, increased clearance at 0.1 ppm and decrease at 1.2 ppm.	Driscoll et al. (1986)
0.1 0.6 1.2	196 1,176 2,352	2 h/day for 1 or 13 days	Rabbit, M (NZW) 2.5-3.0 kg	Acceleration of early alveolar particle clearance at 0.1 and 0.6 ppm for 13 days. After single exposure, increased clearance at 0.1 ppm and decrease at 1.2 ppm	Driscoll et al. (1986)
0.1 0.25 0.6	196 490 1,176	2 h/day for 14 days	Rabbit, M (NZW) 2.5-2.7 kg	Retarded mucociliary particle clearance at 0.6 ppm only following a single 2-h exposure; no effect of 14-day exposure.	Schlesinger and Driscoll (1987)
0.5	980	4 h/day, 5 days/week for 6 weeks	Sheep, F 23-41 kg	Increase of tracheal glycoprotein secretion following acute exposure (4 h/day for 2 days) with a decrease following longer term exposure.	Phipps et al. (1986)
1.0	1,960	24 h/day for 7 days	Ferret	Increased secretion of glycoconjugates by tracheal glands.	McBride et al. (1991)
1.0	1,960	4 h/day for 5 days	Sheep 1st week of life	Retardation of normal morphologic development of the tracheal epithelium. Decreased tracheal mucous velocity. Decreased tracheal mucosa epithelial density. Retardation of developmental decrease of tracheal mucous cells and their carbohydrate composition.	Mariassy et al. (1989, 1990)
1.0	1,960	2 h and 5 h/day for 4 days	Sheep 26-41 kg	Decreased tracheal mucous velocity.	Allegra et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Exposure of rabbits for 2 h/day for 13 days to 0.1 and 0.6 ppm O_3 resulted in acceleration of early alveolar clearance of polystyrene latex particles (Driscoll et al., 1986). After a single exposure to 0.1 ppm, the greatest acceleration occurred over the period shortly after exposure ceased (1 to 4 days), although the effect was still observed at Day 14 postexposure. A single exposure to 0.6 ppm caused no effect, whereas a higher concentration (1.2 ppm) retarded clearance. To investigate the effects of longer term O_3 exposure on alveolobronchiolar clearance, rats were exposed to an urban pattern of O_3 (continuous 0.06 ppm, 7 days/week with a slow rise to a peak of 0.25 ppm and subsequent decrease to 0.06 ppm over a 9-h period for 5 days/week) for 6 weeks and were exposed 3 days later to chrysotile asbestos, which can cause pulmonary fibrosis and neoplasia (Pinkerton et al., 1989). Ozone did not affect the deposition of asbestos at the first AD bifurcation, the site of maximal asbestos and O_3 deposition. However, 30 days later, the lungs of the O_3-exposed animals had twice the number and mass of asbestos fibers as the air-exposed rats.

6.2.3.4 Alveolar Macrophages

Within the gaseous exchange region of the lung, the first line of defense against microorganisms and nonviable particles that reach the alveolar surface is the AM. This resident phagocyte is responsible for a variety of activities, including the detoxification and removal of inhaled particles, maintenance of pulmonary sterility, and interaction with lymphocytes for immunologic protection. Under normal conditions, AMs seek out particles deposited on the alveolar surface and ingest them, thereby sequestering the particles from the vulnerable respiratory membrane. If the particle is insoluble, the AMs serve as a repository for the transport of the particle from the alveolus to the bottom of the mucociliary escalator located at the far distal portion of the conducting airways. Degradable particles are detoxified by powerful lysosomal enzymes, whereas microorganisms are killed by biochemical mechanisms, such as superoxide anion radicals and lysosomal enzymes. To adequately fulfill their defense function, the AMs must maintain active mobility, a high degree of phagocytic activity, and an optimally functioning biochemical and enzyme system.

As discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986), short periods of O_3 exposure can cause a reduction in the number of free AMs available for pulmonary defense, and these AMs are more fragile, less phagocytic, and have decreased lysosomal enzyme activities. The lowest O_3 concentration showing AM effects in this early work was 0.25 ppm; a 3-h exposure of rabbits decreased lysosomal enzyme activities (Hurst et al., 1970).

Since the publication of the previous criteria document, the studies performed have been, in general, confirmatory of previous observations (Table 6-7). Morphologic observations showed that continuous exposure for 7 days to 0.13, 0.25, 0.5, and 0.77 ppm O_3 resulted in concentration-related increases in the number of rat AMs at 5 days postexposure, as well as increased AM size and morphologic changes consisting of surface microvilli and bleb formation (Dormans et al., 1990). Other morphological studies discussed in Section 6.2.4 also show increased numbers of AMs. A 2-h exposure to 0.1 ppm O_3 did not affect the AM number in the BAL when the analyses were made immediately postexposure, but, 7 days later, the total number of AMs increased by about 70% (Driscoll et al., 1987). On repeated exposure for 6 or 13 days, the number of AMs increased on the day after exposure. A single exposure to a higher concentration (1.2 ppm O_3) did not affect the number of AMs when assessed immediately after exposure. It is assumed that, although

Table 6-7. Effects of Ozone on Host Defense Mechanisms: Macrophage Alterations[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.2 0.4	98 196 392 784	16 h	Rat, M (Wistar) 210 ± 10 (SD)g	Increased adherence to nylon fibers at 0.05 and 0.1 ppm, but not at 0.2 and 0.4 ppm.	Veninga and Evelyn (1986)
0.1 0.2	196 392	Continuous for 11 weeks	Rat, M (Wistar) 16-21 weeks old	Increases in enzyme activity.	Mochitate et al. (1992)
0.1 1.2	196 2,352	2 h/day for 1, 2, 6, and 13 days	Rabbit, M (NZW) 2-4 mo old	Single exposure: Decreased phagocytosis at 0.1 and 1.2 ppm immediately and 1 day after exposure; recovery by 7 days after exposure in the 0.1-ppm group. Multiple exposure to 0.1 ppm only: decreased phagocytosis 1 day after 2 and 6 days of exposure.	Driscoll et al. (1987)
0.1 1.2	196 2,352	2 h	Rabbit (NZW)	Increased release of PGE_2 and $PGF_{2\alpha}$ at 1.2 ppm; no effect at 0.1 ppm.	Driscoll et al. (1988)
0.11 to 3.6	216 to 7,056	3 h	Mouse, M (Swiss) Rat, F (S-D)	Concentration-dependent decrease in superoxide anion radical production; mouse more sensitive. No effect on murine AM phagocytosis at 0.42, 0.95, 1.0, and 1.2 ppm.	Ryer-Powder et al. (1988) Amoruso et al. (1989)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344) 12-18 weeks old	At 42 and 66 h PE, concentration-dependent increase in mitotic index beginning at 0.8 ppm; increased size at 1.5 ppm at 18 and 42 h PE.	Hotchkiss et al. (1989b)
0.12 0.27 0.8	235 529 1,568	6 h	Rat, F (F344) 12-14 weeks old	Increase in mitotic index and chromosome damage at 0.27 and 0.8 ppm, no effect at 0.12 ppm.	Rithidech et al. (1990)
0.12 0.25 0.5	235 490 980	20 h/day for 1, 2, 3, 7, and 14 days	Rat, M (F433)	Increase in AM DNA synthesis at 2 and 3 days at 0.25 ppm O_3 and at 1, 2, and 3 days at 0.50 ppm O_3.	Wright et al. (1987)
0.13 to 0.77	250 to 1,500	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Concentration-related effects on number, size, and surface morphology.	Dormans et al. (1990)
0.13 0.26 0.51 0.77	250 500 1,000 1,500	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Decreased phagocytic ingestion (at all concentrations) and intracellular killing (at 0.26 ppm) of *Listeria monocytogenes*.	Van Loveren et al. (1988)
0.2	392	Continuous for 14 days	Rat, M (Wistar) 10 weeks old	Increases in enzyme activity at 3 days. Increased number of AMs by Day 3.	Mochitate and Miura (1989)

Table 6-7 (cont'd). Effects of Ozone on Host Defense Mechanisms: Macrophage Alterations[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.4	784	3, 6, or 12 h; 12 h/day for 1, 3, or 7 days	Mouse, M (NIH) 23-28 g Rat, M (Wistar) 180-200 g	Decreased Fc-receptor mediated phagocytosis of AMs from mice at Days 1 and 7. Decreased phagocytosis at 6 h, increased phagocytosis of AMs from rats on Day 1. Decrease in superoxide anion production at some exposures.	Oosting et al. (1991a)
0.4	784	6 or 12 h, or 12 h/day for 3 or 7 days	Mouse, M (NIH) 25-30 g Rat, M (Wistar) 200-250 g	Increased ATP levels in mouse AMs only after 7 days of exposure.	Oosting et al. (1991b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old	Decreased phagocytosis of *Streptococcus zooepidemicus* (in vivo assay) and latex beads (in vitro assay). C57B1 mice more susceptible than C3H at 0.4 ppm only for bacteria phagocytosis, reverse susceptibility for latex beads.	Gilmour et al. (1993a)
0.4 0.8	784 1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Decreased in vivo phagocytosis of *Streptococcus zooepidemicus* independent of age. Decrease in number of phagocytic cells and number of bacteria/AM.	Gilmour et al. (1993b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old Rat, F (F344)	Decreased phagocytosis of latex beads.	Gilmour and Selgrade (1993)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-25 g	Decreased Fc-receptor mediated phagocytosis of AMs at Days 1-14, with trend towards maximal decrease on Day 3. Effect correlated with increases in PGE. Decreased phagocytosis of peritoneal AMs.	Canning et al. (1991)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-25 g	Decreased Fc-receptor mediated phagocytosis on Days 1, 3, and 7, but not at Day 14.	Gilmour et al. (1991)
0.64	1,254	23 h/day for 27 days	Rat, M (S-D) 130-150 g	Decreased lysozyme enzyme content during chronic *Pseudomonas aeruginosa* bacterial infection.	Sherwood et al. (1986)
1.0	1,960	2 h/day for 3 days	Rabbit, M (NZW) 14 weeks old	Decreased cytotoxicity vs. xenogeneic tumor cells. No effect on TNF- and H_2O_2 production. Depression of superoxide anion production immediately after exposure, with an increase at 24 h.	Zelikoff et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

the lower O_3 concentrations are stimulatory for AMs activity, the higher concentrations are inhibitory because of their ability to produce substantial cellular injury. The number of AMs in BAL is reduced after a single exposure to 0.8 or 1.8 ppm O_3 (Bassett et al., 1988b; Bhalla and Young, 1992; Donaldson et al., 1993). Hotchkiss et al. (1989b) also observed a slight decrease in the number of AMs in the BAL immediately after a 6-h exposure to 0.8 and 1.5 ppm O_3, but the number of AMs increased at 42 and 66 h after the exposure. The results of the studies on AMs in general indicate that, following O_3 exposure, there is a short-term reduction in the number of lavageable AMs, but these cells increase in number over several days following exposure.

Exposure of rats to 0.12, 0.8, and 1.5 ppm O_3 for 6 h also resulted in a concentration-dependent increase in mitotic index at 0.8 ppm at 92 h after postexposure; AM size was only increased at the highest concentration (18 and 48 h postexposure) (Hotchkiss et al., 1989b). In a similar study, exposure of rats for 6 h to 0.12, 0.27, and 0.8 ppm O_3 resulted in AM chromosome damage at the two higher concentrations 28 h after exposure (Rithidech et al., 1990).

Several studies have investigated the effect of O_3 exposure on AM phagocytosis. Exposure of C3H/HeJ and C57Bl/6 mice for 3 h to 0.4 and 0.8 ppm O_3 decreased AM phagocytosis of *Streptococcus zooepidemicus* and latex beads (Gilmour et al., 1993a). In a similar study, a 3-h exposure of 5- and 9-week-old CD-1 mice decreased AM phagocytosis of *S. zooepidemicus*, but there was no effect of age (Gilmour et al., 1993b). Decreased phagocytic ingestion of *Listeria monocytogenes* also was observed following continuous exposure of rats to 0.13, 0.26, 0.51, and 0.77 ppm for 7 days; only the two lower concentrations inhibited intracellular killing (Van Loveren et al., 1988). Although lower O_3 concentrations were not tested, rats exposed to 1.02 ppm O_3 were unable to clear *Listeria* from their lungs. Exposure of rabbits to 0.1 ppm O_3 for 2 h/day resulted in decreased AM phagocytosis of latex microspheres after 2 or 6 (but not 13) days of exposure (Driscoll et al., 1987). In the same study, a single exposure to 0.1 or 1.2 ppm decreased AM phagocytosis immediately after exposure; recovery occurred by 7 days postexposure in the 0.1-ppm group but not in the 1.2-ppm group. That repeated exposures to O_3 results in an initial suppression of AM phagocytosis, which is followed by recovery of phagocytic potential while exposure continues (Driscoll et al., 1987), was confirmed by the studies of Gilmour et al. (1991) and Canning et al. (1991). Using identical exposure systems, it was observed that continuous exposure of mice to 0.5 ppm O_3 decreased AM Fc-receptor-mediated phagocytosis of sheep erythrocytes on Days 1, 3, 5, 7, and 8 of exposure, with return to control phagocytic levels by Day 14. This temporal trend paralleled the pattern of O_3-induced reduction of lung bactericidal activity against *Staphylococcus aureus*.

Interspecies comparisons of AM phagocytic potential were made by Gilmour and Selgrade (1993), who exposed C3H/HeJ and C57Bl/6 mice and Fischer 344 rats to 0.4 and 0.8 ppm O_3 for 3 h. Alveolar macrophage phagocytosis of latex beads was suppressed in all animals immediately after 0.4 ppm O_3 exposure, with the percent suppression greater in both strains of mice as compared to similarly treated rats. No differences in phagocytic suppression were observed between 0.4- and 0.8-ppm-O_3-exposed rats or the C57Bl/6 mice, but phagocytosis by AMs from 0.8-ppm-O_3-exposed C3H/HeJ mice was more suppressed as compared to the 0.4-ppm-O_3-exposed group. In a similar comparative study, Oosting et al. (1991a) exposed mice and rats to 0.4 ppm O_3 for single (3, 6, and 12 h) and repeated (12 h/day for 7 days) regimens. A decrease was observed in rat and mouse AM Fc-receptor mediated

phagocytosis following the single O_3 exposure protocol. With the repeated O_3 exposure protocol, rat AM phagocytosis was increased a day after exposure with no significant changes on Days 3 and 7. In contrast, phagocytosis by mouse AMs was suppressed at Day 1 of exposure and still did not recover at Day 7. In the same study, when mice were allowed to recover for 4 days following 3 days of O_3 exposure, phagocytosis by AMs was increased. These interspecies comparisons on the effect of O_3 exposure on AM phagocytic potential indicate that mice may be more susceptible than rats.

A species comparison of superoxide anion radical production between mouse AMs and rat AMs following a single 3-h exposure to O_3 concentrations ranging from 0.11 to 3.6 ppm showed the O_3 concentration that inhibits superoxide anion radical production by 50% to be 0.41 ppm for mouse AMs and 3.0 ppm for rat AMs (Ryer-Powder et al., 1988). Oosting et al. (1991a) also found a species difference in superoxide anion production using a more varied exposure-duration protocol; mice appeared to be more responsive than rats. This oxygen radical is important in antibacterial activity, and both sets of authors suggest that O_3-induced impairment of pulmonary antibacterial defenses may be related to decreases in superoxide anion radical production. Decreased lysozyme enzyme levels in rat AMs also were observed during chronic *Pseudomonas aeruginosa* bacterial infection following exposure to 0.64 ppm O_3 for 23 h/day for 27 days (Sherwood et al., 1986).

Exposure of rats for 16 h to 0.05, 0.1, 0.2, and 0.4 ppm O_3 increased AM adherence to nylon fibers at 0.05 and 0.1 ppm, but had no effect at 0.2 and 0.4 ppm (Veninga and Evelyn, 1986). Increased metabolic activity of AMs retrieved from rats following continuous O_3 exposure for 14 weeks to 0.1 and 0.2 ppm was observed by Mochitate et al. (1992). In a similar study, long-term O_3 exposure of rats (continuous 0.2 ppm for 11 weeks) continued to increase AM metabolic activity (Mochitate and Miura, 1989). Exposure of mice and rats for 14 h/day for 7 days to 0.4 ppm O_3 also increased adenosine triphosphate (ATP) levels in the mouse AMs, but had no effect on ATP levels in rat AMs (Oosting et al., 1991b).

In addition to their phagocytic function and particle removal, AMs also play several other roles in host defense that include (1) a regulatory role through their release of mediators (soluble substances secreted by the AMs that produce biologic effects on other cells) such as tumor necrosis factor, interleukin-1, and PGs; (2) activities associated with tumor surveillance; and (3) accessory cell function in antigen presentation to lymphocytes in the initiation of the immune response. Investigating the effect of a single 2-h exposure of rabbits to 0.1 and 1.2 ppm O_3, Driscoll et al. (1988) observed an increased release of PGE_2 and $PGF_{2\alpha}$ by AMs following exposure to 1.2 but not 0.1 ppm. Prostaglandin E_2 can depress AM and natural killer cell cytotoxicity to tumor cells. Perhaps this is a mechanism involved in the depression of AM-mediated cytotoxicity toward xenogeneic tumor cells following exposure of rabbits for 2 h/day for 3 days to 1.0 ppm O_3 (Zelikoff et al., 1991). No studies were found on the effects of O_3 on antigen presentation.

6.2.3.5 Immunology

In addition to the above nonspecific defense mechanisms, the respiratory system also has specific immunologic mechanisms that can be initiated by inhaled antigens. There are two types of immune mechanisms: antibody (humoral)-mediated and cell-mediated.
In general, humoral mechanisms neutralize viruses and microbial toxins, enhance the ingestion of bacteria by phagocytes, and play an important role in defense of the lung against fungal and parasitic infections. Cell-mediated mechanisms enhance the microbiocidal capacity of AMs in

defense to intracellular bacteria such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, whereas another arm of the cellular immune response generates a class of lymphocytes that are cytotoxic for virus-infected cells. Both the humoral and cell-mediated responses protect the respiratory tract against infectious agents and operate in three major temporal waves: (1) natural killer cells (nonspecific lymphocytes that can destroy bacteria, viruses, and tumor cells), (2) cytotoxic T lymphocytes (lymphocytes that lyse specifically recognized targets), and (3) antigen-specific antibodies.

Little information was available in the previous criteria document (U.S. Environmental Protection Agency, 1986) on the effects of O_3 on immunologic defenses. However, the data base indicated an immunotoxic effect of O_3 exposure, especially on T-cell populations. For example, Aranyi et al. (1983) found that a 90-day (5 h/day, 5 days/week) exposure of mice to 0.1 ppm O_3 suppressed blastogenesis of splenic lymphocytes to T-cell, but not B-cell mitogens; the ability of these cells to produce antibodies was not affected either. As can be seen from Table 6-8, this database has greatly expanded and also has been recently reviewed (Jakab et al., 1995). Many of the studies include both the pulmonary and systemic immune system, which, to a degree, are compartmentalized; both systems are discussed here.

Studies on the effect of O_3 exposure on the immune system can be divided into three broad categories. These are (1) measurement of lymphoid organ weights and cellular composition, (2) determination of the functional capacity of lymphocytes in the absence of antigenic stimulation, and (3) measurement of the immune response following antigenic stimulation.

Dziedzic and White (1986a) observed that exposure of mice to 0.3, 0.5, and 0.7 ppm O_3 for 20 h/day for 28 days resulted in a concentration-dependent initial depletion of cells in the mediastinal lymph nodes (MLNs) (Days 1 and 2); this was followed by a T-cell hyperplasia peaking about Days 3 and 4. There was an enhanced blastogenic response to the T-cell mitogen concanavalin A (ConA) at 0.7 ppm O_3 (only level tested). There was no effect of O_3 on cell division morphology of B cells. In a similar study, exposure of mice to 0.7 ppm O_3 for 20 h/day for 28 days resulted in an initial thymic atrophy, with return to normal thymus weights by Day 14 of exposure (Dziedzic and White, 1986b). Exposure of rats to 0.5 ppm O_3 for 20 h/day for 14 days also increased bronchus-associated lymph node and MLN cell proliferation at 2 and 3 days of exposure, but not at 1, 7, and 14 days of exposure (Dziedzic et al., 1990). Bleavins and Dziedzic (1990) observed that exposure of BALB/c mice to 0.7 ppm O_3 for 20 h/day for 14 days resulted in decreased spleen and thymus weights at Day 4, with recovery at Day 14. The absolute number of thymocytes decreased following exposure of mice to 0.7 ppm O_3 for 24 h/day for 7 days (Li and Richters, 1991a) and to 0.3 ppm O_3 for 24 h/day for 3 weeks (Li and Richters, 1991b). Although the latter exposure protocol (0.3 ppm for 24 h/day for 3 weeks) decreased the absolute number of thymocytes, an increase in the percentage of thymocytes was observed in the absence of any changes in splenic T cells (Li and Richters, 1991b). Continuous exposure of rats to 0.26 ppm O_3 for 7 days increased MLN T:B lymphocyte ratios immediately and 5 days postexposure (Van Loveren et al., 1988). Bleavins and Dziedzic (1990) observed an increased infiltration of Thy-1.2$^+$ lymphocytes and IgM$^+$ cells into the O_3-induced pulmonary lesion following exposure of mice to 0.7 ppm O_3 for 20 h/day for 14 days. The lesion was defined by quantitative histomorphometric analysis as any lung area with inflammatory cell infiltration, cellular proliferation, consolidation, or edema. Dziedzic and White (1987a) further investigated these T-cell effects by exposing normal and athymic nude

Table 6-8. Effects of Ozone on Host Defense Mechanisms: Immunology[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.06 base, spike rising to 0.25	118 base, spike rising to 490	13 h/day, 7 days/week base; ramped spike 9 h/day, 5 days/week	Rat, M (F344) 60 days old	No effect on splenic NK cell activity or splenic lymphocyte blastogenic response to T-cell mitogens (PHA and ConA) or B-cell mitogen (*S. salmonella* glycoprotein).	Selgrade et al. (1990)
0.1 0.5 1.0	196 980 1,960	23.5 h/day for 10 days	Rat, M (F344) 8-12 weeks old	Decreased lung NK cell activity at 1.0 ppm on Days 1, 5, and 7, with recovery at Day 10; decreased NK cell activity at 0.5 ppm on Day 1 (only day tested).	Burleson et al. (1989)
0.13 0.26 0.51 0.77 1.02	250 500 1,000 1,500 2,000	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	At 0.26 ppm, increased T:B cell ratios in MLN. Decreased T:B cell ratios in MLN and delayed-type hypersensitivity response following *Listeria monocytogenes* immunization.	Van Loveren et al. (1988)
0.2 0.4 0.82	400 800 1,600	24 h/day for 7 days	Rat, M (Wistar) NS	Decreased lung NK cell activity at 0.82 ppm; increased lung NK cell activity at 0.2 and 0.4 ppm.	Van Loveren et al. (1990)
0.3	588	24 h/day for 3 weeks	Mouse, F (BALB/c) 3 weeks old	Increase in percentage of thymocytes with lower absolute numbers. No changes in splenic T-lymphocytes.	Li and Richters (1991b)
0.3 0.5 0.7	588 980 1,372	20 h/day for 28 days	Mouse, F (CD-1)	Concentration- dependent MLN cell depletions (Days 1 and 2) followed by T-cell hyperplasia (Days 4, 7, 14, and 28). Enhanced blastogenic response to T-cell mitogen ConA at 4 and 7 days at 0.7 ppm (other levels not tested).	Dziedzic and White (1986a)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss)	Decreased antiviral serum antibody following influenza virus infection and decreased T-cells in lung tissue.	Jakab and Hmieleski (1988)
0.5	980	20 h/day for 14 days	Rat, M (F344) 11 weeks old	BALT and MLN cell proliferation at 3 days, but not at Days 1, 2, 7, and 14.	Dziedzic et al. (1990)
0.7	1,372	20 h/day for 28 days	Mouse, F (CD-1)	MLN hyperplasia. Thymic atrophy through Day 7 with return to control level at Day 14.	Dziedzic and White (1986b)
0.7	1,372	24 h/day for 7 days	Mouse, F (BALB/c) 5-12 weeks old	Decrease in the absolute number of thymocytes.	Li and Richters (1991a)
0.7	1,372	20 h/day for 7 and 14 days	Mouse, F (Athymic and euthymic)	Compared to euthymic (nu/+) mice, athymic nude (nu/nu) mice have increased lung inflammation and lesion volume, but no MLN hyperplasia.	Dziedzic and White (1987a)

Table 6-8 (cont'd). Effects of Ozone on Host Defense Mechanisms: Immunology[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.7	1,372	20 h/day for 14 days	Mouse, F (BALB/c) 10-12 weeks old	Decreased spleen and thymus weight at Day 4 with recovery at Day 14. Increased infiltration of Thy-1.2$^+$ lymphocytes and IgM$^+$ cells into the O_3-induced pulmonary lesion.	Bleavins and Dziedzic (1990)
0.8	1,568	23 h/day for 14 days	Mouse, F ($B6C3F_1$) 6-8 weeks old	Decreased blastogenesis of MLN and splenic lymphocytes to PHA mitogen on Day 1; no effect on Days 3, 7, and 14. Decreased splenic NK cell activity on Days 1 and 3; no effect on Days 7 and 14. Decreased pulmonary IgG and IgA response to ovalbumin immunization.	Gilmour and Jakab (1991)
0.8	1,568	24 h/day for 14 days	Mouse, M (BALB/c) 2-3 mo old	Suppression of delayed-type hypersensitivity response to SRBCs on Day 7; no effect on Days 1, 3, and 14. Thymic atrophy on Day 7 with decreased Thy-1.2$^+$ cells in thymus.	Fujimaki et al. (1987)
0.8	1,568	24 h/day for 4 weeks	Mouse, M (BALB/c) 7 weeks old	Suppression of serum IgG following ovalbumin immunization.	Ozawa (1986)
1.0	1,960	24 h/day for 4 weeks	Mouse, M (C57B/6 × DBA/2 F1) 11-14 weeks old	Decreased ability of spleen cells to generate a primary antibody response to SRBCs in vitro.	Wright et al. (1989)
1.0	1,960	8 h/day for 7 days	Rat, M (Long-Evans) 7-8 weeks old	No effect on lung and splenic ADCC activity. Enhanced blastogenesis of splenic lymphocytes to PHA, ConA, and lipopolysaccharide mitogens at 1.0 ppm.	Eskew et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

mice to 0.7 ppm O_3 for 7 or 14 days (20 h/day). After 7 days of exposure, the athymic nude mice did not have the MLN hyperplasia seen in the euthymic mice. However, the athymic nude mice had a greater inflammatory response and an increase in lung lesion volumes compared to the euthymic mice. Thus, it appears that T cells have some involvement in protecting the lungs from the morphological effects of O_3.

The above longitudinal studies on the effects of O_3 exposure on lymphoid organ cell numbers provide information on cellular traffic and cell numbers but provide few insights into the functional capacity of the lymphocytes. A number of studies have investigated the effect of O_3 exposure on the blastogenic response of lymphocytes to nonspecific mitogens. These assays measure nonspecific clonal expansion of the lymphocyte population, a critical step during the amplification of the immune response. Exposure of mice to 0.7 ppm O_3 for 20 h/day for 28 days enhanced the MLN cell blastogenic response to the T-cell mitogen ConA at 4 and 7 days of exposure, with return to control levels by Day 14 (Dziedzic and White, 1986a). In a similar manner, Gilmour and Jakab (1991) observed that continuous exposure of mice to 0.8 ppm O_3 decreased the MLN and splenic lymphocyte blastogenic response to the T-cell mitogen phytohemagglutinin (PHA) on Day 1 of exposure, with the effect abrogated after prolonged exposure. An enhanced blastogenic response of splenic lymphocytes to PHA and Con A and a B-cell mitogen (*Escherichia coli* lipopolysaccharide) was observed following exposure of rats to 1.0 ppm O_3 for 8 h/day for 7 days (Eskew et al., 1986).

Natural killer cell activity also has been studied. One such study in rats observed a decreased lung NK cell activity following 1, 5, and 7 days of exposure to 1.0 ppm for 23.5 h/day, with recovery by Day 10 (Burleson et al., 1989). In a similar experiment, Van Loveren et al. (1990) observed decreased lung NK cell activity following 7 days of continuous exposure of rats to 0.82 ppm O_3; however, exposure to 0.2 and 0.4 ppm enhanced lung NK cell activity. Exposure of mice for 23 h/day for 14 days to 0.8 ppm O_3 also decreased splenic NK cell activity on Days 1 and 3, with a return to control values on Days 7 and 14 (Gilmour and Jakab, 1991). Finally, Selgrade et al. (1990) used an experimental protocol designed to mimic diurnal urban O_3 exposure patterns. Rats were exposed to a background level of 0.06 ppm for 13 h (7 days/week), followed by a broad exposure spike (5 days/week) rising from 0.06 to 0.25 ppm and returning to 0.06 ppm over 9 h, and then followed by 2-h downtime. After 1, 3, 13, 52, or 78 weeks of exposure, spleen cells were assessed for NK cell activity and responses to T-cell mitogens (PHA and ConA) and a B-cell mitogen (*Salmonella typhimurium* glycoprotein). Ozone exposure had no effect on NK cell activity, nor were there any O_3-related changes in mitogen responses in splenic or blood leukocytes. There were also no effects of a single 3-h exposure to 1.0 ppm O_3 on spleen cell responses to the mitogens immediately after exposure or at 24, 48, and 72 h thereafter.

Several studies also have investigated the effect of O_3 exposure on the immune response following antigenic stimulation. Fujimaki (1989) observed that exposure of mice to 0.8 ppm O_3 for 24 h/day for 56 days suppressed the primary splenic antibody response to sheep red blood cells (SRBCs; T-cell-dependent antigen) but not to DNP-Ficoll (T-cell-independent antigen). In a similar study, exposure of mice to 0.8 ppm O_3 for 24 h/day for 14 days suppressed the delayed type hypersensitivity response to SRBCs on Day 7, but not on Days 1, 3, and 14 (Fujimaki et al., 1987). Suppression of serum IgG levels on ovalbumin immunization was observed following exposure of mice to 0.8 ppm O_3 for 24 h/day for 4 weeks (Ozawa, 1986). Decreased pulmonary IgG and IgA responses on ovalbumin immunization were also observed in mice during a 2-week O_3 exposure for 23 h/day to

0.8 ppm (Gilmour and Jakab, 1991). Exposure of mice to 0.5 ppm O_3 continuously for 14 days during the course of influenza virus infection also decreased the serum hemagglutinin antiviral antibody response (Jakab and Hmieleski, 1988).

Van Loveren et al. (1988) investigated antigen-specific responses following pulmonary *Listeria* infection and observed no significant changes in the delayed-type hypersensitivity response when O_3 exposure (continuous, 0.77 ppm) was for 7 days prior to infection. However, if the O_3 exposure took place when an infection with *Listeria* was also present (from Days 0 to 7 or from Days 7 to 14), the delayed-type hypersensitivity response was significantly decreased. In a similar manner, no significant changes were observed in the splenic lymphoproliferative response to *Listeria* antigen when the 0.77-ppm O_3 exposure preceded the infection, whereas the response was suppressed when the 0.77-ppm O_3 exposure occurred immediately after infection or from Days 7 to 14 after infection. In the same series of experiments, Van Loveren et al. (1988) observed that continuous exposure to 0.26 ppm O_3 impaired the increase in T:B lymphocyte ratios that occurred in response to the *Listeria* infection.

6.2.3.6 Interaction with Infectious Agents

Because respiratory infections remain one of the most common public health problems, it is important to determine whether or not exposure to air pollutants reduce susceptibility to infectious agents. Measurement of the competence of the host's antimicrobial mechanisms can best be tested by challenging air-pollutant-exposed animals and the clean-air-exposed control animals to an aerosol of viable organisms. If the test substance, such as O_3, decreases the efficiency of the host's integrated protective mechanisms (i.e., physical clearance via the mucociliary escalator, microbicidal activity of the AMs, and associated humoral and cellular immunologic events), the microorganisms are less efficiently killed in the lungs, or the organisms may even multiply, resulting in the demise of the host. The defensive function of the lung is remarkably similar across animals species, and available human data suggests that qualitative findings obtained on functional resistance mechanisms using appropriate animal models may be extrapolated to humans (Green, 1984).

The studies detailed in the previous criteria document (U.S. Environmental Protection Agency, 1986) primarily used the mouse "infectivity model" (Gardner, 1982). Briefly, animals are randomly selected to be exposed to either clean air or O_3. After exposure, the animals from both groups are combined and exposed to an aerosol of microorganisms. The vast majority of these studies have been conducted with streptococcus species. At the termination of the infectious exposure period, the animals are housed in clean air and the mortality rate in the two groups is determined during a 15-day holding period. In this system, the concentrations of O_3 used do not cause any mortality. The mortality in the control group (clean air plus exposure to the microorganism) ranges from approximately 10 to 20% and reflects the natural resistance of the host to the infectious agents. The difference in mortality between O_3-exposure groups and the controls is concentration-related (Gardner, 1982). These studies showed that, depending on the O_3 exposure protocol, a 3-h exposure to concentrations as low as 0.08 ppm O_3 can enhance the increased mortality of CD-1 mice from streptococcus infection (Coffin et al., 1967; Coffin and Gardner, 1972; Miller et al., 1978). However, although a prolonged intermittent exposure (103 days) to 0.1 ppm O_3 increased mortality in this model system, the magnitude of the effect was not substantially greater than that after acute exposure (Aranyi et al., 1983).

Another approach to assess the effect of air pollutants on host defenses is to quantitate rates of pulmonary bacterial inactivation following aerosol infection with microorganisms. In this system, the animals are exposed either to clean air or to the air pollutant and then are exposed to an aerosol of microorganisms in a manner similar to the method used for the infectivity model. However, instead of assessing enhancement of mortality, viable bacteria are quantitated in lung homogenates at various times after inhalation of the microorganisms (Goldstein et al., 1971a,b). In air-exposed control animals, there is a rapid inactivation of the inhaled microorganisms that have been deposited in the respiratory tract. However, O_3 exposure alters the ability of the microbicidal mechanisms of the lungs to function normally and bacterial inactivation proceeds at a slower rate, indicating impairment of host defenses. For example, Goldstein et al. (1971b) showed that a 4-h exposure of mice to 0.6 ppm O_3 after infection with *S. aureus* decreased lung bactericidal activity. Studies appearing in the literature since publication of the previous criteria document (U.S. Environmental Protection Agency, 1986) are described below (also see Table 6-9).

Gilmour et al. (1993a) observed that exposure of C3H/HeJ and C57Bl/6 mice for 3 h to 0.4 and 0.8 ppm O_3 resulted in decreased intrapulmonary killing of *S. zooepidemicus* in both strains of mice. Although both strains were affected, the C3H/HeJ mice appeared to be more susceptible because bactericidal activity was decreased sooner and mortality was enhanced more. Gilmour et al. (1993b) expanded these studies to CD-1 mice of different ages (5 and 9 weeks old) exposed for 3 h to 0.4 and 0.8 ppm O_3. The higher concentration decreased intrapulmonary killing 4 h after infection with *S. zooepidemicus*; there was no effect of age. However, the 5-week-old mice were more susceptible to the infection because mortalities were 9, 41, and 61% in the air, 0.4-ppm, and 0.8-ppm exposure groups, respectively; whereas only 4, 15, and 28% of the older animals died with analogous exposure. Pretreatment of the mice with indomethacin reduced the O_3-induced enhancement of PGE_2 levels in BAL as well as the enhanced mortality in the 5-week-old mice, suggesting an involvement of arachidonic acid metabolites in antibacterial defenses.

Gilmour and Selgrade (1993) studied the interspecies response to experimental *S. zooepidemicus* infection of rats and C3H/HeJ and C57Bl/5 mice following a 3-h exposure to 0.4 and 0.8 ppm O_3. Exposure of rats to O_3 suppressed intrapulmonary bacterial killing, with no differences observed between the 0.4- and 0.8-ppm O_3 exposure groups. Exposure of C57Bl/6 mice to 0.4 ppm O_3 also resulted in a suppression of bactericidal activity, and exposure to 0.8 ppm O_3 led to bacterial proliferation in the lungs, resulting in 60% mortality at Day 4. Exposure of C3H/HeJ mice to both 0.4 and 0.8 ppm O_3 resulted in bacterial proliferation with, respectively, 60 and 80% mortality at Day 4 after exposure. Increased mortality from *S. zooepidemicus* infection following 24 h/day exposure for 5 days/week for 3 weeks also was observed following 0.3 and 0.5 but not 0.1 ppm O_3 exposure (Graham et al., 1987).

To investigate the effect of longer exposures and challenges with bacteria, Gilmour et al. (1991) exposed mice continuously to 0.5 ppm O_3 for 14 days. At 1, 3, 7, and 14 days, intrapulmonary killing was assessed by inhalation challenge with *S. aureus* and *Proteus mirabilis*. Ozone exposure impaired the intrapulmonary killing of *S. aureus* at 1 and 3 days. However, with prolonged exposure, the bactericidal capacity of the lungs returned to normal. In contrast to *S. aureus*, when *P. mirabilis* was the challenge organism, O_3 exposure had no suppressive effect on pulmonary bactericidal activity. The authors attribute this difference to the defense mechanisms involved. Alveolar macrophages are

Table 6-9. Effects of Ozone on Host Defense Mechanisms: Interactions with Infectious Agents[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.3 0.5	196 588 980	24 h/day, 5 days/week for 3 weeks	Mouse, F (CD-1) 4-6 weeks old	Increased mortality from *Streptococcus zooepidemicus* infection at 0.3 and 0.5 ppm.	Graham et al. (1987)
0.13 0.26 0.51 0.77 1.02	250 500 1,000 1,500 2,000	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	Decreased bactericidal activity vs. *Listeria monocytogenes* at 0.77 and 1.02 ppm. Increased mortality at 1.02 ppm.	Van Loveren et al. (1988)
0.25 0.5 1.0	490 980 1,960	3 h/day for 5 days	Mouse, F (CD-1) 3-4 weeks old	Increased mortality from influenza virus infection and increased pulmonary virus titers at 1.0 ppm when infection followed 2 days of exposure, but not at other time points. Histopathologic and pulmonary function changes more severe with this regimen. At 0.5 ppm, increased lung wet weight when virus given after 2 days of O_3 exposure.	Selgrade et al. (1988)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) 30 days old	Decreased bactericidal activity vs. *Streptococcus zooepidemicus*. Increased mortality in both strains, with greater mortality in the C3H/HeJ strain.	Gilmour et al. (1993a)
0.4 0.8	784 1,568	3 h	Mouse, F (CD-1) 5 and 9 weeks old	Decreased bactericidal activity vs. *Streptococcus zooepidemicus*. Increased mortality in both age groups, with greater mortality in the 5-week-old mice.	Gilmour et al. (1993b)
0.4 0.8	784 1,568	3 h	Mouse, F (C3H/HeJ C57Bl/6) Rat (F344) 30 days old	Decreased intrapulmonary killing of *Streptococcus zooepidemicus*.	Gilmour and Selgrade (1993)
0.5	980	24 h/day for 14 days	Mouse, F (Swiss) 20-23 g	Decreased bactericidal activity vs. *Staphylococcus aureus* on Days 1 and 3; no effect on Days 7 and 14. Enhanced bactericidal activity vs. *Proteus mirabilis* on Days 3, 7, and 14; no effect on Day 1.	Gilmour et al. (1991)
0.5	980	24 h/day for 15 days	Mouse, F (Swiss) 20-23 g	No effect on pulmonary virus titers during influenza virus infection. O_3 decreased lung morphological injury due to virus (Day 9).	Jakab and Hmieleski (1988)
0.5	980	24 h/day for 120 days	Mouse, F (Swiss) 20-23 g	Ozone decreased acute lung influenzal injury, but increased pulmonary fibrosis during the course of and period after influenza virus infection.	Jakab and Bassett (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

active against the gram-positive *S. aureus*; AMs and PMNs defend against the gram-negative *P. mirabilis*. The effects of O_3 on bactericidal activity against *S. aureus* paralleled the effects on AM phagocytosis (early decrease, then no change). With *P. mirabilis*, there was more than a 1,000-fold increase in PMNs in the lung that was not altered by O_3, enabling bactericidal activity to occur. In a similar manner, exposure of rats for 24 h/day for 7 days to 0.13, 0.26, 0.51, 0.77, and 1.02 ppm O_3 decreased pulmonary bactericidal activity against *Listeria* at 0.77 and 1.02 ppm, with increased mortality at 1.02 ppm (Van Loveren et al., 1988). These effects were associated with increased pathologic lesions, characterized by multifocal infiltrates of histiocyctic and lymphoid cells, found in the lungs and liver of O_3-exposed and *Listeria* infected animals as compared to *Listeria* infection alone.

Fewer studies of viral infectivity have been conducted. Exposure for 15 days to 0.5 ppm O_3 during the course of murine influenza virus infection had no effect on pulmonary virus titers (Jakab and Hmieleski, 1988). A 5-day exposure for 3 h/day to 1.0 ppm O_3 with influenza virus infection on the second day of exposure had no effect on pulmonary virus titers, but did show increased mortality, increased lung wet weight, and more severe nonsuppurative pneumonitis and epithelial metaplasia and hyperplasia, with changes in lung function consistent with that effect (Selgrade et al., 1988). Lung wet weight also was increased when the mice were infected after the second day of exposure to 0.5 but not 0.25 ppm. When infection occurred on other days during the 5-day O_3 exposure, no such effects were found.

Typically, influenza virus infection causes pneumonitis characterized by severe acute lung damage that eventually resolves to persistent alveolitis and changes in the parenchyma (focal interstitial pneumonia and collagen deposition). Jakab and Bassett (1990) investigated the effect of long-term O_3 exposure (24 h/day for 120 days to 0.5 ppm) on mice administered influenza virus immediately before O_3 exposure started. The authors observed an increase in pulmonary fibrosis with the virus infection, as compared to O_3 exposure alone. During the course of the viral infection, O_3 exposure had no effect on pulmonary virus titers and reduced the virus-induced acute lung injury. However, from Day 30 after infection, increased numbers of AMs, lymphocytes, and PMNs were recovered from animals exposed to virus plus O_3, as compared to virus infection alone or O_3 exposure alone. This increased alveolitis correlated with increases in morphometrically determined lung damage and lung hydroxyproline content, a biochemical marker indicative of pulmonary fibrosis. Ozone exposure administered 10 days after viral infection enhanced lung hydroxyproline content at Day 30, as compared to either virus infection or O_3 exposure alone. Thus, O_3 enhanced postinfluenzal aveolitis and parenchymal changes. From these data, the authors speculated that the mechanism for the postinfluenza lung damage may be related to O_3 impairing the repair process of the viral-induced acute lung injury.

In the studies reported to date, it is clear that the temporal relationships between O_3 exposure and influenzal infection are important. This is not surprising because there are several waves of different antiviral defense mechanisms that might be affected differently by O_3. However, they have not been studied adequately for susceptibility to O_3. Apparently, O_3 does not alter defenses responsible for clearing virus from the lungs, as evidenced by the lack of effect of O_3 on viral titers (Selgrade et al., 1988; Jakab and Hmieleski, 1988). The interaction between virus and O_3 on histological changes in lung tissue can be damaging (Selgrade et al., 1988; Jakab and Bassett, 1990) or beneficial (Jakab and Bassett, 1990), possibly depending on the time of observation relative to the stage of the infectious process.

The exact reasons are not known, but perhaps the induction of interferon production by the virus plays a role. In noninfectious studies, Dziedzic and White (1987b) observed that interferon induction mitigates O_3-induced lung lesions, defined as areas with inflammatory cell infiltration, cellular proliferation, consolidation, or edema, and that anti-interferon treatment exacerbates those lesions.

6.2.3.7 Summary

Exposure to O_3 can result in alterations of all the defense mechanisms of the respiratory tract, including mucociliary and alveolobronchiolar clearance, functional and biochemical activities of AMs, immunologic competence, and susceptibility to respiratory infections. Structural (see Section 6.2.4), functional, and biochemical alterations in the mucociliary escalator occur after O_3 exposure. Mucociliary clearance is slowed in rabbits after a single 2-h exposure to 0.6 ppm, but repeated (up to 14-day) exposures have no such impact (Schlesinger and Driscoll, 1987). Secretions of mucous components are affected by repeated exposure (Phipps et al., 1986; McBride et al., 1991). When lambs were exposed (1.0 ppm O_3, 4 h/day, 5 days) shortly after birth, tracheal mucous components did not develop normally (Mariassy et al., 1989, 1990). In contrast, alveolar clearance of rabbits after acute O_3 exposure (0.1 ppm, 2 h/day, 1 to 4 days) is accelerated (Driscoll et al., 1986). In the same study, a 14-day exposure caused no effects, and a higher concentration (1.2 ppm) slowed alveolar clearance. A similar pattern of slowed tracheobronchial clearance and accelerated alveolar clearance occurs in rats (Phalen et al., 1980; Kenoyer et al., 1981). A subchronic (6-week) exposure of rats to an urban pattern of O_3 increased the retention of asbestos fibers (Pinkerton et al., 1989).

Although AMs have numerous functions, one primary role is to clear the lung of infections and noninfectious particles. Phagocytosis of bacteria, inert particles, and antibody-coated red blood cells (RBCs) is inhibited by acute exposure to O_3. The lowest effective concentration tested was 0.1 ppm O_3 (2 h) in rabbits (Driscoll et al., 1987). If exposures are repeated for several days, phagocytosis returns to control levels (Driscoll et al., 1987; Gilmour et al., 1991; Canning et al., 1991). The ability of AMs to produce superoxide anion radicals (important to bactericidal activity) is inhibited by acute exposure to O_3, especially in mice as compared to rats (Ryer-Powder et al., 1988; Oosting et al., 1991a). The effect is clearly evident after exposure for 3 h to 0.4 ppm, as observed by dysfunction in AM phagocytosis and enhanced susceptibility to experimental respiratory infection (Gilmour et al., 1993a, 1993b). Thus, the evidence indicates that the AM-dependent alveolobronchiolar transport mechanisms are impaired, as are their phagocytic and microbicidal activities, leading to decreased resistance to respiratory infections.

The experimental database also shows that the effects of O_3 on the immune system are complex. These effects are not yet fully evaluated, and the reported effects on immune parameters are dependent on the exposure regimen and the observation period. It appears that the T-cell-dependent functions of the immune system are more affected than B-cell-dependent functions (U.S. Environmental Protection Agency, 1986; Fujimaki, 1989). Generally, there is an early immunosuppressive effect that, with continued O_3 exposure, results in either return to normal responses or immunoenhancement. For example, in mice exposed for 28 days (20 h/day) to 0.3 to 0.7 ppm O_3, there was an early (Days 1 and 2) depletion of cells in the MLN, followed by MLN T-cell hyperplasia and increased blastogenic response to a T-cell mitogen (Dziedzic and White, 1986a). Several investigations have found an initial (Days 1 to 4)

decrease in blastogenic response to T-cell mitogens in the MLN and spleen of mice exposed for a few weeks to 0.7 or 0.8 ppm O_3 that returned to control levels by the end of the exposure (Dziedzic and White, 1986a; Gilmour and Jakab, 1991). There also are changes in cell populations in lymphatic tissues. For example, T:B-cell ratios in the MLN increase when rats are exposed for 7 days to 0.26 ppm O_3 (Van Loveren et al., 1988). Natural killer cells in the lung are affected under some circumstances. Van Loveren et al. (1990) showed that a 1-week exposure to 0.2 or 0.4 ppm O_3 increased NK cell activity, but a higher concentration (0.82 ppm) decreased it. Ozone also alters response to antigenic stimulation. For example, antibody responses to a T-cell-dependent antigen were suppressed after a 56-day exposure of mice to 0.8 ppm O_3, and a 14-day exposure to 0.5 ppm O_3 decreased the antiviral antibody response following influenza virus infection (Jakab and Hmieleski, 1988). The temporal relationship between O_3 exposure and antigenic stimulation is important. When O_3 exposure preceded *Listeria* infection, there were no effects on delayed-type hypersensitivity or splenic lymphoproliferative responses; when O_3 exposure occurred during or after *Listeria* infection was initiated, these immune responses were suppressed (Van Loveren et al., 1988). With experimental viral infections, O_3 exposure decreases the T-lymphocyte responses and the antiviral antibody response (Jakab and Hmieleski, 1988); the latter impairment may pave the way for lowered resistance to reinfection.

The significance of O_3-induced suppression of immune parameters in relation to risk of infectious disease is an example of a generic problem that remains to be clarified. For example, correlative studies between immune parameters and acquired immune deficiency syndrome have provided clear insights on the magnitude of the immune dysfunction and the progression of the disease. However, there is a paucity of such correlative studies on alterations of immune parameters and function resistance mechanisms or disease endpoints. Because the major functions of the immune apparatus are to protect against infectious agents and conduct tumor surveillance, suppression of immune parameters is considered a signal of increased susceptibility to infections and acquisition of tumors. One of the few studies that has addressed this issue (Selgrade, 1995) illustrates how the temporal relationships between exposure to a compound and exposure to infectious agents or tumor cells will have an impact on the risk associated with immune suppression in experimental animal models. The types of immune responses affected by a chemical and their importance to defense against any particular infectious agent, the recovery time of the immune response, the length of exposure, and the time required for mobilization of alternative defenses are among the factors that can impact the risk of enhanced infectious disease that might be associated with an immunosuppressive event.

In addition to a suppressive effect on pulmonary immunity, O_3 exposure also can affect systemic immunity. Although these depressive effects on the systemic compartment occur at approximately twice the O_3 exposure concentrations observed for pulmonary immunosuppression (with the exception of a study by Aranyi et al. [1983] at 0.1 ppm O_3), the observations are important because they show that the effects of O_3 exposure on host resistance are not limited to the lung alone, but may increase susceptibility to systemic infections as well as pulmonary infections. However, Selgrade et al. (1990) found no effects on selected systemic immune functions in rats exposed for up to 78 weeks to an urban pattern of O_3.

Numerous studies have confirmed that acute or short-term exposure to O_3 decreases lung bactericidal activity and increases susceptibility to respiratory bacterial infections. The lowest exposure showing such effects was 0.08 ppm (3 h) in the mouse streptococcal model (Coffin et al., 1967; Coffin and Gardner; 1972; Miller et al., 1978). Further research has

indicated that changes in antibacterial defenses are dependent not only on exposure regimens, but also on species and strain of animal, species of bacteria, and age of animal (e.g., young mice are more susceptible) (Gilmour et al., 1991, 1993a,b; Gilmour and Selgrade, 1993). Furthermore, increasing the duration of an exposure to 0.1 ppm O_3 from a few hours to 3 weeks either causes no effect or does not enhance the streptococcal-induced mortality observed after acute exposure (Graham et al., 1987; Aranyi et al., 1983). In general, the effect of O_3 exposure on antibacterial host defenses appears to be concentration- and time-dependent. Acute exposures result in an impairment of host defenses, whereas the defense parameters become reestablished with more prolonged exposures.

Effects of O_3 on the course of viral infections are more complex and highly dependent on the temporal relationship between O_3 exposure and viral infection. For example, Selgrade et al. (1988) found increases in mortality and lung wet weight in mice infected with influenza only after the second day of O_3 exposure (1 ppm, 3h/day). Jakab and Bassett (1990) found no detrimental effect of a 120-day exposure to 0.5 ppm O_3 on acute lung injury from influenza virus administered immediately before O_3 exposure started. However, O_3 enhanced postinfluenzal alveolitis and lung parenchymal changes. Because O_3 did not affect lung influenza viral titers in any of these studies, it is unlikely that O_3 has an impact on antiviral clearance mechanisms.

Ozone-induced susceptibility to experimental respiratory infections has been correlated with the immunotoxic effects of O_3 by the observation that O_3 exposure increases the severity of *Listeria* infection while concurrently suppressing the antigen-specific immune responses (Van Loveren et al., 1988).

6.2.4 Morphological Effects

6.2.4.1 Introduction

All mammalian species studied react to inhaled concentrations of <1.0 ppm O_3 in a generally similar manner, with species variation in morphological responses depending on the distribution of sensitive cells and the type of junction between gas conducting and exchange areas of the lung (U.S. Environmental Protection Agency, 1986). The cells most damaged by O_3 are the ciliated epithelial cells in airways and Type 1 cells in gas-exchange areas. Both of these cell types have very large surface areas (relative to volume) exposed to inhaled gases. The many factors that influence the distribution of inhaled O_3 within the respiratory system (see Chapter 8) result in some of the largest effective doses to the epithelial cells lining the nose and to epithelial cells located at the junction of the conducting and exchange areas, the CAR, in lungs. The 1986 criteria document did not contain studies of morphological effects of O_3 on the nose. There are species differences both in the basic structure of the CAR and in the epithelial cells that line the CAR (Tyler, 1983; Plopper, 1983). The CAR of humans, other primates, dogs, cats, and a few other domesticated species consists of the last conducting airway, the TB, several generations of RBs, alveoli that open directly into RB lumens, and ADs that branch from RBs (Figure 6-2). In lungs from many other mammals, including those most commonly used for inhalation toxicology (i.e., rats, mice, guinea pigs, and rabbits), RBs are poorly developed or absent and the CAR consists of TBs that open directly into ADs.

Epithelial degenerative changes in TBs and alveoli occur early, 2 to 4 h, in an O_3 exposure (Stephens et al., 1974a; Castleman et al., 1980). Depending on the dose to

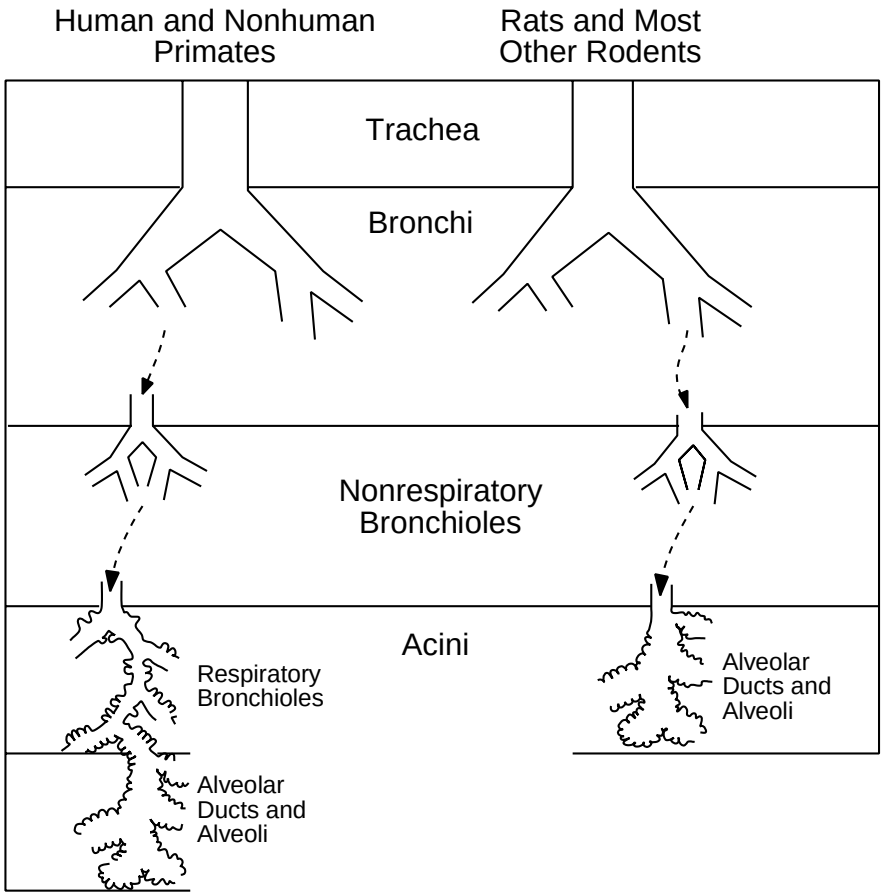

Figure 6-2. Schematic representation of intrapulmonary conducting airways and acini from animals with respiratory bronchioles (RBs) (human and nonhuman primates) and without RBs (rats and most rodents). For simplicity, several generations of bronchi and nonrespiratory bronchioles are not depicted.

Source: Redrawn from Weibel (1963) using information from Tyler and Julian (1991).

individual ciliated cells, they may lose cilia; undergo degenerative changes; or become necrotic and be sloughed into the lumen, leaving bare basement membrane until other cells replace them (Stephens et al., 1974a; Castleman et al., 1980). In the TB of the CAR, sloughed ciliated cells are replaced by nonciliated bronchiolar cells, which may become hyperplastic following longer exposures. Although these changes in TB epithelial cells can be studied readily by light microscopy (LM) or transmission electron microscopy (TEM), the surface views provided by scanning electron microscopy (SEM) provide a more comprehensive understanding of the three-dimensional aspects of CAR changes (Schwartz et al., 1976; Castleman et al., 1980).

Changes in mucus-secreting cells of conducting airways were considered minor in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Schwartz et al. (1976) did not find changes in mucous cells suggesting damage to cell organelles in rats

exposed to 0.2, 0.5, or 0.8 ppm O_3 for 7 days continuously or 8 h/day intermittently. Mellick et al. (1977) reported similar negative findings in mucous cells of monkeys following exposure to 0.5 or 0.8 ppm 8 h/day for 7 days. Wilson et al. (1984) reported more prominent small-mucous-granule (SMG) cells in the tracheas from monkeys exposed to 0.64 ppm O_3 continuously for 3 or 7 days. They speculated that these SMG cells may be related to repair processes.

Following O_3 exposure, Type 1 cells lining alveoli in the CAR, especially those opening into either RBs or ADs, may undergo vacuolization, fragmentation, or necrosis and be sloughed from the surface, leaving bare basement membrane (Stephens et al., 1974a). Although these degenerative changes, called lesions for purposes of this document, can be seen by careful LM of thin plastic sections, they are more reliably identified and the amount of damage can be estimated morphometrically using TEM (Barry et al., 1983; Crapo et al., 1984; Fujinaka et al., 1985). In alveoli, the bare basement membrane that follows O_3 exposure is recovered by Type 2 alveolar epithelial cells. Some Type 2 cells differentiate into Type 1 cells (Evans et al., 1975), but the epithelium remains thickened (Barry et al., 1983; Crapo et al., 1984).

Epithelial replacement in both TBs and alveoli can be followed and the amount can be estimated using radiolabeled thymidine and autoradiography (Evans et al., 1976a,b). Although bare basement membrane in alveoli is usually recovered by multiplication of Type 2 cells, in chronic exposures, bronchiolar cells, especially nonciliated bronchiolar cells, may cover part of the basement membrane formerly occupied by Type 1 or 2 cells. This process, termed bronchiolization (Nettesheim and Szakal, 1972), results in remodeling of CAR airways with the formation of new RBs. New RBs are identified readily by SEM or by LM in lungs from species in which RBs are normally absent or poorly developed (Boorman et al., 1980; Moore and Schwartz, 1981). In animals whose lungs normally have well- developed RBs, the extent of remodeling can be estimated using LM morphometry (Fujinaka et al., 1985).

The above epithelial changes are accompanied by an inflammatory response in the CAR characterized by increased numbers of PMNs in early stages, by increased numbers of AMs in lumens and in tissue at later stages, by hyperemia and interstitial edema, and by a fibrinous exudate (Stephens et al., 1974a; Schwartz et al., 1976; Boorman et al., 1980; Castleman et al., 1980; Fujinaka et al., 1985). As exposure continues, alveolar septa in the CAR thicken due to increased matrix, basement membrane, collagen, and fibroblasts and other cells, as well as by thickened alveolar epithelium (Boorman et al., 1980; Barry et al., 1983; Crapo et al., 1984; Fujinaka et al., 1985).

6 weeks to 0.25 ppm O_3. They reported CAR alveoli had more Type 1 and 2 epithelial cells and more AMs. The Type 1 cells were smaller in volume, covered less surface, and were thicker. They were aware of the results of Stephens et al. (1978) and speculated that the changes they described may have occurred primarily during the last 3 weeks of exposure. Boatman et al. (1983) did not find effects of O_3 on lung growth following pneumonectomy.

Several studies included both an exposure and a postexposure period during which the animals breathed air without O_3. Plopper et al. (1978) reported that CAR epithelial cells returned to normal appearance 6 days after a 72-h exposure to O_3. Incomplete resolution was reported 7 days after a 50-h O_3 exposure of monkeys (Castleman et al., 1980), 10 days after a 20-day O_3 exposure of mice (Ibrahim et al., 1980), and 62 days after a 180-day O_3 exposure of rats (Moore and Schwartz, 1981).

The previous criteria document (U.S. Environmental Protection Agency, 1986) comprehensively evaluated several citations reporting emphysema following O_3 exposure using current definitions of human emphysema (Snider et al., 1985). The morphological changes described in those earlier publications did not meet the current criteria for emphysema of the type seen in human lungs.

6.2.4.2 Sites Affected

The sites in the respiratory system that are affected will be discussed in anatomical sequence, beginning with the upper respiratory tract and proceeding downward. The upper or extrathoracic conducting airways (also referred to as the nasopharyngeal region) include the nasal cavity, pharynx, and larynx. Lower conducting airways begin with the trachea and include the bronchi and nonterminal bronchioles (also referred to as the tracheobronchial region). A summary of available information on these sites in the respiratory system is in Table 6-10, Effects of Ozone on Conducting Airways. Summaries of the information available concerning effects on the CAR, the gas exchange region (also referred to as the pulmonary region), and other pulmonary structures are divided into effects of short-term (<2-week) exposures in Table 6-11 and effects of long-term exposures in Table 6-12.

Nasal Cavity and Nasopharynx

The nasal cavity "conditions" inhaled air and in that process "scrubs" some reactive pollutants from the inhaled air, thereby reducing the concentration to which other portions of the respiratory system are exposed (Yokoyama and Frank, 1972; Miller et al., 1979). Although this scrubbing process is protective of other portions of the respiratory system, it results in a large dose of pollutant to the cells and tissues that line the nasal cavity.

There is a large range of variation in the structure of the nasal cavity among the animals used for inhalation toxicology and between those animals and humans (Schreider and Raabe, 1981). Schreider and Raabe (1981) found a striking similarity between the nasopharyngeal cavities of monkey and humans. They proposed that, with appropriate scaling, the monkey could serve as a model for aerosol and gas deposition in the nasopharyngeal region of humans. Thus, the studies on monkeys by Harkema et al. (1987) provide useful information for extrapolation to humans, as well as information concerning cellular responses in monkeys.

Table 6-10. Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.5 1.0 2.0 4.0 6.0 8.0	98 196 980 1,960 3,920 7,840 11,760 15,680	30 min (In vitro monolayer cultures)	Rabbit (NZW)	Phase contrast LM and trypan blue exclusion of tracheal epithelium. No consistent changes at 0.05 or 0.1 ppm, but 0.5 to 2.0 ppm resulted in cytoplasmic vacuolization without decreased viability. More evidence of cell damage, including gaps in previously confluent cultures and loss of cell viability at 4.0 and 8.0 ppm. Lipid peroxidation also was studied in these cultures.	Alpert et al. (1990)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, F (F344/N) 280-400 g	LM pathology and LM morphometry of PMNs in lung and nasal epithelia. Also nasal and bronchoalveolar lavage. See Section 6.2.2. Nasal epithelium: No necrosis, loss or attenuation of cilia, or hyperplasia at any exposure. Increases in pavemented PMNs at most concentrations and times. Lung: No lesions due to 0.12 ppm. CAR lesions not obvious at 0.8 or 1.5 ppm immediately or 3 h PE, but a progressive increase in CAR lesions at all other times. CAR TBs and proximal alveolar septa thickened and increased inflammatory cells. Increased CAR tissue PMNs in 0.8- and 1.5-ppm groups at 18 and 66 h PE and 1.5-ppm group at exposure end.	Hotchkiss et al. (1989a)
0.12 0.8	235 1,568	6 h/day for 7 days	Rat, F (F344/N) 12-14 weeks old	LM pathology and morphometry of LM histochemistry of nasal epithelia. No LM pathology at 0.12 ppm, but 0.8 ppm caused hyperplasia of transitional nonciliated epithelium. In 0.8-ppm group, shortened cilia in respiratory epithelium of nasopharynx, but not of nasal septum. Increased intraepithelial mucosubstances in all areas at 7 days PE to 0.8 ppm.	Harkema et al. (1989)
0.12 0.25 0.5	235 490 980	20 h/day, 7 days/week for 2 years (4, 12, 26, 52, 78, and 104 weeks)	Rat, M (F344, CrlBR) 42 days old	Smaller body weights after 7 weeks exposure to 0.5 ppm. LM histopathology of nasal epithelia. Nose: At 0.25 ppm, mucous cell and respiratory epithelial hyperplasia. No lesions in mainstem or large bronchi. CAR: Described in Table 6-12.	Smiler et al. (1988)
0.12 0.27 0.8	235 529 1,568	6 h/day for 3 or 7 days	Rat, F (F344/N) 8-12 weeks old	LM pathology and LM BrdU for DNA synthesis by nasal epithelia. No LM pathology in squamous epithelium or in ciliated respiratory epithelium. Hyperplasia of nonciliated cuboidal/transitional epithelium at 0.8 ppm. BrdU uptake (DNA synthesis) increased at end of 3- and 7-day exposure to 0.8 ppm. BrdU uptake decreased in squamous epithelium only after 7 days exposure to 0.8 ppm and 7 days PE.	Johnson et al. (1990)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	Nasal changes limited to nasal transitional nonciliated epithelium at 0.5 and 1.0 but not 0.12 ppm. LM histochemistry of intraepithelial mucosubstances in the nose and bronchi. Epithelial cell hyperplasia; mild to moderate inflammatory cell influx into the mucosa; and increased mucosubstances. Mucous flow rates decreased.	Harkema et al. (1994)

Table 6-10 (cont'd). Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration µg/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	LM morphometry and histochemistry of "short-" and "long-path" conducting airways and CAR. *Trachea*: No changes in epithelial thickness, cell populations or stored glycoconjugate, but a dose-dependent loss of stored glycoconjugate was found. *Bronchi*: No changes in epithelial thickness or cell populations. Rats exposed to 1.0 ppm had increased stored gylcoconjugates in cranial (short-path) and caudal (long-path) bronchi, but not in central (short-path) bronchi. *Bronchioles*: TB of rats exposed to 1.0 ppm had thicker epithelium with increased V_v of nonciliated bronchiolar cells. V_v also increased in caudal (long-path) TB of 0.5-ppm exposed group. Mass ($\mu m^3/\mu m^2$) (V_v) of nonciliated cells increased in caudal (long-path) TBs of all exposed rats, but not in short-path TBs. *CAR*: Increased V_s of bronchiolar epithelium in former ADs in cranial and caudal CARs of rats exposed to 1.0 ppm and in cranial CAR of rats exposed to 0.5 ppm.	Plopper et al. (1994a)
0.12 0.24 0.48	235 470 940	3, 6, 12, or 24 h	Rat, F (F344/N) 11-13 weeks old	LM pathology and semiautomatic image analysis system for DNA synthesis (BrdU uptake) of epithelium in nasal maxilloturbinates. C × T design. No effects at 0.12 or 1.44 ppm·h. For a given C × T, increased DNA synthesis equal at different Cs and Ts. Nonlinear increase in DNA synthesis as C × T increased.	Henderson et al. (1993)
0.15 0.3	294 588	8 h/day for 6 days to 0.15 ppm or for 90 days to 0.15 or 0.3 ppm	*Macaca radiata*, M, F 2-6 years old, 2.3-9.7 kg	LM, SEM, and TEM morphometry of nasal epithelia. Respiratory epithelium: Ciliated cell necrosis, shortened cilia, and increased small mucous granule cells at all exposures, even at 0.15 ppm for 6 days. Transitional epithelium: Decreased nonciliated cells without granules, increased nonciliated cells with granules, and increased small mucous granule cells. Increased intraepithelial leukocytes only at 6 days in both types of epithelium.	Harkema et al. (1987)
0.15 0.25	294 490	8 h/day for 6 days to 0.15 ppm or for 90 days to 0.15 or 0.3 ppm	*Macaca radiata*, M, F 2-6 years old, 2.3-9.7 kg	LM and TEM histochemistry for intraepithelial mucosubstances in nasal mucosa. More mucous cells that had dilated granular endoplasmic reticulum. Also changes in mucosubstances.	Dimitriadis (1992)
0.15 0.25	294 490	8 h/day for 7 days	*Macaca radiata* NS	The O_3 concentration is not clear—the abstract states 0.64 ppm, the text mentions only 0.25 ppm. LM morphometry of vocal fold mucosa. Disruption and hyperplasia of stratified squamous epithelium. Epithelium thickened at 12 h and 7 days PE. Connective tissue of lamina propria thickened at 7 days PE. Basement membrane is undulating rather than smooth. Even though thickened, epithelium appeared normal at 7 days PE.	Leonard et al. (1991)
0.2 0.4 0.8	392 784 1,568	22 h/day for 3 days	Rat, M (Wistar RIVM [TOX]) 150-190 g	LM pathology and tritiated thymidine uptake by nasal epithelia. Observations on mixtures are in Section 6.4. Respiratory epithelium: No changes at 0.2 ppm O_3. Loss of cilia and disarrangement at 0.4 and 0.8 ppm. Some epithelia were hyperplastic or metaplastic or both. Thymidine uptake increased in rostral (anterior) portions.	Reuzel et al. (1990)

Table 6-10 (cont'd). Effects of Ozone on Conducting Airways[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.8	1,568	6 h/day for 3 or 7 days	Rat, F (F344/N) 12-14 weeks old	LM morphometry and histochemistry of nasal nonciliated cuboidal epithelium. Other types of nasal epithelia not examined. After 3 days O_3 and 18 h PE, no changes in cell density or in intraepithelial mucus. After 7 days exposure or after 3 days exposure and 4 days PE, hyperplasia and increased intraepithelial mucosubstances with no change in the ratio of acidic to neutral mucosubstances.	Hotchkiss et al. (1991)
0.8	1,568	6 h	Rat, F (F344/N) 12-16 weeks old	LM pathology and DNA synthesis by BrdU uptake by nasal nonciliated transitional epithelium. Ozone did not result in necrosis, exfoliation, or inflammation, but did increase DNA synthesis.	Hotchkiss and Harkema (1992)
0.96	1,882	8 h/night, 7 nights/week for 3 or 60 nights	Rat, M (S-D) 234-263 g	LM morphometry, histochemistry, autoradiography, and SEM and TEM morphometry of tracheal epithelium. Neither 3 nor 60 days exposure altered the cell density of ciliated, serous, basal, brush, migratory, or unidentified cells in tracheal epithelium. 3 days: Damage to cilia and ciliated cells, including necrosis. Thymidine labeling index increased. Serous cell histochemistry unchanged. 60 days: Less evidence of injury than at 3 days, but more damaged ciliated cells than in controls. Complete recovery of the epithelial changes by 42 days PE.	Nikula et al. (1988a)
0.96	1,882	8 h	*Macaca mulata*, M 2.0-8.5 years old 2.1-6.3 kg	LM and TEM morphometry of trachea, bronchi, and RBs. Increased necrotic cells in trachea and RBs at 1 h PE and in bronchi at 12 and 24 h PE. Decreased ciliated and basal cells in bronchi at 1, 12, and 24 h PE. Basal cells in bronchi also decreased at 72 and 168 h PE. Nonciliated bronchiolar cells in RBs increased only at 24 h PE. In bronchi, smooth muscle increased and amorphous matrix decreased at 24, 72, and 168 h PE. In RBs, smooth muscle increased at 24 h, fibroblasts increased at 24 and 72 h, and amorphous matrix increased at 12 h PE.	Hyde et al. (1992)
1.0	1,960	4 h/day for 5 days (examined at 2 weeks)	Sheep New-born	LM morphometry of tracheal epithelium. Also see Section 6.2.3. Percentage of ciliated and mucous cells remained at newborn levels, rather than ciliated cell percent increasing and mucous cell percent decreasing as in control lambs.	Mariassy et al. (1990)
1.0	1,960	4 h/day for 5 days (examined at 2 weeks)	Sheep New-born	LM morphometry of mucosubstances in tracheal epithelium. No evidence of damage or inflammatory changes. Decreased epithelial cell density, decreased ciliated and basal cells. Lectin-detectable intraepithelial mucosubstances did not undergo the maturation changes seen in control lambs.	Mariassy et al. (1989)
1.0	1,960	96 h (in vitro tracheal explants)	Rat, M (S-D) 250-270 g	Tracheal organ cultures exposed in vitro. Filtered air + O_3 resulted in extensive damage to cilia, and intermediate cells were seen. Cultures exposed to 95% O_2 + O_3 had stratified thickened epithelium with metaplastic cells in a middle zone and no ciliated cells at the surface.	Nikula and Wilson (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-11. Effects of Ozone on Lung Structure: Short-Term Exposures (< 2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.12 0.8 1.5	235 1,568 2,940	Continuous for 6 h	Rat, M (F344/N) 12-18 weeks old	LM histopathology of lungs and LM morphometry of lavaged AMs: No LM histologic effect detected at 0.12 ppm. No LM histologic effect at 0.8 and 1.5 ppm immediately or 3 h PE. At later PE times, there was mild, patchy CAR bronchiolitis and alveolitis. Increase in AMs and PMNs from 18-66 h PE. Progressive thickening of TB walls and CAR AD septa at 18, 42, and 66 h PE.	Hotchkiss et al. (1989b)
0.12 0.8 1.5	235 1,568 2,940	6 h	Rat, M (F344/N) 12-18 weeks old	Observations of nose and CAR. Same rats as in Hotchkiss et al. (1989a). LM histopathology of CAR is the same; new morphometry of PMNs in CAR and nasal mucosa. Emphasis on PMNs in nasal mucosa and nasal lavage compared with PMNs in CAR tissues and BAL at exposure end and at 3-66 h PE.	Hotchkiss et al. (1989a)
0.12 0.5 0.75	250 1,000 1,500	Continuous for 1-7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	LM: Increased AMs in CAR and parenchyma. CAR increase persisted 5 days PE. TEM and SEM: BAL AMs had microvilli and blebs in addition to ruffles characteristic of AMs from controls. Also see Section 6.2.3.	Dormans et al. (1990)
0.15 0.5		Continuous for 3 or 7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	Elastase-induced emphysema and saline control rats. LM histopathology and morphometry for alveolar size. Also see Section 6.2.5. The incidence and severity of CAR LM lesions was the same in elastase- or saline-treated rats exposed to O_3. No change in alveolar size due to O_3.	Dormans et al. (1989)
0.2 0.4	393 784	4 h	Rat, M (S-D) 7 weeks old	LM histopathology. Necropsy 24 h PE. Lung lesions from 0.2 ppm not reported, but 0.4 ppm resulted in increased AMs and increased cellularity of alveolar septa with focal thickening. No increase in DNA synthesis by nasal epithelium. See mixture effects in Section 6.4.	Mautz et al. (1991)
0.2 0.38	393 745	3.75 h, Rest or exercise	Rat (S-D)	LM histopathology and morphometry. No effect from 0.2 ppm at rest. Increased free cells in airspaces at 0.38 ppm at rest. Exposure during exercise resulted in larger areas with free cells and in areas of septa thickened by infiltrating cells.	Mautz et al. (1985b)
0.25 0.8	490 1,568	29 min/day for 2 days, Strenuous exercise	Horse (Thoroughbred) 5-6 years old Gelding	LM and TEM histopathology (see also Section 6.2.5). 0.25 ppm: Lesions limited to vacuoles seen only by TEM in TB ciliated cells. 0.8 ppm: Gross hemorrhage and edema in two of three horses. CAR lesions, visible only by TEM, included edema, necrosis and sloughing of Type 1 cells, slight increase in AMs, shortened cilia, and vacuoles in ciliated and nonciliated bronchiolar cells.	Tyler et al. (1991c)
0.35 0.6	686 1,176	4 h at rest, 3 h with exercise	Rat, M (S-D) 7 weeks old	Examination 48 h PE. Lung: LM morphometry of lesions as a percent of parenchyma section area. No statistical evaluation of groups exposed to O_3 at rest and exercise. The lesion percent of parenchyma appears concentration-dependent and increased by exercise similar to Mautz et al. (1985b). Nasal epithelium: Evaluated percent thymidine labeled cells in respiratory epithelium. No change due to O_3 at rest. Effects of mixtures in Section 6.4.	Mautz et al. (1988)
0.35 0.8	686 1,568	72 h	Rat, F (S-D) 60 or 444 days old	LM morphometry and SEM. V_v of CAR lesions. Exposed adults lost body weight. Adults exposed to 0.8 ppm had smaller fixed lung volumes. Lesion V_v larger in young than adult rats at both concentrations. Free cell (AM) V_v increased in young rats at 0.35 ppm compared to adults. The CAR lesions were similar by SEM, but younger rats had more AMs in the CAR. Younger rats had larger CAR lesions and more AMs, but older rats had greater changes in body weight and fixed lung volume.	Stiles and Tyler (1988)

Table 6-11 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures (< 2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.35 0.5 1.0	686 980 1,960	2.25 h/day for 5 days	Rat, M (F344) 110-120 days old	LM histopathology and morphometry for parenchymal density and alveolar size. No significant changes in these morphometric parameters. Histopathology: Reported only for 0.5 ppm O_3 + CO_2 challenge group, which had maximal CAR tissue damage on Days 4 and 5. Increased AMs on Days 2 and 3 and foci of necrotic TB epithelium. By Day 5, hyperplasia of TB epithelium and increased AMs and other inflammatory cells, which completely filled some CAR alveoli. Morphologic damage continued, whereas pulmonary functional changes attenuated. Also see Section 6.2.5.	Tepper et al. (1989)
0.4	784	Continuous up to 14 days	Rat, M (Wistar Jcl) 5 weeks old	SEM morphometry, immunocytochemistry. See Section 6.2.1 for biochemistry. Number of Clara cells/m^2 increased at 14 days, but not earlier. The length of the Clara cell apical projection was increased after 6 h, decreased at 1 day, and not different at other periods. Cytochrome P-450 was localized to agranular endoplasmic reticulum of Clara cells.	Suzuki et al. (1992)
0.5	980	20 h/day for 1-14 days	Rat, M (F344) 13 weeks old	LM morphometry of thymidine-labeled cells in bronchus-associated lymph node and MLN. Other lung changes not described. Also see Section 6.2.3.	Dziedzic et al. (1990)
0.64	1,254	Continuous, 7 days	Rat, M (S-D) 250-300 g	LM morphometry of CAR (proximal alveolar) lesions. Increased centriacinar lesions. Rats treated with dimethylthioruea (a H_2O_2 scavenger) had smaller lesion volumes.	Warren et al. (1988)
0.7 0.9	1,372 1,764	20 h/day for 4 days	Mouse, F (CD-1 Crl:CD1/ (CR)BR) 20-22 g	LM morphometry for areal density (e.g., volume density) of lesions. No comparison of O_3 and air exposures. Also see Section 6.2.3.	Dziedzic and White (1987b)
0.75	1,470	Continuous for 3 days	Rat, M (Wistar) 200-250 g	LM histopathology. Ozone-exposed rats gained less body weight. Increased cells in TB and CAR AD septa. Number of cells diminished by Day 4 PE, but foci of AMs remained.	Bassett et al. (1988a)
0.8 0.4	1,568 784	Continuous for 7 days	Rat, M (Wistar) 8 weeks old	LM: Clara cell numbers/mm of TB basement membrane unchanged. Also see Section 6.2.3. Cell isolation: Although the number of isolated Clara cells/10^6 cells isolated/lung was increased, the percent Clara cells in the isolate was not changed. The percent Type 2 cells in the isolate was increased. No morphologic observations at 0.4 ppm.	Van Bree et al. (1989)
0.8	1,568	Continuous for 3 h	Rat, M (S-D) 250-300 g	By LM, PMNs in alveolar septa increased three times at 4 h PE. Number of septal PMNs peaked at 8 h PE and then rapidly declined. Free cells, septal thickening, and cellularity increased with increasing time PE. Also see Section 6.2.2.	Bhalla and Young (1992)
0.82	1,600	Continuous for 7 days	Rat, M (Wistar RIV:TOX) 8 weeks old	LM morphometry of histochemically identified Type 2 cells. Increased number of Type 2 cells than in controls.	Dormans (1989)

Table 6-11 (cont'd). Effects of Ozone on Lung Structure: Short-Term Exposures (< 2 Weeks)[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.97	1,901	Continuous for 7 days	Rat, M 225-275 g	Smaller body weights. No other statistical comparison of controls and O_3 alone (see Sections 6.2.6 and 6.4).	Last et al. (1986)
1.0 2.0	1,960 3,920	4 h/day for 5 days	Dog, M, F (Beagle) 6 weeks old	LM morphometry for alveolar size. Mean linear intercepts larger (indicating larger alveoli) in 1.0- but not 2.0-ppm group.	Phalen et al. (1986)
1.0 2.0	1,960 3,920	Continuous for 3 h (Isolated perfused lungs)	Rat, M (S-D) 300-380 g	LM and TEM morphology. Necrosis and sloughing of airway epithelial cells of bronchi and larger bronchioles. TB had less severe lesions, including fewer necrotic cells and less damage to cilia. Fragmentation of some Type 1 cells with some areas of bare basal lamina.	Pino et al. (1992a)
1.0	1,960	Continuous for 8 h	Rat, M (S-D) 10 weeks old	LM and TEM morphometry. Also see Section 6.2.2. Rats received either normal rat serum or rabbit anti-rat PMN serum before the exposure. At exposure end, both exposed groups had a smaller volume of ciliated cells per unit area of epithelial basal lamina (V_s) compared with filtered air controls with similar serum. Ciliated cell V_s was also smaller at 4 and 16 h PE.	Pino et al. (1992b)
1.0	1,960	4, 6, 8, and 24 h	Rat, M (S-D) 63 days old	TEM morphometry. Also see Section 6.2.2. Volume of necrotic cells per area basal lamina (V_s) in the TB larger than controls at the end of 4- and 24-h exposure, but not at other exposure or PE times. With increasing exposure time, there was a shift from necrotic cells on the basal lamina to necrotic cells free in the TB lumen. The V_s of necrotic alveolar cells was increased after 4, 6, and 24 h of exposure. Viable undifferentiated cell V_s in TBs was increased after 6-h exposure followed by 18 h PE, 8-h exposure followed by 16 h PE, and after a 24-h exposure. In alveoli, viable Type 1 cell V_s was increased after a 24-h exposure. Total connective tissue V_s changes only increased in TBs after 8-h exposure followed by 4 h PE and in alveoli at the end of 8-h exposure. The V_s of migratory cells in TB interstitium was only increased 4 h after a 6-h exposure. In alveoli, the V_s of capillaries was increased after 8-h exposure.	Pino et al. (1992c)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-12. Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
Base 0.06; spike to 0.25	Base 118; spike to 490	Base 13 h/day, 7 days/week; Ramped spike 9 h/day, 5 days/week (1, 3, 13, and 78 weeks)	Rat, M (F344) 60 days old	TEM morphometry, data expressed as volume per unit epithelial basement membrane (V_s). Acute response in the CAR (proximal alveolar) region included increased AMs, interstitial edema, and interstitial cell hypertrophy. These changes subsided by 3-weeks exposure. Changes at 13 and 78 weeks include increased V_s, total alveolar tissue, and epithelium. Type 1 cell increases include V_s at 13 weeks; number at 13 weeks, 13 + 6 weeks PE, and 78 weeks; and decreased surface area at 78 weeks. Type 2 cell V_s increased at 78 weeks and after 78 + 17 weeks PE, and number increased at 78 weeks. Total interstitium was increased at 78 weeks, and noncellular interstitium (collagen and basement membrane) was increased at 13 and 78 weeks. Thickened basement membrane had crystalline deposits. No bronchiolization or centriacinar airway remodeling. Changes in terminal bronchiolar cells include a decrease in surface area of ciliated and nonciliated at 78 weeks. No changes in bronchiolar cell numbers. All changes diminished, except the increased volume of Type 2 cells and the thickened basement membrane, 17 weeks PE.	Chang et al. (1992)
A: 0.25 B: Base 0.06; spike to 0.25	490; 118 to 490	6 weeks 3 weeks 13 weeks 12 h/day 22 h/day	Rat (F344) 7 weeks old	Compared effects of A and B exposure regimens. Cumulative doses for A (Barry et al., 1985) were 60.5 and 126.0 ppm-h and, for B, were 45.3 ppm-h at 3 weeks and 196.0 ppm-h at 13 weeks. The B regimen alone (Chang et al., 1992) is described earlier in this table. TEM morphometry of CAR. The pattern of exposure did not affect the degree of injury.	Chang et al. (1991)
0.1	196	2 h/day, 5 days/week for 1 year	Rabbit, M (NZW) 3-3.5 kg	LM morphology and morphometry of intrapulmonary conducting airways. (Also see Section 6.4). No difference in number of airways/area or in distribution of airway size. ESCs in the smallest conducting airways (< 0.30 mm) increased at 4, 6, and 12 mo of exposure to O_3 and decreased at 6 mo PE. ESCs in the next larger airways (0.31-0.49 mm) only increased after 4 mo of exposure and decreased at 6 mo PE. No effect on airways >0.50 mm.	Schlesinger et al. (1992a)
0.12 0.25	235 490	12 h/day for 6 weeks	Rat, M (F344) 1 day or 6 weeks old	TEM morphometry of proximal alveolar region (CAR). Type 1 epithelial cells increased in number and thickness, but decreased in luminal and basal lamina surface area. Some bare basement membrane where Type 1 cells sloughed, but not significantly increased. At 0.25 ppm, Type 2 epithelium increased in number, but not in volume, thickness, or surface area. Interstitium increased in thickness in adults at 0.25 ppm, but not at 0.12 ppm or in juveniles. AMs increased by 0.25 ppm at both ages, but only older rats had increased interstitial AMs. No differences due to age.	Barry et al. (1985)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	LM morphometry of CAR remodeling. Thickened tips of alveolar septa lining ADs (alveolar entrance rings) 0.2 mm from TB in rats exposed to 0.12 ppm and to 0.6 mm in rats exposed to 1.0 ppm. Interstitial changes accompanied these epithelial changes.	Pinkerton et al. (1995)
0.12 0.5 1.0	235 980 1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-8 weeks old	Laser scanning confocal LM immunohistochemistry for CC10 in nonciliated bronchiolar (Clara) cells. Clara cells from rats exposed to 1.0 ppm, but not to 0.12 ppm, had increased cell volume of granule-based CC10, increased CC10 concentration within the granules, and increased number of granules per Clara cell profile.	Dodge et al. (1994)
0.12 0.25 0.5	235 490 980	20 h/week, 7 days/week for 2 years (Examined at 4, 12, 26, 52, 78, and 104 weeks)	Rat, M (F344 CrlBR) 42 days old	Rats exposed to 0.5 ppm had smaller BW after 7-weeks exposure. LM histopathology. Nose: At 0.25 ppm, mucous cell respiratory epithelium hyperplasia; no lesions in mainstem or large bronchi. CAR: 0.25 ppm, TB epithelium hyperplastic and hypertrophic; bronchiolarization and airway remodeling. No changes in 0.12-ppm group after 26 weeks of exposure. Peribronchiolar tissue and AD walls thickened by eosinophilic material after 12 weeks at 0.5 ppm and after 26-weeks at 0.25 ppm. Collagen found in these areas using special stains. Increased AMs at 0.25 ppm.	Smiler et al. (1988) Wright et al. (1989, 1990)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (>2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.15 0.3	294 588	8 h/day for 6 or 90 days	*Macaca radiata*, F, M 2-6 years old	TEM, SEM, and LM morphometry. First generation RBs had epithelial hyperplasia, and alveoli opening into these RBs had increased AMs. RB epithelium thickened, but no difference due to either exposure time or concentration. RB interstitium was thickened in all exposed monkeys, but both cellular and acellular compartments were individually thickened only after 90-days exposure to 0.3 ppm. No differences due to age or gender. No evidence of epithelial cell necrosis nor of inflammatory cell infiltration other than the increased AMs.	Harkema et al. (1993)
0.25	490	12 h/day for 6 weeks	Rat, M (F344) 1 day or 6 weeks old	TEM morphometry of the TBs. Luminal surface area covered by cilia decreased, as did the luminal surface of Clara (nonciliated bronchiolar) cells. Number of brush cells decreased. No differences due to age.	Barry et al. (1988)
0.25	490	8 h/day, 7 days/week, "daily" for 18 mo or "seasonal" O_3 odd months, filtered air even months for 18 mo (9 mo of O_3)	*Macaca fascicularis*, M 6 mo old	LM morphometry. Also see Section 6.2.5. Low-grade respiratory bronchiolitis in both exposed groups. Compared with controls, both groups of exposed monkeys had increased V_v of tissue other than parenchyma and V_v of RBs and their lumens. Both V_v and V of RB wall increased in the "daily" group but not in the "seasonal" group. The only significant morphometric difference between the two exposed groups was the V_v of cells, mostly AMs, free in airspace lumens. This difference and the difference in significance of the RB wall thickness was presumed due to the difference in time after the last O_3 exposure and necropsy. Daily group necropsied the day after the last exposure, whereas seasonal group necropsied after a month of filtered air. Seasonal group had an amount of morphological changes similar to the daily group.	Tyler et al. (1988)
0.25	490	8 h/night, 7 nights/week, "daily" for 18 mo or "seasonal" O_3 odd months, filtered air even months for 18 mo	Rat, M (S-D) 22 days old	LM morphology and morphometry. Monkey data from Tyler et al. 1988 (above) compared with rats exposed to a similar regimen. Rats: Estimated the extent of centriacinar remodeling by counting the number of junctions of bronchioles with ADs per area of lung section (B/A J/cm^2). At the end of the exposure, both exposed groups had more B/A J/cm^2 than filtered air controls. Recovery by 30 days PE. No difference between the two exposed groups, even though the daily group was exposed twice as many days as the seasonal group.	Tyler et al. (1991a)
0.3	588	7 h/day, 5 days/week for 6 weeks	Mouse, M (Swiss-Webster) Newborn	LM morphometry of histochemically identified Type 2 cells. Type 2 cells tended to be larger (longer linear intercepts), and the number per microscope field tended to be greater, but the p values were >0.05 in final data in which the images were edited electronically. However, these values were significant ($p < 0.05$) in unedited data. Exposed mice had larger ($p < 0.05$) body weights at both 3 and 6 weeks.	Sherwin and Richters (1985)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (> 2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.35	686	4.5 h/day, 5 days/week for 4 weeks, 380 mmHg (5,400 m) or sea level	Mouse, M (Swiss-Webster) 32 g	Automated LM morphometry of stainable elastin in alveolar walls. Simulated high altitude (5,400 m) with O_3 (SHA-X) or without O_3 (SHA-C) resulted in larger lung volumes than sea-level controls (SL-C), but not different from each other. Unlike most studies, sea-level, O_3-exposed mice had the smallest lung volumes. Alveolar wall areas, after adjustment to SL-C lung volumes, were increased only in the SHA-X group. Alveolar wall elastin area, adjusted to the SL-C lung volumes, increased in both high-altitude groups compared to SL-C and also were different from each other with the largest amount of elastin area in the SHA-X group. However, if the elastin areas were not adjusted for differences in lung volumes, there were no differences between the groups.	Damji and Sherwin (1989)
0.4 0.64	784 1,254	8 h/day, 7 days/week for 90 days	*Macaca radiata*, M 5-8 years old	LM and TEM morphometry with emphasis on RBs. Respiratory bronchiolitis and peribronchiolar inflammation. RB walls thicker with smaller lumens. Increased wall thickness due both to thicker epithelium (significant only at 0.64 ppm) and interstitial components. Epithelial changes in both O_3 groups include increased nonciliated bronchiolar epithelial cells and decreased Type 1 cells. Interstitial changes in both O_3 groups included increased smooth muscle cells, mast cells, and fibers. Components increased at 0.64 ppm, but not at 0.4 ppm, included interstitial AMs, PMNs, and amorphous ground substance.	Moffatt et al. (1987)
0.5	980	6 h/day, 6 days/week for 2, 3, 5, and 12 mo	Rat, M (Wistar) 100 g	LM, TEM, and LM morphometry of collagen fibers. Bronchitis, peribronchitis, CAR remodeling, and increased stainable collagen in bronchioles. Rats apparently had intercurrent respiratory disease, as 10 of 44 exposed rats died of pneumonia or pulmonary edema. In addition, it appears that only one set of 12 rats maintained in room air served as controls for all exposed groups, even though controls and exposed rats would be of significantly different age and size.	Hiroshima et al. (1989)
0.5	980	20 h/day, 7 days/week for 52 weeks	Rat (F344)	LM histopathology. 6-mo exposure: Inflammation, mononuclear cells, and fibroblasts in AD walls and walls of adjacent CAR alveoli. TB not involved. 12-mo exposure: Similar to 6 mo with possibly a slight increase in AMs, some increased thickening of centriacinar AD and alveolar walls, and a few foci of bronchiolization (CAR remodeling). 12-mo exposure + 6 mo PE: Slight dilation of ADs, minimal inflammatory reaction, slight thickening of AD and CAR alveolar walls, and a few foci of bronchiolization.	Gross and White (1987)
0.5	980	Continuous for 120 days	Mouse, F (Swiss) 20-23 g	LM morphometry and histopathology. Also see Section 6.2.3. More tissue, primarily inflammatory cells, at Day 9. Little change from Days 10 to 120. Thickened airway walls with increased collagen. Increased collagen in alveolar walls along the ADs.	Jakab and Bassett (1990)
0.64 0.96	1,254 1,881	8 h/night, 7 nights/week for 42 nights, "pair" fed	Rat, M (S-D) 28 days old	LM morphometry and SEM. Also see Section 6.2.5. End of 42-night exposure: No difference in BW, hemoglobin, or total serum proteins. At 0.96 but not 0.64 ppm, larger fixed and saline-filled lung volumes, lung volume/BW ratios, and volumes of parenchyma. At both concentrations, increased V_v and V of RB and RB walls and their ratios to BW. By SEM, remodeling of CAR airways with the formation of RBs and thickened CAR septa at both ppm. After 42 days PE: Fixed lung volume at 0.96 ppm increased. V_v and V of RB walls and ratio to BW increased at 0.96 ppm, as did ratios of volumes to BW for parenchyma, alveoli, total RB, and RB wall. SEM revealed persistence of CAR remodeling and thickened septa.	Tyler et al. (1987)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-Term Exposures (> 2 Weeks[a])

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s) [3]	Reference
0.64	1,254	8 h/day for 12 mo	*Macaca faciscularis*, M 6-7 mo old	LM morphometry of CAR airway remodeling. Both V_v and V of RBs, and their walls and lumens, increased at end of exposure and 6 mo PE. RB internal diameters were smaller at exposure end, but not at 6 mo PE. V_v free cells, mostly AMs, increased only at exposure end. No differences in BW or fixed lung volumes.	Tyler et al. (1991b)
0.7	1,372	20 h/day for 4 weeks	Rat, M (F344) 14 weeks old	LM histopathology. Also see Section 6.2.5. Exposure end: CAR inflammation of TB, AD, and CAR (proximal) alveoli characterized by edema with mononuclear and leukocyte infiltration. 4 weeks PE: Few inflammatory foci, edema decreased, and interstitial mast cells. Slight thickening of ADs and septa. 9 weeks PE: Inflammation cleared, TB walls slightly thickened by amorphous matrix.	Gross and White (1986)
0.95	1,862	8 h/day for 90 days	Rat, M (S-D) 61 days old	LM and TEM morphology. RB: Increased volume of total RB and of RB wall and lumen. RB walls thickened by interstitial inflammation with edema, hyperemia, fibrosis, and hypertrophied smooth muscle and by interstitial mononuclear cells, granulocytes, and plasma cells. Epithelial and vascular basal lamina fused. TB: Smaller internal diameter and smaller luminal volume, but no change in total TB volume or in wall volume. Proximal AD: Most severe cell damage and inflammation at alveolar septal tips (alveolar entrance rings). Epithelium at these tips was frequently necrotic or missing, leaving bare basement membrane. Duct walls thicker due mainly to increased interstitial edema, fibrosis, and cellular infiltrates. Basal lamina thickened, split or duplicated, and had granular deposits. Site of most severe injury shifted progressively distally as new segments of RB were formed.	Barr et al. (1988)
0.95	1,862	8 h/day for 90 days or 5-day episodes followed by 9 days PE	Rat, M (S-D) 52 days old	LM and TEM morphometry. Both groups examined at exposure end. The lesions were as previously described by Barr et al. (1988). Both groups had CAR airway remodeling with the formation of new RBs. The only morphometric difference in RBs between the groups was the volume of RB wall, which was greater in the daily group, but both groups were greater than controls. The volume of the total RB and of RB lumen was increased in the daily group. RB epithelium of the daily group was more differentiated. TB interstitium was increased in the episodic group. Alveolar duct/sac lumen volume was increased in both groups with the increase in the episodic group significantly greater than the daily group. Alveolar volume was decreased in the episodic group. The total amount of CAR damage was not different for both episodic (35 exposures) and daily (90 exposures) groups.	Barr et al. (1990)
0.96	1,882	8 h/night, 7 days/week for 3 or 60 nights	Rat, M (S-D) 234-263 g	LM morphometry, histochemistry, autoradiography, and SEM, and TEM morphometry. Neither 3 nor 60 days of exposure altered the cell density of ciliated, serous, basal, brush, migratory, or unidentified cells in tracheal epithelium. 3 days: Damage to cilia and ciliated cells, including necrosis. Thymidine labeling index increased. Serous cell histochemistry unchanged. 60 days: Less evidence of injury than at 3 days, but more damaged ciliated cells than in controls. Complete recovery of the epithelial changes by 42 days PE.	Nikula et al. (1988a)
0.98	1,921	8 h/day, 7 days/week for 90 days	Rat, M (S-D) 65 days old	LM morphometry and SEM of CAR. Remodeling of CAR. Increased thickness of septal edge (tips) of alveoli, which form the walls of ADs (alveolar entrance rings) up to 0.6 mm from TB. Alveolar septa thickened by replacement of Type 1 cells by Type 2 and bronchiolar cells to 0.6 mm from TB.	Pinkerton et al. (1992)

Table 6-12 (cont'd). Effects of Ozone on Lung Structure: Long-term Exposures (> 2 Weeks[a])

Ozone Concentration					
ppm	g/m	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
1.0	1,960	6 h/day, 5 days/week for 20 mo	Rat, M (F344) 6-7 weeks old	LM morphometry, SEM, confocal microscopy, immunocytochemistry, and conventional histochemistry. Remodeling of CAR. Former ADs were converted to RBs. Bronchiolar epithelium in these former ADs consisted of well-differentiated ciliated and nonciliated bronchiolar (Clara) cells.	Pinkerton et al. (1993)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Harkema et al. (1987) exposed bonnet monkeys to 0.15 or 0.30 ppm O_3, 8 h/day for 6 or 90 days. They sampled four regions of the nasal cavity and nasopharynx. Changes were limited to the respiratory and transitional epithelium in the two most rostral (anterior) of the four sections. No changes were reported in the caudal (posterior) two sections, the last of which included the nasopharynx. The respiratory epithelium of the rostral nasal cavity had both qualitative and quantitative changes. Quantitative changes included decreased density of ciliated cells characterized qualitatively by multifocal loss of cilia, necrotic ciliated cells, ciliated cells with attenuated cilia, and others with only microvillar surface. The respiratory epithelium also had an increased density of SMG cells, presumably related to repair processes. Monkeys exposed to 0.30 ppm O_3 for 90 days also had increased abnormal cells with intracytoplasmic lumens containing both cilia and microvilli. Qualitative changes were also seen in mucous (goblet) cells, which appeared to have fewer secretory granules and dilated endoplasmic cisternae. Ozone exposure resulted in more nonciliated cells with secretory granules and with dilated cisternae of the endoplasmic reticulum. Like the respiratory epithelium, the transitional epithelium had an increased density of SMG cells. In both epithelia, inflammatory cells were increased only in the monkeys exposed to 0.15 ppm O_3 for 6 days. Most of the morphometric changes in the respiratory but not the transitional epithelium were as large after 6 days of exposure to 0.15 ppm O_3 as after 90 days of exposure to either 0.15 or 0.30 ppm. The histochemistry and cytochemistry of the nasal epithelia from these monkeys were studied by Dimitriadis (1992). This investigator reported changes in the intraepithelial mucosubstances and the presence of mucous cells with dilated cisternae in the granular endoplasmic reticulum.

Acute changes in nasal epithelia from rats exposed to O_3 concentrations of 0.12 to 1.0 ppm for 6 h to 7 days have been studied extensively (Table 6-10). In general, short-term exposure to 0.2 ppm O_3 results in either no changes detectable by LM or in mild hyperplasia. Higher concentrations for up to 7 days can result in damaged cilia, hyperplasia, and increased stored intraepithelial mucosubstances. Several studies document the hyperplasia using morphometry or DNA synthesis and document the stored mucosubstance by histochemistry and morphometry. In one study, the increased stored intraepithelial mucosubstances reached their largest quantity 7 days postexposure (Harkema et al., 1989). Details of individual studies follow.

Exposure to 0.12, 0.8, or 1.5 ppm O_3 for 6 h followed by postexposure periods up to 66 h resulted in inflammatory changes characterized by increased PMNs, but without LM evidence of necrosis, ciliary loss, or hyperplasia (Hotchkiss et al., 1989a). Hotchkiss and Harkema (1992) reported similar LM findings in rats exposed to 0.8 ppm O_3 for 6 h. They also reported increased DNA synthesis by bromodeoxyuridine (BrdU) uptake in nasal nonciliated transitional epithelium. Exposure to 0.8 ppm O_3 6 h/day for 3 or 7 days, or for 3 days with 4 days postexposure, resulted in hyperplasia of the nasal nonciliated cuboidal (transitional) epithelium with increased intraepithelial mucosubstances without significant changes in histochemical staining characteristics (Hotchkiss et al., 1991). In that study, no changes were reported for rats exposed for 3 days and examined 18 h postexposure.

Reuzel et al. (1990) exposed rats to 0.2, 0.4, or 0.8 ppm O_3, 22 h/day for 3 days. They did not report changes in rats exposed to 0.2 ppm, but those exposed to 0.4 or 0.8 ppm had loss of cilia and disarrangement of the epithelium with hyperplasia and metaplasia. Cell proliferation, as measured by radiolabeled thymidine, was increased at the two higher concentrations. The influence of O_3 C $\times$ T on epithelial cell proliferation in the nasal anterior

maxilloturbinates was measured by BrdU uptake (Henderson et al., 1993). Rats were exposed to 0.12, 0.24, and 0.48 ppm O_3 for 3, 6, 12, and 24 h, resulting in six C × T products. Exposure to 0.12 ppm or C × Ts of 0.72 or 1.44 ppm·h did not cause effects. For a given C × T between 2.88 and 11.52 ppm·h, the increased DNA synthesis was similar; the response did not increase linearly with increasing C × Ts. Generally, above 0.12 ppm O_3 there was a linear increase with increasing C but not T. Thus, exposure duration apparently was responsible for the lack of C × T linearity. Johnson et al. (1990) also used BrdU to study DNA synthesis in rats exposed to 0.12, 0.27, or 0.8 ppm O_3, 6 h/day for 3 or 7 days, and examined 3 or 7 days postexposure. Rats exposed to 0.8 ppm O_3, but not to the lower concentrations, had increased DNA synthesis in the nonciliated cuboidal (transitional) epithelium at 3 and 7 days and increased numbers of labeled cells in the ciliated respiratory epithelium and the olfactory epithelium only at 3 days. No changes were found in squamous epithelia except a decrease in labeled cells 7 days postexposure to 0.8 ppm O_3. Johnson and co-workers reported no LM changes in the ciliated respiratory, olfactory, or squamous epithelia, but hyperplasia occurred in the cuboidal transitional epithelium.

Epithelial mucosubstances were studied in rats exposed to 0.12 or 0.8 ppm O_3, 6 h/day for 7 days or 7 days postexposure (Harkema et al., 1989). They reported no LM pathology in the nasal or nasopharyngeal airways from rats exposed to 0.12 ppm, with the exception of an increase in secretory cells in ciliated epithelium. Rats exposed to 0.8 ppm had attenuation of cilia in the lateral walls of the nasopharynx; 7 days postexposure, an increase in stored intraepithelial mucosubstances was observed. The 0.8-ppm group also had hyperplasia of the nonciliated transitional epithelium accompanied by an increase in PMNs in the lamina propria. Seven days postexposure, rats in the 0.8-ppm exposure group had more stored intraepithelial mucosubstances in some areas of ciliated respiratory and nonciliated transitional epithelia.

In rats exposed to 0.12, 0.25, or 0.5 ppm O_3, 20 h/day for 2 years, Smiler et al. (1988) reported hyperplasia, especially of mucous cells, in the respiratory epithelium over the rostral portion of the nasoturbinate of rats in the 0.25- and 0.5-ppm groups. The respiratory epithelium lining other parts of the nasal cavity were less affected, and no changes were found in the squamous and olfactory epithelia.

Harkema et al. (1994) reported no changes in the amount of mucosubstances in conducting airways, including the nasal cavity, of rats exposed to 0.12 ppm O_3, 6 h/day, 5 days/week, for up to 20 mo. After exposure to 0.5 and 1.0 ppm O_3, however, mucous flow rates were slower and mucous cell metaplasia was evident over the lateral wall and turbinates of the proximal third of the nasal airways. Exposure to 0.5 and 1.0 ppm O_3 also caused epithelial hyperplasia in nasal transitional epithelium, an increase in eosinophilic globules in the surface epithelium lining the distal nasal airways, and mild-to-moderate inflammatory cell influx in the nasal mucosa of the proximal and middle nasal passages.

Larynx

Leonard et al. (1991) reported disruption and thickening of the stratified squamous epithelium over the vocal folds of bonnet monkeys (*Macaca radiata*) exposed to O_3, 8 h/day for 7 days. The basement membrane appeared undulating rather than smooth. At 7 days postexposure, the epithelium appeared thickened, but otherwise normal. The O_3 concentration to which the monkeys were exposed is not clear because different concentrations appear in the summary and text sections of the publication. However, these larynges were from bonnet

monkeys that also were studied by Harkema et al. (1987) and Dimitriadis (1992) and, therefore, most likely were exposed to 0.15 ppm.

Trachea and Bronchi

Several investigators studied effects of 0.96 or 1.0 ppm O_3 on the tracheas of monkeys, rats, and sheep during and after short-term (very brief) or long-term exposures. Hyde et al. (1992) studied the trachea, bronchi, and RBs of rhesus monkeys exposed to 0.96 ppm O_3 for 8 h and examined them at 1, 12, 24, 72, and 168 h postexposure. Although the primary objective of the study concerned inflammation (see Section 6.2.2), the study also provided much new morphometric information concerning reactions to O_3 of tracheal, bronchial, and RB epithelia and their interstitium. Both epithelial and interstitial data were determined as volume per surface area of epithelial basal lamina (V_s). At 1 h postexposure, the major change in the tracheal and RB epithelia was an increase in necrotic cells, whereas in the bronchial epithelium, there were fewer ciliated and basal cells. There were no other changes in tracheal epithelial cell V_s at any of the postexposure times examined. At 12 and 24 h postexposure, the V_s of necrotic cells was increased in bronchi but not in the trachea or RBs. The V_s of ciliated and basal cells was smaller in the bronchial epithelium but not in the trachea. Basal cells in bronchi also were increased at 72 and 168 h postexposure. Respiratory bronchioles had smaller V_s of Type 1 alveolar epithelial cells at all times except 1 h postexposure. In RBs, nonciliated bronchiolar cells were increased only at 24 h postexposure. Epithelial cell DNA synthesis was studied in the filtered air controls and at 1 and 12 h postexposure by radiolabeled thymidine incorporation. The only increase was observed in the bronchial epithelium at 12 h postexposure. Changes in the interstitial components of the trachea were minimal, with a decrease in the amorphous matrix at 24 h postexposure. Bronchi had increased V_s of smooth muscle and decreased amorphous matrix at 24, 72, and 168 h postexposure. Collagen fibers in the bronchial interstitium were decreased at 168 h. In RBs, the arithmetic mean thickness was increased at 12 and 24 h, but not at other times. In RBs, smooth muscle V_s was increased at 24 h, V_s of fibroblasts was increased at 24 and 72 h, and V_s of the amorphous matrix was increased at 12 h postexposure.

Nikula et al. (1988a) exposed rats to 0.96 ppm O_3, 8 h/night for 3 or 60 nights or for 60 nights followed by 7 or 42 days postexposure, and examined the tracheas using LM, TEM morphometry, SEM, LM mucosubstance histochemistry, and DNA synthesis by radiolabeled thymidine incorporation. Ciliated cells with short or damaged cilia were increased after 3 and 60 nights of exposure; cells with short cilia were increased after 60 nights of exposure and 7 days postexposure. Intermediate cells, presumed to be immature ciliated cells, were increased only after 3 nights of exposure. However, the numeric density of total ciliated cells, basal cells, total serous cells, brush cells, and total migratory cells was not different from controls. There were no changes in LM histochemistry for mucosubstances at any time. The only increase in thymidine labeling occurred after 3 days of exposure. Recovery was complete 42 days after 60 nights of exposure.

Mariassy et al. (1989, 1990) exposed newborn lambs to 1.0 ppm O_3, 4 h/day for 5 days, and studied controls at birth and controls and exposed lambs at 2 weeks of age. Tracheal mucous velocity was decreased at 2 weeks and at several additional postexposure times (see Section 6.2.3). In control lambs, the percent of ciliated cells increased and mucous cells decreased in the tracheas from birth to 2 weeks of age. This normal change in cell populations did not occur in the exposed lamb tracheas. In the more detailed morphological

study (Mariassy et al., 1989), epithelial cell density (cells per millimeter), rather than differential cell counts, was reported. In tracheas from control lambs, the density of mucous cells decreased from birth to 2 weeks of age. Ozone exposure resulted in decreased total epithelial cell density, with decreased densities of ciliated and basal cells. Mucous cell density remained at newborn levels. Ozone exposure also prevented the normal maturational changes of lectin-detectable mucosubstances but not of tinctorially stained mucosubstances.

The most comprehensive study of the effects of long-term O_3 exposure on conducting airways of rats is that by Plopper et al. (1994a). They used LM morphometry and tinctorial histochemistry to study conducting airways from the trachea to centriacinar alveoli following two "short" and one "long" pathway by airway dissection of fixed lungs. In rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo, the investigators did not find differences due to O_3 exposure in tracheal or bronchial epithelial thickness, cell populations, or stored glycoconjugates. However, they did find a concentration-dependent loss of stored glycoconjugates in the tracheas and in the caudal long-path bronchi but not in the cranial or central short-path bronchi. Although not significantly different from controls, there was a concentration-dependent thinning of the epithelium in caudal long-path bronchi. Terminal bronchioles from rats exposed to 0.5 and 1.0 ppm O_3 had increased volume fraction (V_v) of nonciliated bronchiolar (Clara) cells, and the epithelium was thicker in TBs from rats exposed to 1.0 ppm. In all exposed rats, the mass (V_s) of nonciliated bronchiolar cells was increased in TBs that had long pathways (caudal) but not in TBs with short pathways (cranial and central).

Centriacinar Region

As described in the previous criteria document (U.S. Environmental Protection Agency, 1986) and in the summary of it above, the CAR varies with the species. By common usage (Weibel, 1963; Schreider and Raabe, 1981; Weibel, 1983; Rodriguez et al., 1987; Haefeli-Bleuer and Weibel, 1988), the acinus consists of a TB, RBs when present, and the ADs and alveoli supplied by that TB. In some species (e.g., humans, monkeys, dogs, and cats), several generations of RBs are found between the TB and ADs. In other species (e.g., rats, mice, guinea pigs, and rabbits), RBs either are absent or very poorly developed and limited to a single, very short generation (Tyler, 1983; Tyler and Julian, 1991). The CAR consists of the TB, RBs if present and alveoli that open directly into RBs, and the initial portions of ADs. Acini that do not have RBs have a smaller volume than those that do. Rodriguez et al. (1987) estimated the acinar volume in rat lungs to be 1.86 mm^3, and Haefeli-Bleuer and Weibel (1988), using the same methods, estimated the acinar volume in the human lungs at 187.0 mm^3. Mercer and Crapo (1989) and Mercer et al. (1991) found that variation in acinar size within an individual lung is an important determinant of the intensity of lesions due to inhaled reactive gases. Thus, the intensity of CAR lesions may vary when animals with differing size acini are compared (Plopper et al., 1991).

The CAR lesion, both in animals with small acini (e.g., rats) and animals with large acini (e.g., monkeys) has been well described, both in the original reports and in the 1986 document (U.S. Environmental Protection Agency, 1986). Some of the reports published since that document contain additional details concerning cellular and interstitial responses in the CAR to short- or long-term O_3 exposure and are presented in this section (Chang et al., 1992; Pino et al., 1992c; Harkema et al., 1993). Most of these studies need TEM levels of resolution and magnification and employ morphometric methods. In other reports,

morphometric estimates of the volume of the CAR lesion were used to study factors that might alter the intensity of the lesion or evaluate the intensity of reaction to specific exposure regimens (Mautz et al., 1988; Warren et al., 1988; Stiles and Tyler, 1988). Due to the size and definition of the CAR lesion, this approach can use LM morphometry to estimate lesion volume. In other studies, the cumulative effect of O_3 on the CAR is estimated by LM morphometry of one of the components, distal airway remodeling, which results in the formation of new RBs (Barr et al., 1988; Tyler et al., 1988; Pinkerton et al., 1993). Examples of each type of study will be presented.

Pino et al. (1992c) exposed rats to 1.0 ppm O_3 for 4, 6, 8, or 24 h, followed by postexposure periods in filtered air for up to 20 h, so that the total exposure and postexposure period did not exceed 24 h. Some of the rats were used for BAL (see Section 6.2.2), others for TEM morphometry. The morphometric data are expressed as V_s values. After a 4-h exposure, necrosis was the dominant morphologic feature, with increases in V_s of necrotic cells in the TB epithelium (ciliated cells) and in CAR alveoli (Type 1 cells). With increasing time of exposure or postexposure, the volume of necrotic cells in TBs shifted from the epithelium to the lumen, with this change being significant at 24 h. In CAR alveoli, increased V_s of total necrotic cells occurred at 4, 6, and 24 h of exposure and at 24 h in the epithelium. Healing in the TBs, evidenced by increased V_s of undifferentiated cells, was underway 18 h after a 6-h exposure, 16 h after an 8-h exposure, and immediately after 24 h of exposure. The only significant change in viable alveolar cells was an increase in V_s of Type 1 cells after 24 h of exposure. This increase appeared predominantly due to swelling of individual Type 1 cells. Increased V_s of total TB interstitium occurred 4 h after an 8-h exposure. In CAR alveoli, total interstitium was increased after 8 h of exposure, with much of the increase due to an increase in capillary volume.

Chang et al. (1992) used TEM morphometry to evaluate cellular and interstitial responses in the CAR, TB, and alveoli (proximal alveoli) of rats exposed to a 9-h peak slowly rising to 0.25 ppm O_3 superimposed on a 13-h background level of 0.06 ppm (the background was 7 days/week, the peak was 5 days/week). Chang and co-workers examined rats after 1, 3, 13, and 78 weeks of exposure; 6 weeks after a 13-week exposure; and 17 weeks after a 78-week exposure. Centriacinar region alveoli had a larger volume of total tissue and total epithelium per area of basement membrane (V_s) only after 13 or 78 weeks of exposure, and these values were not different after postexposure periods. Type 1 cells had a larger volume only at 13 weeks of exposure, increased numbers at 78 weeks, and increased numbers after 13 weeks of exposure plus 6 weeks postexposure. Type 2 cell V_s was increased only after 78 weeks of exposure and 78 weeks of exposure plus 17 weeks postexposure. Macrophages in the CAR alveoli were increased only after 1 week of exposure. In CAR alveoli, both interstitial cells and matrix were increased after 1 week of exposure, and the matrix increased again after 13 and 78 weeks of exposure. This difference was no longer significant after either postexposure period. Although the data are not in the tables or figures in the article, the text indicates that both epithelial and endothelial basement membranes were thickened after 13 and 78 weeks of exposure and after the 17-week postexposure period. Crystalline deposits in the basement membrane are demonstrated in Figure 6-3. In TBs, the luminal surface area of Clara cells was reduced at 1 week of exposure, and both ciliated and Clara cells had smaller luminal surface areas after 78 weeks of exposure; these returned to control values during the postexposure period. However, increased V_s of Type 2 cells persisted for the 17-week postexposure period that followed the

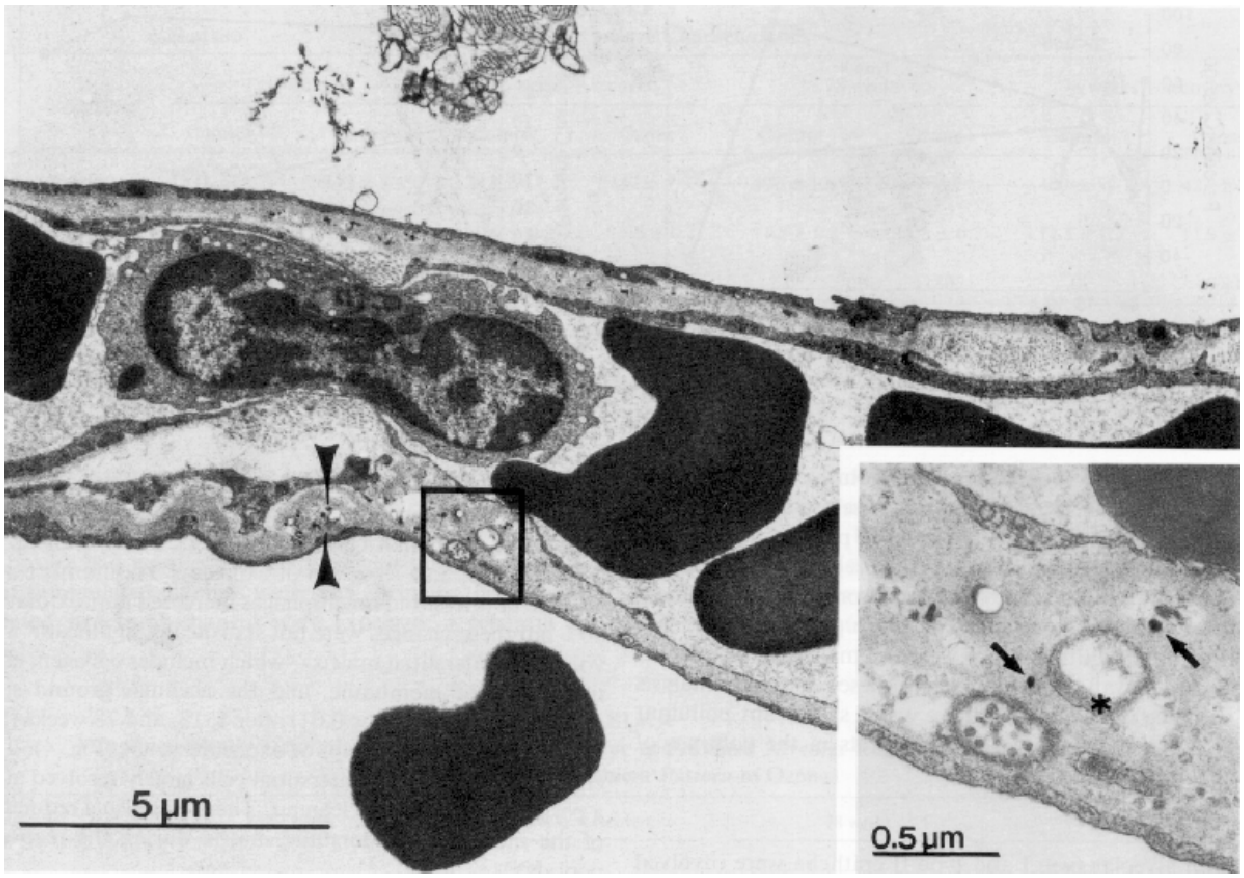

Figure 6-3. Electron micrograph of alveolar septa in the centriacinar region of the lungs from laboratory rats exposed to a simulated pattern of ambient O_3 for 78 weeks, showing thickened basement membrane (arrow heads). Inset micrograph shows dense crystaline deposits (arrows) and cellular extensions (*).

Source: Chang et al. (1992).

78 weeks of exposure. Chang et al. did not find significant bronchiolization of alveoli (i.e., distal airway remodeling). This observation may be due to the way the investigators sampled the CAR proximal alveoli and the strict orientation of the airways to obtain exact cross sections. This study complements those of Barry et al. (1985, 1988), who used similar TEM morphometric methods, by extending the exposure period and adding postexposure periods.

Cellular and interstitial changes were studied in nonhuman primates exposed 8 h/day for 90 days by both Moffatt et al. (1987) and Harkema et al. (1993), using TEM and morphometry. The study by Harkema et al. (1993) is reported here because the concentrations used, 0.15 and 0.3 ppm O_3, were lower than those used by used Moffatt et al. (1987), which were 0.4 and 0.64 ppm. Harkema and co-workers also studied reactions after only 6 days of exposure to 0.15 ppm. There were no major differences among the three exposed groups

(i.e., 6 days to 0.15 ppm, 90 days to 0.15 ppm, and 90 days to 0.3 ppm O_3). All exposed monkeys had thicker RB epithelium and thicker RB interstitium. There were more nonciliated cuboidal epithelial cells per millimeter of basement membrane in all exposed groups and increased squamous cells only in the 6-day group. The thickened total interstitium was due to increases in both acellular (matrix) and cellular components, but both compartments were increased individually only in the 90-day O_3 group. There were no differences in RB smooth muscle. Transmission electron microscopy and SEM observations, which were not studied quantitatively, include increased AMs in alveoli opening into RBs and increased "dome"-shaped nonciliated bronchiolar cells, which had more apical cytoplasm, more agranular and granular endoplasmic reticulum, more mitochondria, and more Golgi with secretory granules. With the exception of increased AMs, there was no evidence of necrosis nor of inflammatory cells. No differences due to age or gender were detected.

Harkema et al. (1993) speculate that their finding of a larger percent increase in RB cuboidal cells in monkeys exposed to a lower concentration of O_3 for the same time than that reported by Moffatt et al. (1987) might be due to the difference in sampling methods. Harkema et al. studied only the first generation RBs, whereas Moffatt et al. (1987) studied a random sample of all generations of RBs. The first generation tends to be more damaged than succeeding generations (Mellick et al., 1977; Eustis et al., 1981). The Harkema et al. (1993) sampling procedure also prevented examination for the increased volume density of RBs and decreased RB diameter reported by Fujinaka et al. (1985) and Moffatt et al. (1987).

Remodeling of Centriacinar Region Airways. This is a less well-known sequela of long-term O_3 exposure. Using SEM, Boorman et al. (1980) and, later, Moore and Schwartz (1981) reported the development in rats of an airway with the appearance of RBs between the TB and ADs. This new segment was longer than those occasionally seen in control rats. Respiratory bronchioles in rats either are absent or developed to only a single, very short segment (Tyler, 1983; Tyler and Julian, 1991).

Barr et al. (1988) examined the development of this new segment using LM and TEM morphometry on lungs from rats exposed to 0.95 ppm O_3, 8 h/day for 90 days. They reported a significant increase in the total volume of RB and of RB lumen and wall. The new RBs reached a maximum length of four alveolar opening rings. They also noted that, in some of these RB segments, the capillary and epithelial basal laminae were fused as they are in TBs, rather than separate as in alveoli. Most Type 1 cell necrosis was found at the tips of alveolar septa immediately adjacent to the RB/AD junction. Thus, the most severe epithelial damage did not occur at the most proximal alveolus in the CAR, but rather in the alveolus immediately distal to the newly formed RB.

Recently, Pinkerton et al. (1993) developed a new LM morphometric method to evaluate remodeling of CAR ADs. In rats exposed to 1.0 ppm O_3 intermittently for 20 mo, the investigators reported well-differentiated ciliated and nonciliated bronchiolar epithelium lining CAR airways that would otherwise be ADs. Some of this epithelium extended five alveoli from the TB. Thus, the Type 1 and 2 cells characteristic of ADs were replaced by both types of bronchiolar cells characteristic of RBs when RBs are present in control rats. Pinkerton et al. (1995) used their new morphometric method to study rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo. They reported significant thickening of alveolar septal tips 200 mm from the TB in rats exposed to 0.12 ppm, which increased with O_3 concentration to 600 m in rats exposed to 1.0 ppm, but they did not describe the type of

epithelium covering these thickened tips. Several studies that did not find CAR remodeling also used a slightly different procedure (Barry et al., 1985; Chang et al., 1992).

Plopper et al. (1994a) examined CARs from rats exposed to the same regimen, but studied CARs from one cranial short pathway and a caudal long pathway. They found nonciliated bronchiolar epithelial cells in remodeled former ADs in short- and long-pathway CARs from rats exposed to 1.0 ppm O_3, but only in short-pathway CARs from rats exposed to 0.5 ppm. Central, short-pathway CARs were not examined. Using the airway dissection method of selecting CARs to be studied, nonciliated bronchiolar cells were not found in ADs from rats exposed to 0.12 ppm O_3.

The same phenomena apparently occurs in animals with several generations of RBs as increases in V_v and volume (V) of RBs have been reported in all O_3-exposed monkeys examined using morphometric methods to estimate V_v or V of RBs (Fujinaka et al., 1985; Moffatt et al., 1987; Tyler et al., 1988, 1991b). Inflammatory changes and CAR remodeling occur concomitantly, and inflammatory changes in an airway may indicate future remodeling. Mellick et al. (1977) noted that, in monkeys exposed to 0.8 ppm, 8 h/day for 7 days, the inflammatory process extended throughout the RBs and into ADs. Eustis et al. (1981) reported that, in monkeys exposed to 0.8 ppm, 8 h/day for 90 days, all generations of RBs contained aggregates of inflammatory cells. Monkeys exposed to lower concentrations for the same or longer time have increased V_v and V of RBs (Moffatt et al., 1987).

6.2.4.3 Considerations of Exposure Regimens and Methods

Recovery During Postexposure Periods

Evidence of healing occurs soon after short-term O_3 exposures cease. In the studies of Pino et al. (1992c), evidence of healing is provided by the increased V_s of viable undifferentiated cells in TBs detected 16 h after the end of an 8-h exposure to 1.0 ppm O_3.

Chang et al. (1992) reported an increased V_s of Type 2 cells 17 weeks after a 78-week exposure to a simulated urban exposure regimen with a peak O_3 concentration of 0.25 ppm; there were no changes detected 6 weeks after a 13-week exposure. Gross and White (1987) examined rats 3 and 6 mo after a 52-week exposure (20 h/day, 7 days/week) to 0.5 ppm O_3. Using LM pathology, the only changes visible 6 mo after a 12-mo exposure were a few areas of bronchiolization, slight dilation of ADs, and slight thickening of AD walls and adjacent alveolar septa. In an earlier study, less complete healing was reported by Gross and White (1986), who used LM pathology to study rats 4 and 9 weeks after a 4-week exposure (20 h/day, 7 days/week) to 0.7 ppm O_3. Four weeks postexposure, Gross and White reported a slight, unevenly distributed inflammatory reaction with condensed eosinophilic material, presumed to be collagen, in the interstitium. Nine weeks postexposure, some AD walls and TBs were thickened. Rats exposed to 0.96 ppm, 8 h/night for 42 nights and examined 42 days later using LM morphometry had increased V_v and V of the RB wall and SEM evidence of CAR remodeling (Tyler et al., 1987). Collagen content of these lungs increased during the postexposure period (Last et al., 1984b).

Centriacinar region remodeling was more persistent in monkeys exposed to 0.64 ppm O_3, 8 h/day for 12 mo followed by 6 mo postexposure (Tyler et al., 1991b). By LM morphometry, the V_v and V of total RB, RB lumen, and RB walls were increased both at exposure end and at 6 mo postexposure. At exposure end, but not at 6 mo postexposure, RB internal diameters were smaller, and AMs in the CAR increased.

One study concerned postexposure recovery of the trachea (Nikula et al., 1988a). Complete recovery of the trachea (as evaluated by LM morphometry, SEM, and TEM) of rats exposed to 60 nights (8 h/night, 7 days/week) to 0.96 ppm O_3 occurred following a 42-day postexposure period.

Effects of Episodic and Seasonal Exposure Regimens

Many investigators have noted that lesions due to O_3 reach a maximum intensity in a very few days and that, with continued exposure, the intensity of the lesion decreases. Eustis et al. (1981) reported half the number of inflammatory cells in the CAR of monkeys exposed to 0.8 ppm for 90 days as found in monkeys exposed to the same concentration for 7 days. Chang et al. (1992) noted that the acute reactions to the 0.06-ppm background O_3 (7 days/week), with a 9-h peak (5 days/week) slowly rising to 0.25 ppm, that they reported at 1 week of exposure had subsided at 3 weeks of exposure. Harkema et al. (1993) reported no difference in first generation RB epithelial thickness or cell numbers among monkeys exposed to 0.15 ppm O_3 for 6 days or to 0.15 or 0.3 ppm for 90 days.

These and other similar observations prompted Chang et al. (1991) to compare effects of two exposure regimens, which were evaluated using the same TEM morphometric approach. The first regimen was a "square wave", 12-h/day, 7-day/week exposure to 0.12 or 0.25 ppm O_3. The second regimen simulated urban O_3 exposures by exposing rats 7 days/week for 13 h to 0.06-ppm background, with a peak slowly rising to 0.25 ppm over a 9-h period (5 days/week). They calculated cumulative O_3 concentration ($C \times T$) for each exposure regimen and concluded that increases in volume of Type 1 and 2 alveolar epithelial cells were linearly related to increasing $C \times T$. The relationship for Type 1 cells was more robust.

Barr et al. (1990) used TEM and LM morphometry to compare effects of 90 days of daily exposure of rats for 8 h/day to 0.95 ppm O_3 with a regimen that modeled 5-day episodes of O_3 exposure. Each 5-day episode was followed by 9 postexposure days of filtered air. The cycle was repeated seven times so that the "episodic" group was exposed a total of 35 days over an 89-day period, and the "daily" group was exposed for 90 days to the same O_3 concentration. Both groups had CAR remodeling with the formation of RBs. The volume of RBs formed was not different when the two exposure groups were compared. The absolute volume of parenchymal lesion was the same in both groups. The RB epithelial thickness was increased in the daily group but not in the episodic group; conversely, the interstitium of both TBs and ADs was thickened in the episodic group but not in the daily group. Thus, rats exposed to the same concentration of O_3 for 35 days over an 89-day period in an episodic regimen had lesions as severe as those rats exposed daily for 90 days.

Effects of "seasonal" and "daily" exposure of young monkeys to 0.25 ppm O_3 were reported by Tyler et al. (1988). The daily group was exposed every day (8 h/day) for 18 mo, whereas the seasonal group was exposed only during odd months for the 18 mo. Thus, the daily group was exposed twice as many days to the same concentration as the seasonal group. By LM morphometry, both groups had increased V_v of total RB and RB lumen, but RB wall thickness was increased only in the daily group. The only significant morphometric difference between the two groups was an increase in CAR AMs in the daily group. This difference, and the difference in significance of the RB wall thickness in the seasonal group, was presumed due to the daily group being exposed to O_3 the day before necropsy, whereas the seasonal group breathed filtered air for 30 days preceding necropsy. This final 30 days of filtered air

apparently allowed the more acute inflammatory changes in the seasonal group to regress. The seasonal group, but not the daily group, had increased lung collagen (Section 6.2.1) and increased chest wall compliance (Section 6.2.5). Exposure to the same concentration of O_3 for half as many days in a seasonal regimen resulted in morphometric effects similar to daily exposure and in physiological and lung collagen changes not found in the daily group.

Tyler et al. (1991a) exposed rats to seasonal and daily regimens similar to those used for the monkeys described above. The concentration used in both studies was 0.25 ppm O_3 and the total length of exposure was 18 mo. Rats were exposed nights, during their natural period of activity. Both groups of rats were studied at the end of the 18-mo exposure cycle and 30 days postexposure. The lungs were evaluated using a simplified LM morphometric method for CAR airway remodeling, estimating the number of junctions of bronchioles (TB and RB) with ADs per surface area of section. At exposure end, the number of junctions of both exposure groups was increased compared to filtered-air controls; the O_3 groups were not different from each other. Neither group was different from the controls at 30 days postexposure.

Ex Vivo and In Vitro Exposures

Results obtained from studies of isolated perfused lungs and organ culture explants were consistent with some of the findings from in vivo studies (Pino et al., 1992a; Nikula et al., 1988b; Nikula and Wilson, 1990).

6.2.4.4 Considerations of Degree of Susceptibility to Morphological Changes

Species Differences in Degree of Response

Plopper et al. (1991) reviewed data from nonhuman primates and rats that had been exposed to O_3 and evaluated using TEM morphometry. The data were generated in several laboratories and the exposure and evaluation methods were somewhat different, but the data were expressed in similar terms. In the CAR, the results were expressed as total epithelial thickness or numbers of cells per square millimeter of basal lamina. Exposure of rats to 0.25 ppm O_3, 8 h/day for 42 days, resulted in an increase of less than 100% in either parameter compared to controls (Barry et al., 1985, 1988). Exposure of monkeys to 0.15 ppm O_3, 8 h/day for 6 days, resulted in a 230% increase in thickness and a 700% increase in cell number compared to controls (Harkema et al., 1993). As noted earlier (Section 6.2.4.2), the CARs of rats and monkeys are structurally different (Tyler, 1983), and the CAR cells are also different (Plopper, 1983).

There was also a difference when Plopper et al. (1991) compared stored secretory product per square millimeter of basal lamina in the nasal septum and lateral wall of the nasal cavity of O_3-exposed rats and monkeys. Data from the exposure of rats to 0.12 ppm, 6 h/day for 7 days, resulted in a $<10\%$ increase in the nasal septum and a $<100\%$ increase in the lateral wall. Exposure of monkeys to 0.15 ppm, 8 h/day for 6 days, resulted in a 300% increase in the nasal septum and a 125% increase in lateral wall. As in the CAR, there are major morphological differences in the nasal cavities of these two species (Schreider and Raabe, 1981).

Plopper et al. (1991) also compared collagen metabolism in rats and monkeys exposed to 1.5 ppm O_3, 23 h/day for 7 days, using the uptake of tritium-labeled proline. In rats, there was an increase of 200% above controls, whereas the increase was 800% in monkeys.

From these data, it appears that the respiratory system of monkeys is much more responsive than that of rats to near-ambient concentrations of O_3. The mechanisms responsible for these species differences in response to O_3 remain to be elucidated.

Effects of Age

Several studies published since the previous criteria document (U.S. Environmental Protection Agency, 1986) have addressed the effects of age on the intensity of O_3 morphological changes. The study by Stephens et al. (1978) and the initial report by Barry et al. (1983) were cited. Briefly, Stephens et al. exposed rats ranging in age from 1 to 40 days old to 0.85 ppm O_3 for 24, 48, or 72 h and examined their lungs by LM and TEM. Stephens and co-workers reported that, prior to 20 days of age, they did not find damage to TB-ciliated cells or to CAR Type 1 cells and that the amount of injury increased from 21 to 35 days when a plateau in response was reached.

Barry et al. (1985, 1988) exposed 1-day-old and 6-week-old rats to 0.12 or 0.25 ppm O_3, 12 h/day for 6 weeks. The 1985 study emphasized TEM morphometry of CAR (proximal) alveoli. The investigators did not find differences in response due to age. In both age groups, they found Type 1 cells increased in number and thickness, but decreased in both luminal and basement membrane surface area. They found bare basement membrane where Type 1 cells had been sloughed, but the amount was not increased in exposed groups. In the 0.25-ppm groups, but not in the 0.12-ppm groups, Type 2 cells were increased in density per square millimeter basement membrane but not in volume. Alveolar interstitium was increased only in adults exposed to 0.25 ppm. Macrophages in alveoli were increased in both age groups exposed to 0.25 ppm, but not in adults exposed to 0.12 ppm. Interstitial AMs were increased only in adults exposed to 0.25 ppm. The TEM morphometry of TBs from these rats did not include adults exposed to 0.12 ppm (Barry et al., 1988). There were no differences due to age at start of exposure. In both juvenile and adult rats exposed to 0.25 ppm, Barry and co-workers found that the luminal surface covered by cilia and by nonciliated bronchiolar (Clara) cells was reduced. The number of brush cells was also decreased.

Stiles and Tyler (1988) studied effects in a wider range of ages using LM morphometry and SEM. They exposed 60- and 444-day-old female rats to 0.35 or 0.8 ppm O_3 continuously for 72 h. Body weights of the 444-day-old rats, but not of those 60 days old, decreased during exposure. Fixed lung volumes of 444-day-old rats exposed to 0.8 but not 0.35 ppm were smaller than same-age controls. The V_v of CAR lesions was larger in 60-day-old rats than in the 444-day-old rats exposed to either concentration. The V_v of cells free in lumens (AMs) was increased in young rats exposed to 0.35 ppm compared to the older rats, but was not different for rats exposed to 0.8 ppm. Young rats exposed to either concentration had larger CAR lesions than the older rats, and young rats exposed to the lower concentration had more AMs. Older rats had greater changes in body weight and, in those exposed to the higher concentration, in fixed lung volume.

Effects of Exercise

Exercise increases the dose of inhaled toxicants delivered to sensitive cells (see Chapter 8). Mautz et al. (1985b) studied the effects of 0.2 and 0.38 ppm O_3 on rats at rest and during several treadmill exercise protocols. They found increased percent of lung parenchymal area containing free cells (AMs) in exercised rats exposed to both concentrations compared to

rats exposed at rest. At the higher concentration, there was also an increase in the percent of parenchymal area with thickened ADs and alveolar septa.

Tyler et al. (1991c) exposed thoroughbred horses (trained to a treadmill) to 0.25 or 0.8 ppm O_3 for 29 min on 2 consecutive days using a protocol that included 9 min of graded exercise (3 min at maximum speed) and 20 min of "cool out". During maximal exercise, horses increase their rate of oxygen consumption more than other species. Two of three horses exposed to 0.8 ppm O_3 had significant areas of hemorrhage and edema, and one of them refused the second day's exercise and exposure. By TEM, all horses exposed to 0.8 ppm had CAR lesions including necrosis of Type 1 cells. Lesions in those exposed to 0.25 were limited to CAR ciliated cells. No horses were exposed at rest for comparison.

Elastase-Induced Emphysema

Rats with elastase-induced emphysema and saline-instilled controls were exposed to 0.15 or 0.5 ppm for 3 or 7 days (Dormans et al., 1989). Mean linear intercepts, a measure of alveolar size, were determined using LM. The incidence and severity of CAR inflammatory changes were the same in O_3-exposed elastase-treated and saline-control rats. There were no changes in mean linear intercepts due to the O_3 exposure.

6.2.4.5 Summary

Research since the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) continues to support the concept that all mammalian species respond to O_3 concentrations <1.0 ppm in a similar manner, but with significant differences in intensity of reactions among the species studied (Plopper et al., 1991). Dungworth (1989) provided a schematic overview of morphological reactions of the CAR from mammalian lungs to continuous exposure to low concentrations of O_3 as a series of time-response profiles (Figure 6-4). Bronchoalveolar exudative processes are the predominate early response, but the magnitude decreases rapidly with increasing duration of exposure and continues to decline during postexposure periods. Epithelial hyperplasia also starts early and increases in magnitude for several weeks, after which a plateau is reached until the exposure ends. Epithelial hyperplasia declines slowly during postexposure periods. Interstitial fibrosis has a later onset and may not be apparent for a month or more. The magnitude of this response, however, continues to increase throughout the exposure and, at least in some cases (Last et al., 1984b), continues to increase after exposure ends.

Nonhuman primates appear to respond more than rats to O_3 at concentrations <1.0 ppm. However, the mechanisms responsible for these differences in response have not been elucidated. Differences in cell, tissue, and circulating levels of several antioxidants are being studied, as are differences in in vitro responses to O_3 by cultures of cells from the various species. Basic morphological differences in the structure of the most injured portion of the lung, the CAR, and the size (volume) of the basic structural unit, the acinus, may also be factors in the greater response of monkeys to O_3. Both human lungs and lungs from nonhuman primates have CARs characterized by several generations of RBs, whereas rats have no RBs, or only a single poorly developed generation (Tyler and Julian, 1991). Within an individual lung, acinar volume is directly related to the intensity of CAR lesions (Mercer and Crapo, 1989; Mercer et al., 1991). The volume of individual acini in human lungs is 100 times larger than individual acini in rat lungs (Rodriguez et al., 1987; Haefeli-Bleuer and Weibel, 1988).

Acinar volume of the monkeys used in O_3 studies is not known but, on the basis of the CAR structure, is assumed to be more like that of human lungs than rat lungs.

Another morphological factor that may be responsible in part for the greater response to O_3 of nonhuman primates than of rats may be differences in the complexity of the nasal cavity. Schreider and Raabe (1981) studied the cross-sectional morphology of the nasal-pharynx in rats, beagle dogs, and a rhesus monkey. They concluded that the

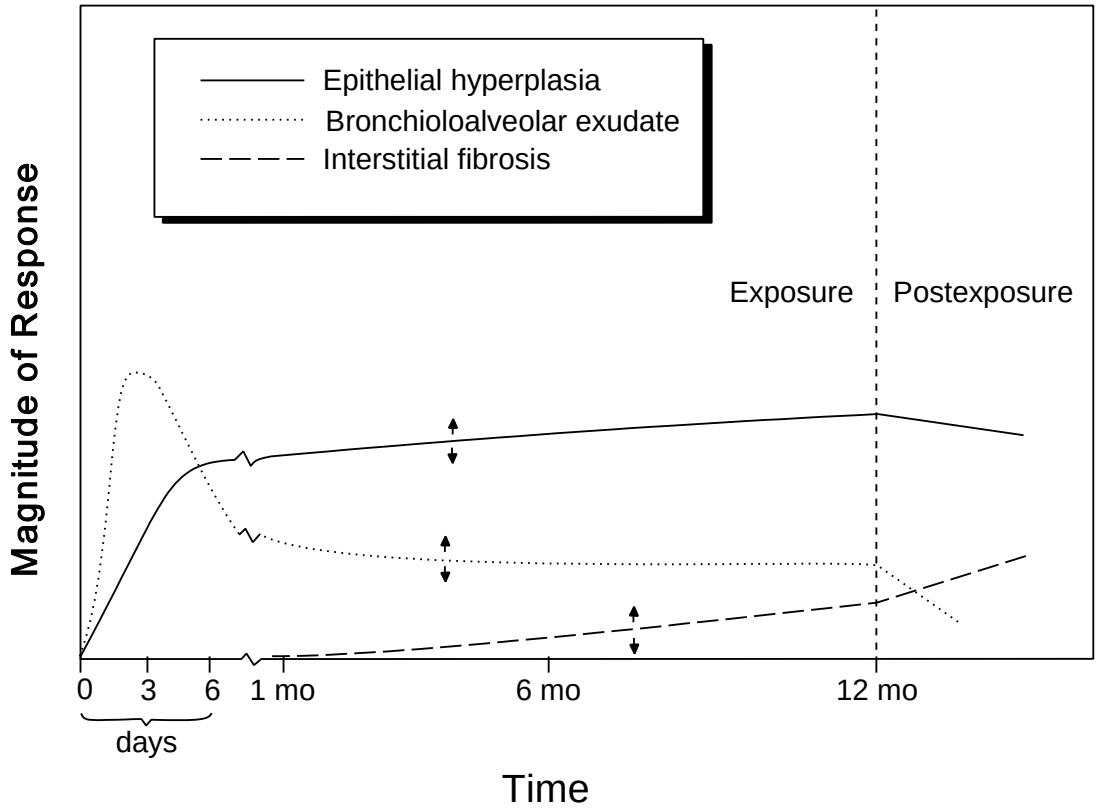

Figure 6-4. Schematic comparison of the duration-response profiles for epithelial hyperplasia, bronchoalveolar exudation, and interstitial fibrosis in the centriacinar region of lung exposed to a constant low concentration of ozone.

Source: Dungworth (1989).

complexity of the nasal cavity, and therefore the "scrubbing" effect (Yokoyama and Frank, 1972; Miller et al., 1979), which reduces the concentration of inhaled O_3 delivered to the lower respiratory tract, would be greater in rats than in monkeys. Schreider and Raabe (1981) proposed that, with appropriate scaling, the monkey could serve as a model for aerosol and gas deposition in the nasopharyngeal region of humans. However, the sensitivity of the nasopharyngeal epithelium may be different because changes in the nasal epithelium that follow O_3 inhalation, like those in the CAR, are more severe in monkeys than in rats (Plopper et al., 1991).

The effect of age at start of exposure on O_3-induced lung injury has not been resolved. Barry et al. (1985, 1988) reported no differences in TEM morphometry of CAR and TB lesions due to age at start of exposure. However, they studied a narrow range of ages, 1 and 42 days old at the start of a 42-day exposure. Barry et al. (l985, 1988) speculate that much of the CAR response in the rats that began exposure at 1 day of age might have occurred during exposure Days 21 to 42 because, as the earlier studies of Stephens et al. (1978) found,

rats are not sensitive to morphological effects of O_3 until weaning at 21 days of age. Thus, the rats that were 1 day old at the beginning of exposure may have developed the same intensity of lesions during the last 21 days of exposure as the older rats did in 42 days of exposure. In studies using a wider range of ages (60- and 444-day-old rats), Stiles and Tyler (1988) reported larger CAR lesions in the younger rats but greater changes in body weight and fixed lung volume in the older rats following a 3-day exposure to O_3.

The effects of exposure regimen and duration were evaluated in more recently published studies. Exposure of young monkeys to 0.25 ppm O_3 in a "seasonal" regimen (i.e., exposure in odd months and postexposure in even months) for 18 mo resulted in the same quantity of CAR lesions as daily exposure to the same concentration for 18 mo (Tyler et al., 1988). A similar quantity of CAR lesions was reported in rats exposed to 0.95 ppm O_3 in a 35 day, "episodic" regimen (units of 5-day exposures and 9 days without exposure) for a total of 89 days, as those exposed to the same concentration each day for 90 days (Barr et al., 1990). Chang et al. (1991) calculated the cumulative O_3 concentration for a "square wave" exposure to 0.12 or 0.25 ppm for 12 h/day, 7 days/week, with a simulated "urban" exposure regimen of 0.06 ppm for 13 h/day, 7 days/week and then raising that background 5 days/week to a peak of 0.25 ppm over a 9-h period. Using TEM morphometry of the CAR, they found no difference due to the pattern of exposure. Thus, it appears that the pattern of daily exposure does not influence the intensity of CAR lesions, but that episodic and seasonal patterns of exposure, with multiple days of clean air between days of exposure, are equivalent to daily exposure.

It has become clear that remodeling of centriacinar airways is cumulative. Using a stereological approach, Barr et al. (1988) reported an increase in the total volume of RB wall and lumen in rats exposed to 0.95 ppm O_3, 8 h/day for 90 days. Barr and co-workers also reported continuing Type 1 cell necrosis at the tips of alveolar septa (alveolar opening rings) immediately distal to the newly formed RB/AD junction (rather than in the TB/AD junction). It appears that some of the necrotic Type 1 cells were replaced by bronchiolar epithelium, rather than by Type 2 cells as previous studies indicated. This was confirmed by Pinkerton et al. (1993), who reported fully differentiated ciliated and nonciliated bronchiolar epithelium lining alveolar tips along a former AD up to 1 mm from the TB in lungs from rats exposed to 1.0 ppm O_3 for 6 h/day, 5 days/week for 20 mo. Remodeling of centriacinar airways appears to be a general phenomena, as increases in the V_v and V have been reported in lungs from all exposed rats and monkeys examined using stereological or morphometric methods that could detect this change (Fujinaka et al., 1985; Moffatt et al., 1987; Tyler et al., 1987; Tyler et al., 1988; Barr et al., 1990; Pinkerton et al., 1992, 1993). Centriacinar region remodeling has been demonstrated to persist in monkeys 6 mo after a 12-mo exposure to 0.64 ppm O_3 (Tyler et al., 1991b) and in rats 42 days after a 42-night exposure to 0.96 ppm O_3 (Tyler et al., 1987).

Several studies have confirmed and extended the earlier reports of epithelial degenerative changes followed by sloughing (i.e., leaving bare basement membrane that is recovered by other cell types), thus altering epithelial cell populations and increasing cell density (hyperplasia) in TB and centriacinar alveoli (Barry et al., 1985, 1988; Moffatt et al., 1987; Chang et al., 1988, 1992; Harkema et al., 1993). Epithelial replacement, a reparative process, occurs very early (Pino et al., 1992c), even though degeneration and necrosis continues (Barr et al., 1988). In specific airways, these processes appear to reach a maximum early in the exposure, as reported by Harkema et al. (1993), who found no difference in the intensity of lesions in first generation RBs, as measured by RB epithelial cell thickness and

numbers, among monkeys exposed to 0.15 ppm O_3 for 6 days or to 0.15 or 0.3 ppm for 90 days. However, it is important to note that there may have been differences in response if more distal generations of RBs or random generation RBs had been selected for study. The interstitium in the CAR also thickens by the addition of cells and matrix. Thickening of the basement membrane and the presence of granular material in it were reported by Barr et al. (1988) and Chang et al. (1992). Chang et al. (1992), using TEM morphometry, reported that some changes in epithelial cell populations persist in rats for 17 weeks after a 78-week exposure to a model urban profile with a peak of 0.25 ppm O_3. At the LM level, Gross and White (1987) reported that 6 mo following a 12-mo exposure to 0.5 ppm O_3, 20 h/day, CAR inflammation had all but disappeared, and only a slight dilation and thickening of some ADs and adjacent alveoli remained.

The epithelia of the nasal cavity respond rapidly to O_3. In ciliated regions, cilia are attenuated, and intraepithelial mucosubstances increase. Hyperplasia and increased intraepithelial mucosubstances are reported in areas of nonciliated transitional epithelium (Harkema et al., 1989). These effects persisted throughout a 20-mo intermittent exposure of rats to 0.5 or 1.0 ppm O_3, but were not seen in rats exposed to 0.12 ppm for that period (Harkema et al., 1994). After acute exposure, DNA synthesis of the epithelium of the anterior maxilloturbinates of rats increases according to a given C × T product at 2.88 ppm·h, but the increase is not linear with increasing C × T (Henderson et al., 1993). Changes have not been reported in the olfactory epithelium or in the squamous epithelium of the nasal cavity.

Respiratory epithelia in other conducting airways, especially the trachea, appear to react in a manner similar to early necrosis of ciliated cells (Hyde et al., 1992). Cell replacement starts early (Hyde et al., 1992), and, after 60 nights of exposure of rats to 0.96 ppm O_3, numeric density of specific cell types was not different from controls (Nikula et al., 1988a). In newborn lambs exposed to 1.0 ppm O_3, 4 h/day for 5 days and examined 9 days later, the normal change in epithelial cell population that occurs by 2 weeks of age did not occur (Mariassy et al., 1990).

6.2.5 Effects on Pulmonary Function

6.2.5.1 Introduction

Numerous studies have been published on the effects of O_3 exposure on pulmonary function in animal models. This work has been reviewed by the U.S. Environmental Protection Agency (1986) and Tepper et al. (1995). The evaluation of pulmonary function after exposure may help provide a more integrated assessment of the severity of health effects by indicating the magnitude, location, and duration of functional disability. In an attempt to summarize the literature here, only key studies employing multiple concentrations or studies demonstrating a particular functional effect, testing a different species or strain, or showing the relationship with a unique variable such as age or sex will be discussed. Because purely descriptive pulmonary function studies now are rarely reported, newer studies will be discussed within the context of the study hypothesis. To enable discussion of the full range of studies, some exposures greater than 1 ppm O_3 will be discussed. For example, many of the airway reactivity mechanism studies were conducted at higher concentrations.

This section is organized by duration of exposure (brief, acute, repeated, and long-term). Within each of these sections, there are subsections on different types of pulmonary function measures. These subsections include a discussion of ventilatory patterns, breathing

mechanics, airway reactivity, and more extended characterizations of lung function, whenever such data are available.

Ventilation

Evaluation of the sinusoidal breathing pattern includes the measurement of tidal volume (V_T) and frequency of breathing (f) and their product, $\dot{V}_E$. Such measurements have proven to be sensitive indicators of O_3 effects. Numerous animal and human studies have shown that O_3 exposure increases f and decreases V_T (tachypnea) (U.S. Environmental Protection Agency, 1986). Although there is evidence indicating that tachypnea may serve to protect the deep lung from exposure, other evidence indicates that this sign of pulmonary irritation represents deep lung toxicity and is of greater concern than breathing pattern changes indicative of upper airway irritation.

Breathing Mechanics

Measurement of breathing mechanics (dynamic compliance [Cdyn] and total pulmonary resistance [R_L]) in animals has an advantage over simple measures of ventilation in that these parameters can assess the mechanical effort required to breathe and can help localize the site of dysfunction to the airways (resistance) or the parenchyma (compliance). With sufficient O_3 exposure, increases in R_L and decreases in Cdyn have been observed (U.S. Environmental Protection Agency, 1986). Changes in R_L and Cdyn typically reverse rapidly after high ambient O_3 exposures; however, these alterations can signal underlying inflammatory or lung permeability changes.

Airway Reactivity

Increased airway reactivity, an exaggerated response of the lung to an exogenously administered bronchoconstrictor, has been observed with O_3 (U.S. Environmental Protection Agency, 1986). Typically in humans, heightened airway responsiveness is determined using progressively increasing concentrations of aerosolized bronchoconstrictors, such as methacholine or histamine (see Chapter 7). Although bronchoprovocation protocols employing doubling doses of inhaled bronchoconstrictors have been relatively standardized for human experiments, no such standardization exists for animal studies, making comparisons between animal and human studies difficult. Increased airway reactivity is a hallmark of asthma and occurs in many other lung diseases, yet the long-term pathological consequences of hyperreactive airways are unknown.

Extended Functional Characterizations

A more complete assessment of the nature and magnitude of functional changes related to O_3 exposure includes an extended characterization of the lung using a battery of human clinical pulmonary function test analogs. Such tests include measurement of static lung volumes, volume-pressure and flow-volume relationships, as well as evaluation of inhomogeneity of ventilation and problems associated with oxygen diffusion across the epithelial barrier. Although these latter measurements are technically complex, they may contribute to a more in-depth understanding of the nature and severity of the physiological impairment and may provide in vivo evidence to suggest the anatomical localization of the functional abnormality.

6.2.5.2 Brief Ozone Exposures (Less Than 30 Minutes)

Few experiments have evaluated the effects of brief exposures (<30 min) to O_3. Most of these brief exposure studies have examined changes in regional breathing mechanics through exposures to the lower respiratory tract via a tracheal tube, thus eliminating any scrubbing by the nasal or oropharynx and thereby increasing the effective dose of O_3 delivered to that region of the lung. The relevance of this method of delivering the exposure, as compared to the typical inhalation route, is uncertain. However, positive effects have been observed, indicating that very rapid reflex responses occur with brief, direct O_3 exposure.

Ventilation and Breathing Mechanics

No studies have evaluated the effects of brief O_3 exposure on ventilatory pattern; however, breathing mechanics (Cdyn and R_L) have been evaluated. The previous criteria document (U.S. Environmental Protection Agency, 1986) described two experiments by Gertner et al. (1983a,b,c), which demonstrated increased collateral resistance within 2 min of exposure to 0.1 ppm O_3 in anesthetized dogs exposed via a fiber-optic bronchoscope wedged into a segmental airway. The response rapidly attenuated with exposure to 0.1 but not 1.0 ppm. Atropine or vagotomy blocked the increase in collateral flow resistance to 0.1 ppm, indicating that vagal postganglionic stimulation was involved, but the response to the 1.0-ppm O_3 exposure was blocked only partially.

More recently, Kleeberger et al. (1988), using a technique similar to Gertner et al. (1983a), exposed the segmental airways of mongrel dogs to 1.0 ppm O_3 for 5 min through a wedged bronchoscope (Table 6-13). As previously described, collateral resistance increased, and this increase was reproducible even when four 5-min exposures over a 3-h period were performed. Thus, no immediate tolerance was observed. Furthermore, this response could be blocked partially by administration of a cyclooxygenase inhibitor (indomethacin) and a H_1-receptor blocker (chlorpheniramine), whereas a thromboxane synthetase inhibitor was ineffective. This study suggests that histamine or cyclooxygenase products released from resident cells directly or via the parasympathetic nervous system may mediate the increase in collateral resistance. However, because collateral resistance probably makes up only a small proportion of pulmonary resistance, these results may not be generalizable to more prolonged exposures and to larger airway responses.

Airway Reactivity

Baboons were exposed via an endotracheal tube to 0.5 ppm O_3 for 5 min after a baseline methacholine inhalation challenge test (Fouke et al., 1988) (Table 6-14). Lung resistance increased with O_3 exposure, and the baboons showed an enhanced response to methacholine. This enhanced methacholine response was due almost exclusively to the post-O_3 increase in R_L and, thus, resulted in no change in the provocative dose that increased R_L by 50%. The experiment was repeated 5 to 14 days later, except that before O_3 exposure, cromolyn sodium was administered. In the presence of cromolyn, baseline R_L after O_3 exposure was less (not significant), but the response to methacholine challenge was significantly lower. In a follow-up study (Fouke et al., 1990) using a similar O_3 exposure protocol (no methacholine challenge), cromolyn partially blocked the O_3-induced increase in R_L; however, post-O_3 exposure analysis of BAL indicated that cromolyn did not affect the level of several measured prostanoids (6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$), suggesting that these mediators were not related to the change in R_L.

Table 6-13. Effects of Ozone on Pulmonary Function[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Drugs	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
Base 0.06 spike 0.25	Base 118 spike 490	Base 13 h/day, 7 days/week; ramped spike 9-h/day, 5 days/week, for 1 week, 1, 3, 12, and 18 mo		Rat, M (F344) 60 days old	Increased expiratory resistance observed at all time points, but mostly at 78 weeks.	Tepper et al. (1991)
0.13 0.22 0.45	255 431 882	3 h Through tracheal tube	Pentobarbital Gallamine	Dog (Foxhounds) 16-20 kg	Positron camera indicated nonuniform distribution of ventilation in small airways; no change in R_L, Cdyn, or forced expiratory flow.	Morgan et al. (1986)
0.2- 0.8	392- 1,568	3 h		Rat, M (S-D) 7 weeks old	Maximum O_2 consumption decreased at 0.2 ppm, tachypnea observed at 0.4 ppm, and ventilation and core temperature decreased at 0.6 ppm.	Mautz and Bufalino (1989)
0.25 0.5 1.0	490 980 1,960	2 h; 2, 4, 6, and 8% CO_2 alternating 15 min		Rat, M (F344) 90 days old	Concentration response-related increase in f and flow at zero pleural pressure, decrease in V_T, no change in $\dot{V}_E$, R_L, or Cdyn.	Tepper et al. (1990)
0.25 0.5 1.0	490 980 1,960	3 h for 5 days	Pentobarbital	Mouse, F (CD-1) 3-4 weeks old	O_3 alone had no effect, but, in combination with virus, a decreased DL_{CO}, N_2, and lung volumes were observed more often than in virus alone at 6, 9, and 14 days PE.	Selgrade et al. (1988)
0.5	980	2 h	Chloralose	Dog (Mongrel) 15 ± 0.9 kg	Increased R_L, decreased Cdyn. No change in BAL prostanoids.	Fouke et al. (1991)
0.35 0.5 1.0	686 980 1,960	2 h/day for 5 days, 8% CO_2 alternating 15 min		Rat, M (F344) 110 days old	Attenuation of tachypnea with consecutive exposures; BAL antioxidants and protein did not adapt with exposure, histopathology increased in severity.	Tepper et al. (1989)
0.5 0.8	980 1,568	2 or 7 h, 8% CO_2 alternating 15 or 45 min/h	Halothane	Rat, M (F344) 90 days old	FVC, DL_{CO}, and N_2 slope all decreased with increasing C × T products. The magnitude of the decrement depended on the both the duration and concentration of O_3 exposure and the measured parameter.	Costa et al. (1989)
0.6	1,176	2 h exercise, muzzle		Dog, F (Beagle) 2-7 years old	Tachypnea, $\dot{V}_E$, O_2 consumption, CO_2 output, and R_L increased; Cdyn decreased.	Mautz et al. (1985b)
0.7	1,372	20 h/day for 28 days	Halothane	Rat, M (F344) 14 weeks old	Decreased forced expiratory flow and DL_{CO} and increased FRC immediately PE, no effect at 4 weeks PE, decrease in forced expiratory flow at 9 weeks PE.	Gross and White (1986)

Table 6-13 (cont'd). Effects of Ozone on Pulmonary Function[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration	Drugs	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
1.0	1,960	5 min Through tracheal tube	Pentobarbital	Dog, M (Mongrel) 20.2 ± 0.8 kg	Ozone-induced increase in collateral resistance blocked by indomethacin and histamine antagonist, not by thromboxane synthetase inhibitor.	Kleeberger et al. (1988)
1.0	1,960	1 h	Ketamine Xylazine	Guinea pig, M (Hartley) 250-300 g	Decreased TLC, VC, FRC, RV, and R_L. Indomethacin and cromolyn blocked change in FRC and RV at 2 and 24 h PE. DL_{CO} increased, blocked by cromolyn.	Miller et al. (1988)
1.0	1,960	1 h	Ketamine Xylazine	Guinea pig, M (Hartley) 250-300 g	R_L increased at 2 but not 8 h. Lung volumes, DL_{CO}, and alveolar ventilation increased at 8 and 24 h PE.	Miller et al. (1987)
1.0	1,960	2 h Through tracheal tube		Dog (Mongrel) 22-25 kg	Tachypnea with inspiratory time and expiratory time equally shortened. No increase in ventilatory drive 1 and 24 h PE.	Sasaki et al. (1987)
1.0	1,960	6 h/day, 5 days/week for 12 weeks	Ketamine Xylazine	Monkey, M (Cynomolgus) 4.5 ± 0.1 kg	No R_L, Cdyn, or forced expiratory flow changes associated with exposure.	Biagini et al. (1986)
1.0 2.0	1,960 3,920	3 h	In vitro	Rat, M (S-D) 300-380 g	R_L increased and Cdyn decreased in rat isolated perfused lung preparation.	Pino et al. (1992a)
1.0 2.0	1,960 3,920	6 h/day for 7 days (1 ppm) or 3 days (2 ppm)	Pentobarbital	Rabbit, M (Albino) 2.5 kg	At 17 h PE, 1 ppm increased R_L; 2 ppm trapped air, decreased Cdyn and forced expiratory flows, and increased R_L.	Yokoyama et al. (1989a)
1.0	1,960	24 h	Pentobarbital	Rat, M (Wistar) 6 weeks old	Decreased Cdyn, Cst, $\dot{V}_{max50\%TLC}$; increased FRC, RV; no additional effect of elastase pretreatment in O_3 exposed rats.	Yokoyama et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Table 6-14. Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m³	Exposure Duration	Challenge[c] Agent	Challenge[c] Route	Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
0.5	980	2-7 h Intermittent 8% CO_2	Ach	IV	Urethane	Rat, M (F344) 70 days old	No increase in airway reactivity despite use of CO_2 during O_3 exposure.	Tepper et al. (1995)
1.0	1,960	4 h	Mch	INH IV		Rat (S-D)	Reactivity in INH- but not IV-challenged rats.	Uchida et al. (1992)
1.0	1,960	2 h	Mch Ach	INH IV	Urethane	Rat, M (F344) 70 days old	Reactivity in INH- but not IV-challenged rats.	Tepper et al. (1995)
1.0	1,960	8 h	Mch	IV	Urethane	Rat, M (F344, S-D, Wistar)	No reactivity in any of three strains.	Tepper et al. (1995)
1.0	1,960	31 h	Ach	IV	Urethane	Rat, M	Increased reactivity.	Tepper et al. (1995)
4.0	7,840	2 h	Ach	INH	Methohexital Pancuronium	Rat, F (Long-Evans) 190-210 g	Reactivity increased immediately, but not 24 h PE, but not accompanied by PMN influx or vascular protein leakage.	Evans et al. (1988)
0.15	294	4 h/day, 5 days/week for 18 weeks	Hist	INH		Guinea pig, M (Hartley) 4-5 weeks old	Increase in sensitivity, but no change in reactivity.	Kagawa et al. (1989)
0.5	980	2 h	Ach	IV	Urethane	Guinea pig, M (Hartley)	Increased reactivity before PMN influx.	Tepper et al. (1990)
0.8	1,568	2 h	Sulfuric acid	INH		Guinea pig, F, M (Hartley) 2 mo old	No increased reactivity to acid; O_3 alone increased gas trapping.	Silbaugh and Mauderly (1986)
1.0 3.0	1,960 5,880	0.5-2 h	Mch	INH	Propranolol	Guinea pig, F (Hartley) 300-400 g	Increased reactivity with 90 min at 1 ppm and with 30 min at 3 ppm. At 2 h, 3 ppm reactivity occurred at 0 and 5 but not 24 h PE.	Nishikawa et al. (1990)
1.0	1,960	2 h	Hist Ach	INH IV	Urethane	Guinea pig, M (Hartley)	Reactivity in INH Hist, but not IV Ach.	Tepper et al. (1990)
3.0	5,880	0.25 h	Ach	IV	Propranolol	Guinea pig, M (English) 550-700 g	Reactivity blocked by lipoxygenase inhibitors but not by indomethacin.	Lee and Murlas (1985)

Table 6-14 (cont'd). Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration	Challenge[c] Agent	Challenge[c] Route	Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
3.0	5,880	0.25-2 h	Mch Hist 5-HT SP	IV INH	Pentobarbitone	Guinea pig, M (Dunkin Hartley) 450-550 g	Mch, Hist, 5-HT, and SP caused increase in reactivity INH, but not IV. Ascorbic acid blocked reactivity to Hist and SP. NEP inhibitors increased control response without changing O_3 response (i.e., O_3 inhibits NEP). Atropine and vagotomy did not or only partially reduced reactivity. Neither cyclooxygenase or 5-lipoxygenase inhibitors affected reactivity.	Yeadon et al. (1992)
3.0	5,880	2 h	Mch	INH	Ketamine Xylazine	Guinea pig, M (Dunkin Hartley) 494 ± 39 g	Platelet activating factor antagonist did not inhibit reactivity or eosinophils.	Tan and Bethel (1992)
3.0	5,880	2 h	Ach	IV		Guinea pig, M (Hartley) 550-750 g	Reactivity occurs with leukocyte depletion using cyclophosphamide.	Murlas and Roum (1985b)
3.0	5,880	2 h	Ach	INH		Guinea pig, M (Hartley) 600-700 g	With increased reactivity, increased lysosomal hydrolase observed in BAL.	Lew et al. (1990)
3.0	5,880	2 h	Ach	INH	Propranolol	Guinea pig, M (Hartley) 600-750 g	In vitro reactivity to SP and Ach, but not KCl. Phosphoramidon blocked SP effect, but not Ach reactivity; no increased reactivity was observed when mucosa was removed.	Murlas et al. (1990)
0.5	980	2 h Trach Tube	Mch	INH	Chloralose	Dog (Mongrel) 15 ± 0.9 kg	Increased reactivity, no change in BAL prostanoids.	Fouke et al. (1991)
1.0	1,960	5 min Bronchoscope	*Ascaris suum*	INH	Pentobarbital Succinylcholine	Dog, M (Mongrel) 21.2 ± 0.5 kg	Increased resistance to flow through the collateral system after antigen challenge attenuated with O_3 exposure 1-3 h and 24 h PE; effect independent of PMNs.	Kleeberger et al. (1989)
3.0	5,880	2 h	Ach	INH	Thiopental Chloralose	Dog (Mongrel) 18-23 kg	Cyclo- and lipoxygenase inhibitor BW755C blocked reactivity.	Fabbri et al. (1985)
3.0	5,880	2 h	Ach FS	INH	Thiopental Chloralose	Dog (Mongrel) 16-25 kg	In vivo but no in vitro reactivity; in vitro, trachea showed reactivity to FS.	Walters et al. (1986)
3.0	5,880	1 h	Ach	INH	Pentothal Chloralose	Dog (Mongrel) 24 ± 9 kg	Reactivity blocked with Ambroxyl, but PMN increased; ambroxyl inhibits arachidonic acid products from PMN.	Chitano et al. (1989)
1.0	1,960	2 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 15-17 kg	Collateral resistance of small airways showed persistent reactivity 15-h PE; no effect on PMN, monocyte, or mast cell numbers at PE time.	Beckett et al. (1988)

Table 6-14 (cont'd). Effects of Ozone on Airway Reactivity[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration	Challenge[c] Agent	Challenge[c] Route	Drugs	Species, Sex (Strain) Age[d]	Observed Effect(s)	Reference
3.0	5,880	0.5 h Trach tube	FS Cch		Pentobarbital	Dog (Mongrel)	In vitro increases in reactivity suggested pre- and postjunctional inhibition (PGE_2) and postjunctional excitation (TXA_2).	Janssen et al. (1991)
3.0	5,880	0.5 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel)	Reactivity was not blocked by TX antagonists.	Jones et al. (1990)
3.0	5,880	0.5 h Trach tube	Ach Hist 5-HT	INH	Pentobarbital	Dog (Mongrel) 18-30 kg	In vitro reactivity not altered by epithelial removal, indicating O_3 did not effect epithelial-derived relaxing factor.	Jones et al. (1988b)
3.0	5,880	0.5 h Trach tube	Ach Hist	INH	Pentobarbital	Dog (Mongrel)	Ganglionic blocker hexamethonium did not alter reactivity.	Jones et al. (1987)
3.0	5,880	0.5 h Trach tube	Cch TX	INH	Pentobarbital	Dog (Mongrel)	Airways not responsive to TX mimetic (U46619) but were to carbachol.	Jones et al. (1992)
3.0	5,880	0.5 h Trach tube	Ach FS KCl	INH	Pentobarbital	Dog (Mongrel) 18-30 kg	In vitro hyperresponsiveness of airway smooth muscle was observed with FS and Ach, but not KCl. FS not associated with increased excitatory junction potentials.	Jones et al. (1988a)
3.0	5,880	0.5 h Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 21-27 kg	CD11b/CD18 monoclonal antibody prevented PMN influx, but not reactivity.	Li et al. (1992)
3.0	5,880	20 min Trach tube	Ach	INH	Pentobarbital	Dog (Mongrel) 18-32 kg	Allopurinol and desferoxamine inhibited reactivity without inhibiting PMN influx.	Matsui et al. (1991)
3.0	5,880	0.5-2 h Trach tube	Ach	INH	Thiopental Chloralose	Dog (Mongrel) 26-32 kg	Thromboxane synthase inhibitor blocked reactivity without influencing PMN influx.	Aizawa et al. (1985)
0.5 0.6	980 1,176	5 min	Mch	INH	Ketamine Fluorodiazepam	Baboon, M 25-40 kg	Brief exposure caused increased reactivity that was blocked by cromolyn.	Fouke et al. (1988)
0.5 0.6	980 1,176	5 min	Mch	INH	Ketamine Fluorodiazepam	Baboon, M 25-40 kg	Reactivity partially blocked by cromolyn, but no effect on stable prostanoids.	Fouke et al. (1990)
1.0	1,960	6 h/day, 5 days/week for 12 weeks	Ach/Pt	INH	Ketamine Xylazine	Cynomolgus, M 4.5 ± 0.1 kg	No increased reactivity in O_3-only group, but increase with platinum mixture.	Biagini et al. (1986)
1.0	1,960	2 h, 1/week for 19 weeks	Mch	INH	Pentobarbital	*Rhesus Macaca*, F 5-7 kg	5-lipoxygenase inhibitor blocked the development of reactivity.	Johnson et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Table ordered according to animal species.
[c]Mch = methylcholine, Ach = acetylcholine, Hist = histamine, 5-HT = 5-hydroxytryptamine, SP = substance P, FS = field stimulation, Cch = carbachol, TX = thromboxane, KCl = potassium chloride, Pt = platinum; Route: IV = intravenous, INH = inhalation.
[d]Age or body weight at start of exposure.

The response to antigen-induced bronchoconstriction, an animal model of allergy, also has been evaluated recently. After a 5-min exposure to 1.0 ppm O_3 via a wedged bronchoscope, collateral resistance in dogs increased for 1 to 3 h (Kleeberger et al., 1989). After the O_3-induced resistance returned to baseline, the typical increase in collateral resistance observed in dogs challenged with *Ascaris suum* antigen, to which the dogs were natively sensitive, was attenuated both 1 to 3 h and 24 h post-O_3 exposure. The attenuated antigen response appeared to be independent of PMNs in the airways. In a follow-up study, the late-phase response to antigen (bronchoconstriction 2 to 12 h postantigen challenge) also was blocked in allergic dogs when O_3 exposure (1.0 ppm, 5 min, via a bronchoscope) preceded antigen challenge (Turner et al., 1989). These studies suggest that, at least in the dog, brief local administration of O_3 to the airways may inhibit allergic responses.

6.2.5.3 Acute Ozone Exposures (Less Than One Day)

Ventilation

Alteration of the ventilatory pattern has long been established as a hallmark of acute O_3 exposure. Several animal studies evaluated tidal breathing changes during and after O_3 exposure (U.S. Environmental Protection Agency, 1986). For most species, a tachypneic response (rapid and shallow breathing) has been observed. For example, Murphy et al. (1964) studied unanesthetized guinea pigs exposed for 2-h to 0.34, 0.68, 1.08, or 1.34 ppm O_3 via nose cones, and measured tidal breathing using a constant volume plethysmograph. A similar experimental preparation was used by Amdur et al. (1978) to evaluate the respiratory response of guinea pigs to 0.2, 0.4, and 0.8 ppm O_3. In both experiments, a monotonic increase in f was observed. In the Amdur et al. (1978) study, decreases in V_T were not observed concomitantly.

Lee et al. (1979, 1980) showed that the tachypneic pattern observed in conscious dogs exposed to 0.56 to 0.85 ppm was not altered by bronchodilator pretreatment or atropine administration. These manipulations would suggest that the rapid, shallow breathing was not caused by bronchoconstriction. The response, however, was blocked by vagal cooling, which was interpreted by the authors to suggest that vagal sensory afferent transmission had been blocked. Thus, the authors suggested that increased vagal afferent impulses produced tachypnea and that the response was independent of vagal efferents (increased smooth muscle tone).

Several new studies evaluating ventilation after acute O_3 exposure have appeared in the literature (Table 6-13). Mautz and Bufalino (1989) measured ventilation ($\dot{V}_E$) as well as oxygen consumption and rectal temperature in awake rats exposed for 3 h to 0.2, 0.4, 0.6, and 0.8 ppm O_3. Concentration-related increases in f were significantly different from controls beginning at 0.4 ppm, with a maximal response observed up to 0.6 ppm. Tidal volume was similarly reduced, whereas $\dot{V}_E$ and rectal temperature were less sensitive to O_3 exposure, showing decreases at 0.6 and 0.8 ppm. Oxygen consumption was decreased at all concentrations tested. The authors concluded that the O_3-induced change in breathing pattern did not cause a decrease in metabolic rate or impose a condition of hypoxia. The changes in ventilation and O_2 consumption appeared coincident or possibly preceded the irritant reflex change in breathing pattern.

Tepper et al. (1990) exposed awake rats to 0.12, 0.25, 0.5, and 1.0 ppm O_3 for 2.25 h in head-out pressure plethysmographs. During exposure, CO_2-stimulated breathing was incorporated to augment ventilation, similar to the use of exercise in human studies.

Frequency increased and V_T decreased monotonically between 0.25 and 1.0 ppm during a 2.25-h exposure. No decrease in $\dot{V}_E$ was observed. This difference from the Mautz and Bufalino (1989) study could be due to their restraining rats in a tightly fitting plastic flow plethysmograph with the face sealed by an aluminum nose cone; in the Tepper et al. (1990) study, the rats were exposed in oversized, steel, head-out plethysmographs and were intermittently challenged with CO_2, which may have overridden the metabolic depressant effect.

Mautz et al. (1985b), using exercising dogs exposed to 0.6 ppm O_3 for 140 min, showed tachypnea, increased $\dot{V}_E$, and elevated ventilation equivalents for O_2 and CO_2 compared to dogs exposed to air while exercising. Pulmonary resistance fell in air-exposed, exercising dogs, but climbed toward the end of the exposure in the O_3-exposed dogs.

In a follow-up study to Lee et al. (1979, 1980), Sasaki et al. (1987) performed similar experiments on two awake dogs that were trained to run on a treadmill. Dogs were exposed to 1.0 ppm O_3 for 2 h and evaluated before O_3 exposure and at either 1 or 24 h postexposure. In all studies with or without exercise, O_3 increased f and decreased V_T, without affecting $\dot{V}_E$. Vagal blockade diminished, but did not abolish, the tachypneic response, indicating that both vagal and nonvagal mechanisms were important. The O_3-induced change in f was due to equal reductions in inspiratory and expiratory times with no, or small, diminution (only during CO_2 rebreathing experiments) of ventilatory drive (V_T/inspiratory time). Additionally, O_3 did not affect functional residual capacity (FRC) or core temperature in resting, exercising, or vagally blockaded dogs. The authors speculate that the change in f is due to a vagally mediated lowering of the volume threshold of the pulmonary stretch receptor for inspiration and expiration with a concomitant increase in flow rate, thus leaving the FRC constant. Furthermore, the authors speculated that increased sensitization of rapidly adapting receptors, C-fiber nerve endings, and nonvagal mechanisms also may be contributors to the tachypneic response.

Two recent studies provide further insight into the mechanism of O_3-induced changes in ventilatory patterns. In the first study, Schelegle et al. (1993) showed that O_3-induced (3 ppm O_3 for 40 to 70 min) tachypnea in anesthetized, spontaneously breathing dogs largely could be abolished by cooling the cervical vagus to 0 but not 7 °C. This would indicate that large myelinated fibers were not involved in this reflex response, but nonmyelinated C fibers, whose activity is decreased only at the lower temperature, are important. In a companion study, Coleridge et al. (1993) measured the responses of five types of single vagal nerve fibers: (1) bronchial C-fibers, (2) pulmonary C-fibers, (3) rapidly adapting receptors, (4) slowly adapting pulmonary stretch receptors, and (5) unclassified fibers. During exposure to O_3, bronchial C-fibers were most affected. Because discharge of these fibers was not immediate with the onset of exposure, but took time to develop, the authors suggested that O_3 may not directly stimulate these receptors and that autacoid mediators released in the lung, which previously have been shown to stimulate these fibers, were probably responsible for fiber activation. Rapidly adapting receptors were shown to play a small part in this reflex response; although, surprisingly, pulmonary C-fibers and slowly adapting receptors were found to be unimportant. Ozone also stimulated several unidentified vagal fibers that may be responsible for residual effects not abolished by 0 °C cooling of the vagus. Thus, early inflammatory changes, as well as direct stimulation of bronchial C-fibers, may be responsible for the tachypnea seen with O_3 exposure in animal experiments. Results from these studies, together with the amelioration of spirometric changes found after

indomethacin in humans (see Chapter 7), suggest that tachypnea, inspiratory pain, and the reduction in forced vital capacity (FVC) could reflect early inflammatory lesions as well as neurogenic stimulation.

Breathing Mechanics

Although changes in breathing mechanics have been observed in laboratory animals, these changes are not observed consistently and tend to be reported more frequently at higher exposure concentrations (U.S. Environmental Protection Agency, 1986; Table 6-13).

The previously discussed studies by Murphy et al. (1964) and Amdur et al. (1978) evaluated breathing mechanics in unanesthetized guinea pigs. The Murphy et al. (1964) study showed an increase in flow resistance only at concentrations >1 ppm O_3. Pulmonary compliance was not measured. Amdur et al. (1978) observed a decrease in Cdyn after exposure to 0.4 and 0.8 ppm O_3, but no significant change in R_L was noted.

In an attempt to expose unanesthetized rats using regimens analogous to human clinical studies, Tepper et al. (1990) observed no significant changes in R_L or Cdyn after a 2.25-h exposure to 0.12, 0.25, 0.5, or 1.0 ppm O_3, in spite of intermittent 15-min periods of exercise-like hyperventilation induced by CO_2. Similarly, no changes in breathing mechanics were observed by Yokoyama et al. (1987) when they evaluated anesthetized rats exposed to 1.0 ppm O_3 for 24 h. However, when Cdyn was normalized for differences in FRC, the resulting specific compliance was decreased compared to air-exposed controls. Furthermore, when the animals were paralyzed and ventilated between 40 and 200 breaths/min, O_3-treated animals showed a frequency-dependent decrease in Cdyn as f increased above 120 breaths/min. The authors conclude that because R_L was not affected, the effect of O_3 was to obstruct peripheral airways.

Pulmonary mechanics were evaluated in anesthetized, paralyzed dogs acutely exposed to 0.12, 0.22, and 0.45 ppm O_3 for 3 h via a stainless steel tracheal tube. No changes in R_L or Cdyn were observed at any concentration (Morgan et al., 1986).

In papers by Miller et al. (1987, 1988), the effect of a 1-h exposure to 1.0 ppm O_3 was evaluated. Two hours after exposure, anesthetized, tracheostomized guinea pigs showed a significant increase in R_L that resolved by 8 h postexposure. Both indomethacin and cromolyn sodium partially blocked the increase in R_L at 2 h postexposure (Miller et al., 1988). These results suggest that eicosanoids produced from an inflammatory response in the lung may be responsible for the increase in R_L. However, plasma levels of $PGF_{2\alpha}$ and 6-keto PGF_{α} were not affected by O_3 or drug treatment, and PGE_1 was not affected by O_3. In an attempt to understand the involvement of eicosanoids in the increase in R_L observed with O_3 exposure, Fouke et al. (1991) showed that exposure to 0.5 ppm O_3 for 2 h caused an increase in R_L and a decrease in Cdyn in anesthetized dogs. Bronchoalveolar lavage fluid from these dogs did not have any increase in 6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$, suggesting that these cyclooxygenase products were not involved in the changes in breathing mechanics. Similar findings were observed by these authors after brief exposures to baboons (see Section 6.2.5.2).

Gas trapping in the excised guinea pig lung was evaluated by water displacement in guinea pigs challenged with acid aerosol exposure after a 2 h, 0.8-ppm O_3 exposure (Silbaugh and Mauderly, 1986). Ozone exposure followed by air exposure increased gas trapping to roughly the same extent as O_3 followed by a sulfuric acid challenge (1 h, 12 mg/m^3) when compared to the air-only control response. These data indicate that O_3 causes an acute

peripheral airway obstruction, but no additive or synergistic effect of sulfuric acid aerosol was observed.

Airway Reactivity

Probably, the most extensive amount of laboratory animal research has been conducted on the role of O_3 in producing acute airway injury resulting in an increase in airway reactivity (U.S. Environmental Protection Agency, 1986; Table 6-14). Much of this research has used O_3 exposures that are never encountered in the ambient environment (3 ppm for 30 min); thus, the relevance of these studies may be questioned. However, the studies are the most thorough mechanistic account of such O_3 effects and have shown some agreement with human O_3 exposure studies (Chapter 7); therefore, these studies are summarized briefly here. The literature is focused around five primary issues that in recent years have been more thoroughly evaluated.

Concentration and Peak Response Time. Easton and Murphy (1967) were the first to demonstrate an increased responsiveness in unanesthetized guinea pigs post-O_3 exposure (2 h, 0.5 to 7 ppm). In their study, responsiveness was assessed by increased mortality due to severe histamine-induced bronchoconstriction, as well as by increased R_L and decreased Cdyn. Lee et al. (1977) examined anesthetized dogs exposed to O_3 (0.7 to 1.2 ppm, 2 h) via a tracheal tube and determined that increased airway reactivity to inhaled histamine occurred at 24 but not 1 h postexposure. A similar experiment, done in unanesthetized sheep by Abraham et al. (1980), indicated that airway responsiveness was increased at 24 h, but not immediately after a 2-h exposure to 0.5 ppm O_3. When the exposure was increased to 1 ppm O_3, an increase in baseline R_L was reported, and reactivity increased immediately and at 24 h postexposure. In apparent contradiction, Holtzman et al. (1983a) showed that airway reactivity increased markedly 1 h after dogs were exposed to 2.2 ppm O_3 for 2 h and was less evident at 24 h postexposure. Gordon and Amdur (1980) also reported that airway reactivity in guinea pigs was maximal 2 h after a 1-h exposure to 0.1, 0.2, 0.4, or 0.8 ppm O_3, as defined by a significant increase in R_L or decrease in Cdyn after a single subcutaneous challenge of histamine. The effect on R_L was concentration dependent, but was significant only at 0.8 ppm. For Cdyn, there was no concentration-related response, but all O_3 exposures exacerbated the decrease in Cdyn after histamine relative to the air-exposed group. The site of bronchoconstriction was suggested to be the conducting airways, rather than the parenchyma, because dynamic compliance was affected and static compliance was not (Gordon et al., 1984).

To examine the role of duration of exposure on experimental outcome, Nishikawa et al. (1990) exposed guinea pigs to C × T products of 30 (1 ppm × 30 min), 90 (1 ppm × 90 min), 90 (3 ppm × 30 min), and 360 (3 ppm × 120 min) ppm · min O_3. After exposure, specific airway resistance (SR_{aw}) during an inhaled methacholine challenge was measured in unanesthetized animals at 5 min, 5 h, and 24 h. In all but the 1-ppm, 90-min exposure group, there was an increase in baseline SR_{aw} at 5 min, but the response was neither concentration nor C × T dependent. At 5 min postexposure, no increase in airway responsiveness was observed at 30 ppm · min. Airway hyperresponsiveness was observed at 90 ppm · min, using either exposure scenario (1 ppm for 90 min or 3 ppm for 30 min), and the response to the 360 ppm · min was greater than that observed with 90 ppm · min exposure. Significant increases in airway responsiveness at both 5 and 24 h postexposure were observed only in the 360 ppm ·

min group. The authors concluded that exposure duration was an important determinant of O_3-induced airway hyperresponsiveness.

Uchida et al. (1992) reported increased airway reactivity in rats to inhaled methacholine after a 1.0-ppm (2-h) O_3 exposure. These results conflict with other published studies in rats also using inhaled methacholine, which reported the inability to produce consistent increases in airway reactivity after exposure to less than 4 ppm O_3 (Evans et al., 1988). Tepper et al. (1995) reported that airway hyperresponsiveness in rats challenged with iv acetylcholine occurred only at 1 ppm O_3 or higher. In these latter studies, exposure durations ranged from 2 to 7 h, and, in some tests, CO_2 was added to the exposure to increase ventilation. Although guinea pigs are more responsive than rats, they are not as responsive as humans to O_3-induced increased airway reactivity, even under optimal conditions (Tepper et al., 1995).

Inhaled Versus Intravenous Challenge. In a follow-up study using a similar exposure protocol as described above, Abraham et al. (1984) observed increased responsiveness to iv carbachol in unanesthetized sheep 24 h after a 2-h, 0.5-ppm O_3 exposure; inhaled carbachol did not produce a similar response. The authors interpreted this result to indicate a decreased penetration of the carbachol aerosol in O_3-exposed animals compared with the direct stimulation of smooth muscle by the iv route. Roum and Murlas (1984) observed that O_3-induced hyperresponsiveness was similar for inhaled versus iv acetylcholine or methacholine challenge through 14 h postexposure, but after that time, only iv administration revealed a persistent O_3-related response. In contrast, Yeadon et al. (1992) reported that guinea pigs exposed to 3 ppm for 30 min were hyperresponsive to inhaled histamine, serotonin, acetylcholine, and substance P, but were not hyperresponsive to O_3 after iv administration of the same agonists. Tepper et al. (1995) and Uchida et al. (1992) also showed that rats were more sensitive to inhaled methacholine than to iv administration of the agonist.

Neurogenic Mediation. Lee et al. (1977) reported increased airway responsiveness to histamine in dogs exposed to 0.7 or 1.2 ppm O_3 for 2 h. Atropine and vagal blockade were effective in reducing the O_3-induced hyperresponsiveness to histamine, suggesting that heightened vagal activity was responsible. Katsumata et al. (1990) also showed that in the cat, airway hyperresponsiveness to histamine could be attributed to cholinergic reflex. This is in apparent contrast to the increased O_3-induced (1.0 to 1.2 ppm, 2 h) responsiveness to histamine (subcutaneous) that was not blocked by atropine or vagotomy, indicating minimal vagal involvement in guinea pigs (Gordon et al., 1984). In agreement, Jones et al. (1987) found that hexamethonium, a ganglionic blocker, did not prevent O_3-induced hyperresponsiveness in dogs exposed to 3 ppm O_3 for 0.5 h via an endotracheal tube. Similarly, Yeadon et al. (1992) showed that atropine or bilateral vagotomy only partially reduced the hyperresponsivenss in guinea pigs exposed to 3 ppm for 120 min but did not block the response in animals exposed for only 30 min.

A role for prejunctional muscarinic receptors has been demonstrated by Schulteis et al. (1994). The M_2 receptor, which is inhibitory for acetylcholine release, was shown to be defective immediately after a 4-h, 2-ppm O_3 exposure in guinea pigs. Fourteen days after exposure, M_2 receptor function and vagally stimulated responsiveness were normal. Thus, the role of the cholinergic system in O_3-induced airway hyperresponsiveness has yet to be firmly established.

Peptidergic mediators also have recently been suggested as important modulators of this response. Murlas et al. (1992) demonstrated that phosphoramidon, an inhibitor of neutral endopeptidase (NEP), increased the responsiveness to substance P in air-exposed (but not O_3-exposed [3 ppm, 2 h] guinea pigs). Substance P-induced bronchoconstriction in air-exposed animals was increased after phosphoramidon because NEP degrades substance P. The finding was associated with a decrease in tracheal NEP in O_3-exposed animals. Additionally, the increased airway responsiveness in O_3-exposed animals was reversed by inhalation of partially purified NEP. Taken together, these results suggest that O_3 inactivates NEP, thus increasing the response to endogenous tachykinin release. A similar result was obtained by Yeadon et al. (1992) in guinea pigs exposed to 3 ppm O_3 (30 or 120 min) and challenged with aerosolized substance P after pretreatment with the NEP inhibitors phosphoramidon, thiorphan, and bestatin. Tepper et al. (1995) depleted guinea pigs of substance P, using multiple doses of capsaicin, and found that airway reactivity, after a 2-h exposure to 1 ppm O_3, was partially blocked. However, although tracheal vascular permeability also was blocked by capsaicin pretreatment, protein influx into the BAL and tachypnea were not blocked. On the other hand, Evans et al. (1989) did not find increased tracheal vascular permeability in rats exposed to 4 ppm O_3 for 2 h. These studies suggest, at least for the guinea pig, that enhancement of the substance P response, by inhibition of NEP, may be important in O_3-induced hyperresponsiveness.

Inflammation. Holtzman et al. (1983b) found a strong association between increased airway responsiveness and increased PMNs present in the tracheal biopsy of dogs 1 h after a 2-h O_3 exposure to 2.1 ppm. Fabbri et al. (1984) extended these findings, showing an association between increased airway reactivity and increased lavageable inflammatory cells from the distal airways of dogs. Further support for this hypothesis was engendered by the demonstration that in PMN-depleted dogs (produced by administration of hydroxyurea), O_3-induced airway hyperresponsiveness was blocked. This is in contrast to Murlas and Roum's (1985a) findings in guinea pigs exposed to 3 ppm O_3 for 2 h, which indicate that increased airway reactivity, mucosal injury, and mast cell infiltration occur before PMN influx. The authors speculate that PMN influx is a response to the damage, not a cause of the increased airway reactivity. Furthermore, Murlas and Roum (1985b) showed that PMN depletion in the guinea pig with cyclophosphamide did not prevent O_3-induced airway hyperresponsiveness. Similar results were obtained by Evans et al. (1988), who reported that airway hyperresponsiveness was not accompanied by airway PMN influx in rats, and by Joad et al. (1993), who showed that adding human PMNs to the pulmonary circulation of the rat lung during a 3-h, 1.0-ppm exposure to O_3 did not further enhance O_3-induced airway reactivity. Beckett et al. (1988) evaluated dogs exposed for 2 h to 1 ppm O_3 directly to the peripheral airways via a wedged bronchoscope. Fifteen hours postexposure, the exposed peripheral airway segments were hyperresponsive to aerosolized acetylcholine. However, at the site of increased responsiveness, there was no association with increased PMNs, mast cells, or mononuclear cells. Such studies agree with perhaps the most definitive study of this hypothesis (Li et al., 1992), which used monoclonal antibodies (CD11b/CD18) to prevent PMN influx into the airways. When PMNs were present in the circulation, but prevented from entering the lung, the dogs were still hyperresponsive after a 30-min exposure to 3 ppm O_3. Thus, in three species, it appears that PMN influx may be associated with O_3 exposure but is not necessary for producing airway hyperresponsiveness.

Several studies have suggested that arachidonic acid metabolites may be important in O_3-induced airway hyperresponsiveness. Although the primary source of arachidonic acid metabolites is suspected to be inflammatory cells in the lung, cells other than PMNs could be responsible for the liberation of arachidonic acid metabolites. Only one study in dogs indicates that blockage of cyclooxygenase products with indomethacin can protect animals from developing airway hyperresponsiveness (O'Byrne et al., 1984). However, several, more recent studies have found that cyclooxygenase inhibitors were ineffective in blocking this response (Lee and Murlas, 1985; Holroyde and Norris, 1988; Yeadon et al., 1992). Two papers indicated the importance of LTs, as demonstrated by the inhibition of hyperresponsiveness with prior administration of 5-lipoxygenase inhibitors to guinea pigs (Lee and Murlas, 1985; Murlas and Lee, 1985). In contrast, Yeadon et al. (1992) found that a specific 5-lipoxygenase inhibitor did not block the response in guinea pigs. One study with dogs showed that TX generation may be important in this phenomenon (Aizawa et al., 1985), but, more recently, two papers from the same investigators have dispelled that notion (Jones et al., 1990, 1992). Furthermore, exposure to 0.5 ppm O_3 for 2 h caused a decrease in the provocative dose of methacholine necessary to cause a 50% increase in R_L in anesthetized dogs (Fouke et al., 1991). Bronchoalveolar lavage on these dogs did not show any increase in 6-keto $PGF_{1\alpha}$, PGE_2, TXB_2, or $PGF_{2\alpha}$, suggesting that these cyclooxygenase mediators of inflammation were not involved in the changes in airway reactivity. In summary, the initial hypothesis of the role of PMNs or PMN-derived products in O_3-induced airway hyperresponsiveness is questionable because most newer studies, using more specific inhibitors of PMNs, cyclooxygenase, and 5-lipoxygenase, and studies blocking TX receptors indicate the lack of a protective effect.

Interactions with Antigen and Virus. In mice, Osebold et al. (1980) showed that an increased number of animals became sensitized to ovalbumin after 3 to 5 days of continuous exposures to 0.5 and 0.8 ppm O_3. Matsumura (1970) and Yanai et al. (1990) made similar findings in guinea pigs and dogs exposed acutely to higher O_3 concentrations, suggesting that O_3 may enhance either sensitization or response to antigen. These results appear to agree with recent findings in humans (see Chapter 7).

Ozone (1 ppm, 2 h) also may increase hyperreactivity associated with virus exposure. Tepper et al. (1995) exposed rats to O_3 either before or during an influenza virus infection. Rats exposed to O_3 before virus infection were more hyperresponsive to inhaled methacholine 3 days later (at a time when there was no hyperresponsiveness to O_3 alone) than were rats exposed to only the virus. An additive effect was observed in virus-infected rats when O_3 exposure was immediately before methacholine challenge.

Extended Functional Characterizations

Extended characterizations of pulmonary function in laboratory animals indicate that the general pattern of functional impairment reported in human studies also is observed in animal studies of acute O_3 exposure. Anesthetized and ventilated cats showed a general decline in vital capacity (VC), static lung compliance, or diffusing capacity for carbon monoxide (DL_{CO}) with exposures up to 6.5 h of 0.26 to 1.0 ppm O_3 (Watanabe et al., 1973). Inoue et al. (1979) observed functional evidence of premature airway closure, as indicated by increases in closing capacity, residual volume (RV), and closing volume, after rabbits were exposed to 0.24 or 1.1 ppm O_3 for 12 h. The volume-pressure curve indicated increased lung volume at low

distending pressures; additionally, nonuniform distribution of ventilation was observed. The effects were most prominent 1 day following exposure and had mostly subsided by 7 days postexposure.

Most studies of O_3 in experimental animals make little effort to mimic human study designs, thereby further confounding the extrapolation of their results to humans. Recently, however, rat studies involving periods of intermittent CO_2-induced hyperventilation to enhance the delivered dose of O_3 have attempted to capitalize on the qualitative similarity of the rat and human maximum expiratory flow-volume curves as a potentially sensitive endpoint of toxicity (Costa et al., 1988b; Tepper et al., 1989). In the rat, FVC decreases acutely with O_3 exposure, and this response has been mathematically modeled for O_3 concentrations between 0.35 and 0.8 ppm with exposure durations between 2 and 7 h (Tepper et al., 1989). The magnitude of response is apparently less than that observed in humans (Tepper et al., 1995), although the extent to which anesthesia mitigates the rat response or that there are inherent species differences in dosimetry or sensitivity is not clear from these studies (see Chapter 8).

In addition to changes in the flow-volume curve, changes in lung diffusion also are observed. In a study that examined concentration, duration, and ventilation factors, rats were exposed for 2 or 7 h to 0.5 or 0.8 ppm O_3 with intermittent 8% CO_2 to hyperventilate (□2 to 3 times resting $\dot{V}_E$) the animals as an exercise analogue to human exposures (Costa et al., 1988a). The DL_{CO} values were reduced by 10% at both 0.5-ppm time points and by 12% with a 2-h exposure to 0.8 ppm. Exposure to 0.8 ppm for 7 h, however, greatly exacerbated the alveolar effect, with a resultant 40% reduction in the DL_{CO}. Static compliance was affected only at this latter exposure concentration and duration. This O_3-induced reduction in DL_{CO} appeared to correlate with the degree of lung edema in affected animals. Yokoyama et al. (1987) found decreases in rat lung volumes (FRC and RV), static compliance (from the volume-pressure curve), and maximal flow at 50% of VC after a 24-h exposure to 1 ppm O_3.

Flow-volume curves and measurements of regional distribution of ventilation, using a positron camera, were evaluated in anesthetized, paralyzed dogs acutely exposed via a stainless steel tracheal tube to 0.12, 0.22, and 0.45 ppm O_3 for 3 h (Morgan et al., 1986). No changes in the flow-volume curve were observed at any concentration, but a less uniform distribution of ventilation was noticed, with the greatest difference occurring between the central and more peripheral regions. The authors conclude that the initial effect of O_3 appears to be obstruction of the small airways.

Miller et al. (1987, 1988) evaluated the effect of a 1-h exposure to 1.0 ppm O_3 on changes in the lung function of anesthetized, tracheostomized guinea pigs. Decreases in lung volumes were noted at 2 h postexposure and were maximum between 8 and 24 h postexposure, after which time they began to resolve. Alveolar ventilation ($\dot{V}_A$) and DL_{CO} also were decreased by exposure. The initial (2 h postexposure) reduction in DL_{CO} may have been caused by a bronchoconstriction-related decrease in $\dot{V}_A$. After this time, disproportionate ratios of DL_{CO} and $\dot{V}_A$ suggest that different mechanisms were responsible for the decreased DL_{CO}. The authors speculate that this latter response probably involves the development of a peripheral inflammatory response (8 to 24 h postexposure) because plasma concentrations of 6-keto $PGF_{1\alpha}$ and PGE_1 also were elevated in guinea pigs exposed for 1 h to 1 ppm O_3 (Miller et al., 1987). Significant increases in the plasma and BAL concentrations of TXB_2 also were observed following acute exposure of guinea pigs to 1 ppm O_3 (Miller et al., 1987) and humans to 0.4 or 0.6 ppm O_3 (see Chapter 7). Both indomethacin and cromolyn sodium partially

blocked the reduction in lung volumes at 2 and 24 h postexposure (Miller et al., 1988). Indomethacin was ineffective in blocking the O_3-induced decrease in DL_{CO} at either 2 or 24 h postexposure, but cromolyn sodium blocked this O_3 response. Both drugs were effective in blocking the O_3-induced decrease in $\dot{V}_A$ at the same examination periods. These results suggest that eicosanoids produced from an inflammatory response in the lung may be responsible for the observed changes in lung function in guinea pigs. However, as noted above the role of eicosanoid mediators in O_3-induced lung injury is controversial.

6.2.5.4 Repeated Acute Exposure Experiments (More Than Three Days)

To date, few physiological studies have examined the attenuation that occurs in humans repeatedly (3 to 7 days) exposed to O_3 (U.S. Environmental Protection Agency, 1986; Table 6-13), despite the fact that this exposure scenario most closely mimics a high oxidant pollution episode.

Ventilation

In the only laboratory animal study using a similar exposure protocol and an experimental design analogous to human repeated-exposure studies, Tepper et al. (1989) showed that rats displayed an initial pulmonary irritant response (tachypnea) that attenuated after 5 consecutive days of exposure in a manner quite similar to the response pattern of humans (see Section 7.2). Exposures were for 2.25 h and included challenge with CO_2 during alternate 15-min periods to augment ventilation (2 to 3 times $\dot{V}_E$—equivalent to light exercise in humans). The functional changes were largest on Day 1 or 2, depending on the parameter and the O_3 concentration (0.35, 0.5, and 1.0 ppm were evaluated). Additionally, lung biochemical and structural consequences were examined at 0.5 ppm O_3 and indicated that several indices of lung damage increased (histopathology) or did not adapt (lavageable protein), despite the loss of the functional response over the 5-day exposure period. Functional attenuation, however, did not occur in the 1.0-ppm O_3 group; such a nonreversing effect has not been observed in humans. It is likely that this lack of reversal was attributable to the high concentration of O_3 and, thus, may be predictive of the human response under similar conditions.

Breathing Mechanics

In rats exposed to 1.0 ppm O_3 for 6 h/day for 7 days, the only change in breathing mechanics was an increase in R_L (Yokoyama et al., 1989a). Whether attenuation occurred cannot be ascertained because functional measurements were obtained only after the end of exposure.

Extended Characterizations

Selgrade et al. (1988) evaluated mice exposed to 1.0 ppm O_3 for 5 days (3 h/day), with and without the inoculation of influenza virus on Day 2 of exposure. Ozone alone did not cause an untoward effect on lung volumes, volume-pressure, and flow-volume relationships when mice were evaluated 1, 4, and 9 days postexposure. Mice exposed to the combination of virus and O_3 showed a decrease in DL_{CO} that persisted for 9 days.

A portion of the Tepper et al. (1989) study, discussed above, was conducted using groups of animals that were exposed between 1 to 5 days to 0.5 ppm O_3. Changes in the shape of the flow-volume curve (as indicated by the change in forced expiratory flow [FEF] at

25% of VC) were maximal on Day 2 but gradually returned to baseline with further repeated exposures.

6.2.5.5 Longer Term Exposure Studies

The question of degenerative or irreversible lung damage when O_3 exposure is extended over periods of days to years remains paramount to the assessment of health risk. Several new studies since the previous criteria document (U.S. Environmental Protection Agency, 1986) have been published using more integrated approaches (structure, function, and biochemical techniques) for understanding this problem. This is especially true for studies evaluating near-lifetime O_3 exposures in rodent species.

Ventilation and Breathing Mechanics

Tepper et al. (1991) evaluated ventilation and breathing mechanics in rats exposed for 1, 3, 13, 52, and 78 weeks to a simulated urban profile of O_3 (Table 6-13). The exposure consisted of a 5-day/week, 9-h "ramped spike" exposure that had an integrated average of 0.19 ppm O_3 and a maximum concentration of 0.25 ppm. During other periods (13 h/day, 7 days/week), the exposure remained at a 0.06-ppm O_3 background level. Pulmonary function measurements were evaluated after 1, 3, 13, 52, and 78 weeks of O_3 exposure in response to a postexposure challenge with 0, 4, and 8% CO_2. Overall, there was a significant increase in expiratory resistance, but only at 78 weeks was resistance significantly different than the time-matched filtered-air control. At all evaluation times, V_T was reduced compared to control rats; this was especially true during challenge with CO_2. Frequency of breathing was significantly decreased when the analysis included all evaluation times, but at no single evaluation time was the reduction significant.

Other evidence of peripheral airflow abnormalities from extended exposures to O_3 is limited. Costa et al. (1983) exposed rats to 0.2 or 0.8 ppm O_3 for 6 h/day, 5 days/week for 12 weeks and did not find a concentration-related increase in pulmonary resistance measured immediately after exposure. Yokoyama et al. (1984) measured increased central resistance in rats exposed for 30 days to 1.0 ppm, but found increased peripheral airway resistance when exposure was for 60 days to 0.5 ppm. Pulmonary resistance was measured at different elastic recoil pressures. Increased R_L at low distending pressures was interpreted as of peripheral origin, whereas uniform increases across all distending pressures were described as originating from the central airways. These changes were consistent with morphological findings of mucus in the large bronchi of rats exposed to 1.0 ppm compared to the rats exposed to 0.5 ppm. These data also agree with a study by Wegner (1982) that suggests the occurrence of airflow obstruction, revealed in terms of small increases in peripheral airways resistance (as measured by oscillation harmonics) that were observed in monkeys after 1 year of exposure to 0.64 ppm O_3 (8 h/day, 7 days/week).

Airway Reactivity

No studies of airway reactivity after long-term exposures were reported before 1985. Since then, several studies have reported no increase in reactivity with daily O_3 exposure (Table 6-14). Biagini et al. (1986) observed no changes in breathing mechanics, FEF parameters, or methacholine and platinum airway responsiveness in a group of monkeys (cynomolgus) exposed to 1 ppm O_3 for 6 h/day, 5 days/week for 12 weeks in a study designed to examine the effects of combining O_3 exposure with the respiratory sensitizer platinum.

Kagawa et al. (1989) exposed guinea pigs 4 h/day, 5 days/week for 4 mo to 0.15 ppm O_3. Baseline total respiratory resistance and response to increasing concentrations of inhaled histamine were assessed every 3 weeks, but did not change in response to O_3. The only exception is the Johnson et al. (1988) study that evaluated airway responsiveness in female rhesus monkeys just before a 2-h single weekly exposure to 1 ppm O_3 delivered via an endotracheal tube. After 19 weeks of exposure, increased responsiveness to inhaled methacholine was observed compared to the animal's historic control. The hyperresponsiveness persisted approximately 15 weeks after exposures were discontinued. Hyperresponsiveness to O_3 was reinstated after a similar 7-week exposure to the same animals. After this exposure regimen, animals recovered in approximately 9 weeks, but hyperresponsiveness was again reinstated with four, once-per-week exposures. The investigators described the effect of a 5-lipoxygenase inhibitor on certain portions of this sequence; however, the descriptions of methods and results were insufficient for evaluation of the effect of treatment on exposure.

Extended Functional Characterizations

The previous criteria document (U.S. Environmental Protection Agency, 1986) cataloged several investigators that reported marginal increases in total lung capacity (TLC) or its component volumes in rats after intermittent or continuous exposures to 0.25 ppm O_3 for 4 to 12 weeks (Bartlett et al., 1974; Costa et al., 1983; Raub et al., 1983). In contrast to these significant results, Yokoyama and Ichikawa (1974) previously had reported no effects on rat static volume-pressure curves after a 6-week exposure to 0.45 ppm (6 h/day, 6 days/week). More recent studies are summarized in Table 6-13.

Exposures of rats to 0.7 ppm O_3 for 28 days (20 h/day) showed an obstructive-type lung function abnormality characterized by a significant reduction in FEFs, lung volumes, and DL_{CO}, and a significant increase in FRC (Gross and White, 1986). These effects largely reversed after an additional 9 weeks of clean air, but some airflow abnormalities persisted.

Tyler et al. (1988) exposed young monkeys to 0.25 ppm O_3 for 8 h/day, 7 days/week for 18 mo or for alternate months of the 18-mo period and observed increased chest wall compliance (C_w) and inspiratory capacity. Because C_w did not decrease with age, as expected, the authors speculated that perhaps O_3 interfered with respiratory system maturation. This effect was greater in monkeys exposed during alternate months than in animals exposed every month of the 18-mo period.

To address the issue of cumulative exposure over a near-lifetime, several rodent studies have been performed using various exposure concentrations. With exposure of rats to 0.5 ppm O_3 for 52 weeks (20 h/day, 7 days/week), increases in RV and FRC were apparent, as was a fall in DL_{CO} (Gross and White, 1987), suggesting substantial end-airway damage and gas-trapping. After a 3-mo period in clean filtered air, these measurements were not different than similarly treated, but air-exposed control rats. In partial contrast, 12 or 18 mo of exposure to a daily urban profile of O_3 (9-h time-weighted average of 0.19 ppm, 5 days/week; a background of 0.06 ppm for 13 h/day, 7 days/week) resulted in small reduction in lung volumes (RV and VC) and an enhanced nitrogen (N_2) washout pattern consistent with a stiffer, restricted lung (Costa et al., 1995). Interestingly, in spite of mural remodeling of small airways (which was concentration dependent), no evidence of airflow obstruction was apparent in this study. However, in a cohort group of animals exposed at the same time, R_L was increased at all time points in unanesthetized animals, as described previously (Tepper et al.,

1991). Harkema and Mauderly (1994) exposed rats for 6 h/day, 5 days/week for 20 mo to either filtered air or 0.12, 0.5, or 1.0 ppm O_3. Within 3 days of the end of exposure, an extended functional evaluation was performed; O_3 caused little impact on respiratory function. However, RV was decreased (between 21 and 36%), a finding similar, but of greater magnitude, to the Costa et al. (1995) study. The existing morphological data in monkeys at the higher concentrations noted above appear consistent with end-airway remodeling, but no clear functional evidence of obstruction has been described (Eustis et al., 1981; Wegner, 1982).

6.2.5.6 Summary

Alterations in the pulmonary function of laboratory animals after exposure to O_3 have been reported by numerous investigators. These changes appear to be homologous with the changes in pulmonary function observed in humans exposed acutely to O_3 (see Chapter 7). Although there are apparent differences in sensitivity among species, it is not clear whether these differences are due to the use of anesthesia or restraint or variances in tissue sensitivity or dosimetry.

Brief exposures to O_3 of less than 30 min have been shown to produce reflex responses (increased collateral resistance) and airway hyperresponsiveness. In the dog, these changes appear to be related, in part, to parasympathetic stimulation and release of inflammatory mediators. However, the relevance of these studies must be questioned because O_3 was delivered to a specific lung region via a bronchoscope and the contribution of collateral resistance to total lung resistance was small.

With exposures lasting greater than an hour, a wide variety of effects has been observed. Most notably, tachypnea (increased frequency of breathing and decreased tidal volume) has been noted in several species at exposures as low as 0.25 to 0.4 ppm (Tepper et al., 1990; Mautz and Bufalino, 1989). In addition to changes in breathing pattern, changes in breathing mechanics (compliance and resistance) and increased airway reactivity have been observed, but, generally, these effects have been reported at concentrations of 1 ppm or greater. In dogs, R_L increased and Cdyn decreased after a 2-h exposure to 0.5 or 0.6 ppm O_3 (Fouke et al., 1991; Mautz et al., 1985b). Enhanced reactivity to bronchoconstrictors has been reported in guinea pigs at 0.5 ppm O_3 (Tepper et al., 1990). The mechanisms that may be responsible for the O_3-induced increase in airway reactivity have been investigated extensively; however, no firm conclusion can be drawn. The most consistent evidence suggests a role for sensory afferent fibers, their associated mediators (tachykinins), and the enzyme responsible for tachykinin degradation (NEP). However, many studies suggest that the parasympathetic nervous system and inflammatory cells and mediators also may play a role in O_3-induced increase in airway reactivity.

Extended characterizations of pulmonary function indicate that the general pattern of functional impairment seen in humans acutely exposed to high concentrations of O_3 (decreased lung volumes, diffusional disturbances, and inhomogeneity of ventilation) also is observed in animals exposed to high ambient O_3 (0.5 to 2.0 ppm). For example, FVC, DL_{CO}, and N_2 slope decreased with increasing $C \times T$ products (0.5 and 0.8 ppm O_3, 2 and 7 h) in rats (Costa et al., 1989).

With daily repeated exposure to O_3, Tepper et al. (1989) showed attenuation of lung function changes (tachypnea and flow volume curve) over 5 days (2 h/day to 0.35 to 1.0 ppm, with CO_2 stimulation of breathing) similar to what is observed in repeatedly exposed humans (Chapter 7). It is of interest that, in this study, morphological changes showed a progressive

increase in severity, and other biochemical indicators of lung injury (lavageable protein and antioxidants) did not show attenuation of the response over the same time period. The findings from long-term exposures of O_3 to laboratory animals are even more difficult to summarize. Various findings in rats ranged from no or minimal effects (Biagini et al., 1986; Kagawa et al., 1989; Chang et al., 1992; Harkema and Mauderly, 1994) to obstructive (Gross and White, 1986; Tepper et al., 1991) or restrictive (Costa et al., 1995) lung function abnormalities. However, in all cases where recovery was evaluated, no severe lung injury was detected, and the physiological alterations that were observed resolved several months after termination of exposure.

6.2.6 Genotoxicity and Carcinogenicity of Ozone

6.2.6.1 Introduction

Ozone is a very reactive molecule and a strong oxidizing agent that can dissolve in aqueous solutions and generate superoxide, hydrogen peroxide, and hydroxyl radicals and can oxidize and peroxidize cellular macromolecules (reviewed in Menzel, 1970; Hoigne and Bader, 1975; U.S. Environmental Protection Agency, 1986; Mustafa, 1990; Victorin, 1992; Pryor, 1993). Early studies of the effects of O_3 on purines, pyrimidines, nucleosides, nucleotides, and nucleic acids showed that O_3 rapidly degraded these compounds in vitro (Christensen and Giese, 1954; reviewed in Menzel, 1984). Ozone-generated hydroxyl radicals can abstract hydrogen from organic molecules, leading to further complex free-radical reactions (reviewed in Menzel, 1970, 1984; Mustafa, 1990; Victorin, 1992). In addition, O_3 initiates radical reactions, resulting in ozonolysis of alkenes to form ozonides, which decompose on reaction with water to form peroxyl radicals, peroxides, and aldehydes. Ozone also can oxidize amines to amine oxides and react with PUFA to form products of lipid peroxidation (reviewed in Menzel, 1970, 1984, 1992; Mustafa, 1990; Pryor, 1978, 1991, 1993). Ozone also has been shown to cause a reduction in plaque formation by bacteriophage f2, to release RNA from phage particles, to inactivate RNA, and to degrade protein (Kim et al., 1980). Hence, because O_3 generates hydroxyl radicals in aqueous solution and degrades DNA, RNA, protein, and fatty acids in vitro, it poses a potential genotoxic hazard by virtue of its ability to generate reactive intermediates that can oxidize nucleic acid bases (reviewed in Victorin, 1992). However, the precise reactions that occur in living cells exposed to O_3 have not yet been defined completely. As the ensuing discussion shows, the genotoxic potential of O_3 is, at most, weak.

This section reviews the information available on the genotoxicity of O_3 since the last air quality criteria document (U.S. Environmental Protection Agency, 1986) was published, although earlier reports are cited to create a historical and scientific perspective for the reader. The areas covered in this review are the ability of O_3 to induce DNA damage, mutagenesis, cell transformation, carcinogenesis, co-carcinogenesis, and tumor promotion. Although modulation of the tumorigenic response by indirect effects of O_3 on the immune system is theoretically possible, no evidence for such modulation has been reported (see Section 6.2.3). Unfortunately, experimental data to evaluate whether O_3 is genotoxic are very limited. Hence, relevant data on genotoxic effects of O_3 above 1 ppm also have been included to ensure discussion of the full array of effects as they currently are understood. Although data at points far above 1 ppm of O_3 are not directly relevant to human health, such high-concentration data serve to address (1) whether ozone is genotoxic at all, (2) whether

concentration-response relationships exist for the specific genotoxicity endpoint studied, and (3) what maximum sensitivity is required for discovering genotoxic effects.

A further caveat is that in many experiments utilizing in vitro systems, O_3 was added to bacteria or to mammalian cells covered by cell culture medium. In all such experiments, the reactivity of O_3 makes it highly likely that the reaction products of O_3 with culture fluid, not O_3 itself, actually reach and interact with cells. This complicates interpretation of the results, making it extremely difficult to extrapolate in vitro results to in vivo results and making it extremely difficult, if not impossible, to extrapolate in vitro results to potential genotoxicity in humans.

6.2.6.2 Ozone-Induced Deoxyribonucleic Acid Damage

Studies utilizing a wide range of O_3 concentrations (0.1 to 20 ppm) have been performed to determine whether O_3 is genotoxic. Hamelin (1985) showed by a combination of agarose gel electrophoresis and electron microscopy that ozonation at 5 to 20 ppm caused single- and double-strand DNA breaks, nicking, relaxation, linearization, and then degradation of double-stranded plasmid pATl53 DNA molecules in solution. Hamelin also showed that ozonation of plasmid DNA reduced the transforming ability of this plasmid, and that *Escherichia coli* strains with mutations in DNA repair pathways (lexA, ozrA, and recA, but not uvrA) were less able to support the transforming ability of the ozonated plasmid. Hence, the lexA, ozrA, and recA gene products participate in repairing O_3-induced DNA breaks.

Similarly, Sawadaishi et al. (1985) showed that ozonolysis of supercoiled pBR322 DNA resulted in conversion of closed-circular DNA molecules to open-circular DNA and caused single-strand cleavage at specific sites. The concentrations of O_3 employed were not listed. Sawadaishi et al. (1986) further explored the specificity of O_3-induced damage to supercoiled plasmid pBR322 DNA by utilizing DNA sequencing techniques. The mechanistic data obtained, showing preferential degradation of thymine bases, are very interesting chemically, but the O_3 concentrations used were far too high (25,600 ppm) to be useful in assessing biologically relevant effects of O_3.

Exposure of naked DNA from HeLa cells to 2 ppm O_3 for 24 h resulted in the formation of hydroxymethyluracil, thymine glycol, and 8-hydroxyguanine (Cajigas et al., 1994). These results indicate a potential mutagenicity for O_3, although the question of the penetration of O_3 to the DNA of intact cells has not been explored carefully. Mura and Chung (1990) also studied the biological consequences of ozonation of DNA. They exposed phage T7 DNA to 5 ppm O_3 for periods of 5 to 15 min and found that O_3 decreased the template activity of the DNA. Both the rate of initiation of transcription and the length of the RNA chains transcribed were reduced. They concluded that O_3 induced abnormal changes in the structure of the phage T7 DNA and that these changes interfered with the ability of the DNA to be transcribed. In mammalian cells, Van der Zee et al. (1987) demonstrated that ozonation of murine L929 fibroblasts caused DNA strand breaks, DNA interstrand cross-links, and DNA protein cross-links, but the O_3 concentrations used were far too high (615 ppm) to be relevant to the ambient exposures that are the focus of this document.

Kozumbo and Agarwal (1990) conducted in vitro studies in which specific arylamines contained in tobacco smoke (l-naphthylamine, 2-naphthylamine, aniline, p-toluidine, o-toluidine, and m-toluidine) were exposed to 0.1 to 1.0 ppm O_3 for 1 h. When the reaction products were added to human lung fibroblasts and transformed human Type 2 cells in vitro, DNA damage occurred. This raises the possibility that smokers could incur

DNA damage in their lung cells due to the interaction of O_3 with arylamines contained in tobacco smoke, but there are no data on whether such reactions occur in vivo.

A logical consequence of these findings (Table 6-15) is that O_3 could inhibit DNA replication in mammalian cells and induce cytotoxicity to these cells, and this was found by Rasmussen (1986). Rasmussen observed that DNA replication was inhibited in a concentration-dependent manner in Chinese hamster V79 cells by O_3 concentrations from 1 to 10 ppm following a 1-h exposure. These exposure regimens also induced cytotoxicity.

Table 6-15. Effects of Ozone on Deoxyribonucleic Acid Damage[a]

Ozone Concentration		Exposure Duration	Exposure Conditions	Cells	Observed Effects	Reference
ppm	g/m³					
0.1	196	1 h	15 M l-napthylamine	Diploid human lung fibro-blasts and transformed Type 2 cells	DNA breaks	Kozumbo and Agarwal (1990)
2.0	3,920	24 h	Phosphate buffered saline	Naked DNA from HeLa cells	Formation of hydroxymethyluracil, thymine glycol, and 8-hydroxyguanine in DNA	Cajigas et al. (1994)
1.0-10	1,960-19,600	1 h	Culture	Hamster (Chinese V79 cells)	Inhibition of DNA replication; cytotoxicity	Rasmussen (1986)
5.0	9,800	5-15 min	DNA at 50 g/mL; O_3 at 0.5 L/min, room temperature	Ozonated T7 phage DNA	Decreased in vitro transcription	Mura and Chung (1990)
5.0-20	9,800-39,200	5-15 min	10 mM Tris/HC1 1 mM EDTA	Plasmid pAT153	Single-/double- strand breaks in DNA	Hamelin (1985)

[a]See Appendix A for abbreviations and acronyms.

Therefore, the available data show that O_3 causes single- and double-strand breaks in plasmid DNA in vitro, damages plasmid DNA so that its ability to serve as a template for transcription is decreased, and inhibits DNA replication and causes cytotoxicity in Chinese hamster V79 cells (Table 6-15).

6.2.6.3 Induction of Mutation by Ozone

Consistent with its ability to induce DNA damage, the very high concentration of 50 ppm O_3 also induced mutation to streptomycin resistance in *E. coli*, via both direct mechanisms and indirectly by the rec-lex error-prone DNA repair system, by a factor from two- to 35-fold in an exposure-time dependent manner (Table 6-16). However, no statistical analysis was performed on these data (Hamelin and Chung, 1975a,b; L'Herault and Chung, 1984). In assays designed to detect base substitution mutations by gases in *Salmonella typhimurium*, Victorin and Stahlberg (1988a) showed that O_3 alone at concentrations of 0.1 to 3.5 ppm did not induce mutation to histidine auxotrophy in Ames' strains TA100, TA102, or TA104. Ozone concentrations 2 ppm were cytotoxic. Victorin and Stahlberg (1988b) also showed that 0.5 and 1.0 ppm O_3, in combination with 1% vinyl chloride, and 1 ppm O_3, plus 0.1 or 1% butadiene, gave rise to a slight (approximately twofold) increase in mutation frequency. In these in vitro studies, as in many studies reviewed in this section, it must be pointed out that because O_3 is so reactive, placing vinyl chloride and butadiene in the experiments would result in exposure of the bacteria to reactive intermediates and reaction products resulting from the mixture, not exposure of the bacteria to O_3 alone. Further, these increases in mutation were small (no more than twofold) and not statistically analyzed, and the authors did not test strictly for concentration-dependent effects (Table 6-16). Thus, it is not clear that these small effects are reproducible.

More recently, Dillon et al. (1992) studied the ability of O_3 to induce mutation in the Ames' strains of *Salmonella* (Table 6-16). Ozone caused no mutation in *Salmonella* strains TA1535, TA98, TA100, and TA104. These authors found that 0.024- and 0.039-ppm O_3 exposures caused small increases in the mutation frequency in *Salmonella* tester strain TA102, which is uniquely sensitive to detecting mutation induced by oxygen radicals. These increases in mutation frequency were significant; however, the authors did not observe consistent concentration-dependent increases in the mutation frequency. At the higher concentrations of O_3, there appeared to be an inverse dependence for induction of mutation by O_3. The authors indicated that the cytotoxicity of O_3 complicated attempts to obtain a clear concentration response for mutagenicity. The presence of Arochlor 1254-induced rat liver S-9 metabolic activation did not affect the mutational responses in any of the strains tested. These authors did not observe reproducible increases in mutation frequency in *Salmonella* strains TA98, TA100, TA104, or TA1535. Dillon et al. (1992) therefore concluded that O_3 is a weak bacterial mutagen only under specific conditions utilizing noncytotoxic concentrations in TA102. However, because clear concentration-dependent responses for mutation could not be achieved, it is not clear that O_3 is definitively mutagenic in these studies.

Gichner et al. (1992) investigated whether O_3 could induce mutation in two mutagenicity assays in plants. The investigators found no induction of mutation in the *Nicotiana tabacum* leaf color reversion assay or in the *Tradescantia* stamen hair assay at 0.1 to 0.3 ppm O_3 (Table 6-16).

Dubeau and Chung (1979) showed that mutants of the yeast of *Saccharomyces cerevisiae* deficient in repair of single- and double-strand DNA breaks were more sensitive to the cytotoxicity of O_3 than wild-type cells, indicating that O_3 kills cells partly by generating these types of breaks. Dubeau and Chung (1982) also showed that treatment of *S. cerevisiae* with 50 ppm O_3 for 30 to 90 min resulted in (1) an 11- to 14-fold increased frequency of forward mutations, (2) an increase in reversions at six different loci by two- to threefold,

Table 6-16. Summary of Findings on the Mutagenicity of Ozone

Ozone Concentration ppm	Ozone Concentration $\mu g/m^3$	Exposure Duration	Exposure Conditions	Cells	Observed Effects	Reference
0.024	47	35 min	Culture	*S. typhimurium* TA102	At 0.024 ppm: 2.4-fold increase in mutation frequency	Dillon et al. (1992)
0.039	76	35 min			At 0.039 ppm: 1.6-fold increase in mutation frequency	
0.39	764	35 min			At 0.39 ppm: 1.3-fold increase in mutation frequency; no effects seen in *S. typhimurium* TA98, TA1535, TA100, or TA104	
0.1-0.3	196-588	5 or 11 h/day for 1-15 or 18 days		*Nicotiana tabacam Tradescantia*	No mutation at color locus	Gichner et al. (1992)
0.1-3.5	196-6,860	6 h	Culture	*S. typhimurium* TA100, TA102, or TA104	No mutation with or without metabolic activation	Victorin and Stahlberg (1988a)
0.5 1.0	980-1,960	6 h	Culture 1% vinyl chloride	*S. typhimurium* TA100	170% increase in mutation; statistical analysis not conducted	Victorin and Stahlberg (1988b)
1.0	1,960	6 h	Culture 0.1 or 1.0% butadiene	*S. typhimurium* TA100	170% increase in mutation; statistical analysis not conducted	Victorin and Stahlberg (1988b)
50	98,000	1-20 min	Culture	*E. coli*	Exposure-time-dependent mutation to streptomycin resistance, up to 35-fold increases in mutation frequency	L'Herault and Chung (1984)
50	98,000	30-90 min	Culture	*Saccharomyces cerevisiae*	Forward mutations, reversions, gene conversion, mitotic crossing over; no statistical analysis conducted	Dubeau and Chung (1982)

(3) an increase in gene conversions by two- to threefold, and (4) an increase in mitotic crossover by 1.3-fold. No statistical analysis was performed on these data, nor were concentration-response curves generated by the authors. These authors, therefore, demonstrated that O_3 was a mutagen and a recombination-inducing agent in *S. cerevisiae*. However, they also showed that its genotoxic activity was weak (20- to 200-fold less activity in terms of the frequency of mutants or recombinants induced) compared to the known mutagens ultraviolet light, X rays, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

In this section, the inclusion of data on bacterial mutation utilizing exposures up to 50 ppm O_3 can be justified in order to help determine whether O_3 is genotoxic at all. The available information shows that O_3 is not mutagenic in four *Salmonella* tester strains and may

cause weak mutagenicity in *Salmonella* strain TA102, but these positive results are weakened by the lack of a concentration-response effect. The data also show O_3 is mutagenic in *E. coli*, is weakly mutagenic in *S. cerevisiae*, but is not mutagenic in *N. tabacam* or *Tradescantia*. Hence, because O_3 is mutagenic in three assays, but not in six others, and is weakly mutagenic in assays where the results are positive compared to known strong mutagens, O_3 should be considered a weak mutagen, at most (also reviewed in Victorin, 1992).

6.2.6.4 Induction of Cytogenetic Damage by Ozone

A number of investigators have studied whether O_3 induces cytogenetic damage. The previous air quality criteria document (U.S. Environmental Protection Agency, 1986) described a number of in vitro and in vivo studies in which O_3 exposure produced cytotoxic effects on cells and cellular components, including genetic material; very few newer studies have been reported. Cytogenetic and mutational effects of O_3 have been reported previously in isolated cultured cell lines, human lymphocytes, and microorganisms (Fetner, 1962; Hamelin et al., 1977a,b; Hamelin and Chung, 1975a,b; Scott and Lesher, 1963; Erdman and Hernandez, 1982; Guerrero et al., 1979; Dubeau and Chung, 1979, 1982). One of the earliest studies by Fetner (1962) demonstrated that in vitro exposure of human KB cells to 8 ppm O_3 for 5 and 10 min induced two- and sixfold increases in the number of chromatid deletions. Shiraishi et al. (1986) found that treatment of Chinese hamster V79 cells with 0.1 to 1.0 ppm O_3 inhibited growth of V79 cells by 10 to 70% and also induced a concentration-dependent increase in the number of sister chromatic exchanges (SCEs) per cell, up to a maximum of fourfold of that in untreated, control cells. The results indicate that if cells in culture are exposed to sufficiently high concentrations of O_3 for sufficiently long periods, chromosome damage will result.

In vivo exposure studies are of greater potential interest. Cytogenetic and mutational effects of O_3 in laboratory animals and humans are controversial. Lymphocytes isolated from animals exposed to O_3 were found to have significant increases in the numbers of chromosome (Zelac et al., 1971a,b) and chromatid (Tice et al., 1978) aberrations, after 4- to 5-h exposures to 0.2 and 0.43 ppm O_3, respectively. Single-strand breaks in DNA of mouse peritoneal exudate cells were measurable after a 24-h exposure to 1 ppm O_3 (Chaney, 1981). Gooch et al. (1976) analyzed the bone marrow samples from Chinese hamsters exposed to 0.23 ppm O_3 for 5 h and the leukocytes and spermatocytes from mice exposed for up to 2 weeks to 0.21 ppm O_3. No effect was found on the frequency of chromosome aberrations, nor were there any reciprocal translocations in the primary spermatocytes. These authors did show that there was a slight, but significant increase in the frequency of chromatid aberrations in human peripheral leukocytes exposed in vitro to 7.2 and 7.9 ppm O_3. The small increases observed in chromatid abnormalities in peripheral blood lymphocytes from humans exposed to 0.5 ppm O_3 for 6 to 10 h were not significant, possibly because of the small number ($n = 6$) of subjects studied (Merz et al., 1975). Subsequent investigations with improved experimental design and more human subjects, however, did not show cytogenetic effects after exposure to O_3 at various concentrations and for various times (McKenzie et al., 1977; McKenzie, 1982; Guerrero et al., 1979). Guerrero et al. (1979) showed no elevation in the frequency of SCEs in circulating lymphocytes of humans exposed to 0.5 ppm of O_3 for 2 h. However, these authors did find that exposure of diploid human fetal lung (WI38) cells to 0.25, 0.50, 0.75, and 1.0 ppm O_3 for 1 h in vitro led to a concentration-dependent increase in SCEs in these cells. In addition, epidemiological studies have not shown any evidence of chromosome

changes in peripheral lymphocytes of humans exposed to O_3 in the ambient environment (Scott and Burkart, 1978; Magie et al., 1982). Evidence now available, therefore, fails to demonstrate any cytogenetic or mutagenic effects of O_3 in humans when the exposure regimens are representative of exposures that the population might actually experience.

Finally, a study conducted by Erdman and Hernandez (1982) showed that treatment of *Drosophila virilis* with 30 ppm O_3 for 2 to 6 h resulted in an exposure time-dependent accrual of dominant lethals.

Therefore, O_3 does induce chromosomal aberrations in cultured cells, but the results in animals exposed to O_3 for chromosomal breakage are, at most, weak and their biological significance is controversial.

6.2.6.5 Induction of Morphological Cell Transformation by Ozone

Ozone has been studied in a number of mammalian cell culture systems to determine whether it can induce cell transformation (Table 6-17). The cell transformation assay in C3H/10T1/2 (10T1/2) mouse embryo cells is a standard assay that has been used by many investigators to detect cell tranformation activity as a potential indicator of carcinogenicity and to study molecular mechanisms of cell transformation induced by organic chemicals, carcinogenic metals, and radiation (reviewed in Landolph, 1985, 1989, 1990, 1994). Syrian hamster embryo (SHE) cells also are widely used to detect cell transformation by many classes of chemical carcinogens and radiation (Borek et al., 1986, 1989a,b). Borek et al. (1986) demonstrated that exposure of SHE cells and 10T1/2 mouse embryo cells to 5 ppm O_3 for 5 min inducted morphological transformation in both cell types. Also, in both cell types, there was a synergistic induction of morphological transformation when the cells were treated with 3 Grays of gamma radiation and 5 ppm O_3. These authors therefore concluded that O_3 acts as a direct cell transforming agent and as a co-cell transforming agent in the presence of gamma radiation. Borek et al. (1989a) also observed an additive amount of transformation when these cell types were treated with 6 ppm O_3 and 4 J/m^2 of ultraviolet light. A further study by Borek et al. (1989b) showed that exposure of 10T1/2 mouse embryo cells to 1 ppm O_3 for 5 min did not result in morphological transformation, but that increasing exposure to 5 ppm increased the transformation frequency by a factor of 15. Ozone and gamma radiation caused a synergistic increase in morphological transformation when O_3 was added to cells after the gamma radiation. When O_3 was added to cells before the gamma radiation, the transformation was not increased over that due to gamma radiation. These authors also showed by DNA transfection experiments that three O_3-induced transformed cell lines possessed dominantly acting transforming genes. In these studies, cells were incubated in phosphate-buffered saline during O_3 treatment and, hence, likely were exposed to reaction products of O_3 rather than O_3 itself.

Thomassen et al. (1991, 1992) exposed rats by inhalation to 0.14, 0.6, or 1.2 ppm O_3 for 6 h/day, 5 days/week, for a total of 1, 2, or 4 weeks. There was no increase in the frequency of preneoplastic transformation in cells removed from the tracheas and subsequently cultured. Cells incubated in serum-free medium and exposed to 0.7 or 10 ppm O_3 for 40 min in vitro also did not show an increased frequency of preneoplastic transformation. When rat tracheal epithelial cells were exposed in vitro to 0.7 ppm O_3,

Table 6-17. Effects of Ozone on Morphological Cell Transformation[a]

Ozone Concentration ppm	Ozone Concentration μg/m³	Exposure Duration	Species, Sex (Strain/Cells) Age[b]	Observed Effects	Reference
0.14, 0.6, 1.2	274, 1,176, 2,352	6 h/day, 5 days/week for 1, 2, or 4 weeks	Rat, M (F344/N) 7-9 weeks old	No induction of preneoplastic variants in cultured tracheal epithelial cells	Thomassen et al. (1991)
0.7	1,372	40 min twice weekly for 5 weeks (in vitro)	Rat (Tracheal epithelial cells)	Twofold increase in frequency of preneoplastic variants; additive effects with MNNG	Thomassen et al. (1991)
0.7 10	1,372 19,600	40 min (in vitro)	Rat (Tracheal epithelial cells)	No induction of preneoplastic variants	Thomassen et al. (1991)
1.0 5.0	1,960 9,800	5 min (in vitro)	Mouse (C3H/10T1/2 embryo cells)	At 1.0 ppm: No morphological transformation alone; increased transformation by 0.4-Gray radiation by 1.7-fold like a co-carcinogen. At 5.0 ppm: 15-fold increase in morphological transformation and synergism with 4-Gray gamma radiation transformation.	Borek et al. (1989b)
5.0	9,800	5 min (in vitro)	Hamster (Primary diploid cells) Mouse (C3H/10T1/2 embryo cells)	In both cell lines, induction of morphological transformation and synergism with gamma rays	Borek et al. (1986)
6.0	11,760	10 min (in vitro)	Hamster (Syrian primary embryo cells) Mouse (C3H/10T1/2 embryo cells)	In both cell lines, induction of morphological transformation; additive transformation with UV light	Borek et al. (1989a)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

two times a week for 5 weeks, there was approximately a twofold increase in the frequency of preneoplastic variants detected. These authors also showed that treatment of rat tracheal epithelial cells with MNNG followed by exposure of cells to 0.7 ppm O_3 twice weekly for 5 weeks resulted in an approximately additive increase in the frequency of preneoplastic variants of the cells. The results of these studies should be interpreted cautiously because the changes are very small and because O_3 exposure followed by MNNG treatment yielded negative results (Thomassen, 1992). In addition, it is likely that the culture conditions may significantly affect these results, particularly the volume of culture medium above the cells,

and this variable has not been explored in sufficient detail. Further, the results of these experiments are somewhat variable. Recently, a system has been developed to culture human tracheobronchial epithelial cells and expose them to consistent and reproducible levels of O_3 (Tarkington et al., 1994).

In all these cell transformation experiments, the reactivity of O_3 makes it likely that secondary reaction products of O_3 formed in the aqueous medium, not O_3 itself, induced the cell transformation. Therefore, O_3 is able to induce morphological transformation in C3H/10T1/2 mouse embryo cells and in SHE cells at high concentrations (1.0, 5.0, and 6.0 ppm) but causes no significant effects in rat tracheal epithelial cells in vitro or in vivo.

6.2.6.6 Possible Direct Carcinogenic, Co-carcinogenic, and Tumor-Promoting Effects of Ozone as Studied in Whole Animal Carcinogenesis Bioassays

To investigate whether O_3 has carcinogenic, co-carcinogenic, or tumor-promoting effects, a number of investigators have conducted in vivo carcinogenesis bioassays with O_3 (Table 6-18). Some of the studies have used strain A mice (reviewed in Mustafa, 1990). The advantages and disadvantages in using strain A mice as a general screen for carcinogens by the ip route have been discussed in the literature (Stoner and Shimkin, 1985; Maronpot et al., 1986; Stoner, 1991; Maronpot, 1991). Strain A mice only rarely have been used in inhalation carcinogenesis assays. In addition, the A/J strain of mice has a high spontaneous incidence of benign pulmonary tumors (adenomas). This strain of mice has been shown to be very sensitive to tumor induction by polycyclic aromatic hydrocarbons (PAHs), carbamates, and aziridines and insensitive to aromatic amines, metal salts, and halogenated organic compounds administered by the ip route (Maronpot et al., 1986). In addition, carcinogenicity results in strain A mice did not correlate well with 2-year mouse and rat carcinogenicity results when the results of chemical testing in strain A/St mice (59 chemicals tested) were compared with strain A/J mice (30 chemicals tested) in a 2-year chronic bioassay (Maronpot et al., 1986). The chemicals chosen were heavily weighted with aromatic amines. The author concluded that "carcinogenicity test data are relevant only to the test model employed since there is no absolute reference for carcinogenicity." Maronpot (1991) also demonstrated a poor concordance between results of testing chemicals in the strain A assay and testing them in 2-year rat and mouse carcinogenicity assays at the National Cancer Institute (NCI). Stoner (1991), using the strain A mouse pulmonary assay, indicated that ip injection of PAHs, nitrosamines, nitrosureas, carbamates, aflatoxin, metals, and hydrazines induces tumors, but that the assay is not responsive to aromatic amines, aliphatic halides, and certain compounds carcinogenic in rodent liver or bladder. In this assay, an increase in lung tumor multiplicity (average number of lung tumors per mouse) caused by a chemical is considered as evidence for the carcinogenicity of a chemical.

Hassett et al. (1985) used inbred strain A/J mice, which are very sensitive to induction of pulmonary adenomas by chemical carcinogens. Exposure of A/J mice to 0.31 ppm O_3 for 103 h per week, every other week, for 6 mo, resulted in a 1.3-fold increase (not statistically significant) in the percent of mice with tumors (tumor incidence) and a statistically significant 1.4-fold increase in the number of tumors per mouse (tumor multiplicity). In this experiment, O_3 did not promote the carcinogenicity of urethane when

Table 6-18. Summary of Results on the Possible Carcinogenicity of Ozone[a]

Ozone Concentration ppm	Ozone Concentration μg/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effects	Reference
0.05 (sine wave from 0 to 0.1)	98 (sine wave from 0 to 96)	10 h/day for 13 mo	Rat, M (Wistar) 4 weeks old	Lung tumor response increased from 0% in BHPN- or O_3-treated animals, to 8.3% in animals treated with 0.5 g/kg BHPN + 0.05 ppm O_3 (not significant).	Ichinose and Sagai (1992)
0.31	608	103 h/week, every other week for 6 mo	Mouse, F (A/J) 7 weeks old	1.33-fold increase in percent of mice with adenomas, 1.42-fold increase in number of tumors per mouse. No promotion of carcinogenicity of urethane (2 mg/mouse before O_3).	Hassett et al. (1985)
0.5	980	102 h/first week of each month for 6 mo	Mouse, F (A/J) 7 weeks old	2.11-fold increase in percent of mice with tumors, 3.42-fold increase in number of tumors per mouse, interaction between O_3 and urethane (2 mg/mouse after each O_3 week).	Hassett et al. (1985)
0.4 0.8	784 1,568	8 h/day, 7 days/week for 18 weeks	Mouse, M (Swiss Webster and A/J) 8 weeks old	Urethane treatment before O_3 started. In Swiss Webster mice: No increase in lung tumor incidence; nonsignificant decrease in tumors per lung in urethane-treated animals. In A/J strain: No effect at 0.4 ppm. At 0.8 ppm: Threefold increase in percent mice with tumors, and 4.2-fold increase in number of tumors per mouse. Both 0.4 and 0.8 ppm O_3 decreased yield of tumors per mouse in urethane-treated mice, but had no effect on tumor incidence.	Last et al. (1987)
0.8	1,568	23 h/day 7 days/week for 6 mo	Hamster, M (Syrian Golden) 7-11 weeks old	No tumors observed in animals treated with 0.8 ppm O_3 only. In animals treated with 20 mg/kg DEN sc twice/week, 0.8 ppm O_3 did not increase tumors of lung, bronchus, trachea, or nasal cavity. Tumors of lung were decreased 50% (N.S.)	Witschi et al. (1993a,b)
0.12 0.5 1.0		6 h/day 5 days/week for 104 weeks (2 years)	Rat, M, F (F344/N)	No increase in neoplasms at any concentration tested.	National Toxicology Program (1994) Boorman et al. (1994)
0.5 1.0		6 h/day 5 days/week for 124 weeks (lifetime)	Rat, M, F (F344/N)	No increase in neoplasms at any concentration tested.	National Toxicology Program (1994) Boorman et al. (1994)
0.12 0.5 1.0		6 h/day 5 days/week 105 weeks (2 years)	Mouse, M, F ($B6C3F_1$)	No effects in males. In females: Increase in number of mice with neoplasms at 1.0 ppm (combined alveolar/bronchiolar adenoma or carcinoma in lung).	National Toxicology Program (1994)
0.5 1.0		6 h/day 5 days/week 130 weeks (lifetime)	Mouse, M, F ($B6C3F_1$)	In males: Increase in number of mice with carcinoma at 0.5 and 1.0 ppm, but not significant for change in number of mice with total neoplasms. In females: increase in number of mice with adenomas, but not carcinomas or total neoplasms.	National Toxicology Program (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

O_3 exposure began 1 week after a single injection of animals with a total dose of 2 mg urethane/mouse. In a second experiment, exposure to 0.50 ppm O_3 (102 h during the first week of every month for 6 mo) caused a nonsignificant 2.1-fold increase in tumor incidence and a 3.2-fold increase in tumor multiplicity (statistics not shown) (Hassett et al., 1985).

These authors reported that exposure to 0.5 ppm O_3, followed by urethane treatment (2 mg after each O_3 exposure set), resulted in an interaction between O_3 and urethane such that there were more animals with more than 16 lung tumors each. These authors concluded that exposure to 0.31 and 0.5 ppm O_3 increased the yield of pulmonary adenomas in A/J mice and that O_3 interacted with urethane to produce more lung tumors than urethane alone when O_3 was added before urethane.

The pulmonary adenomas induced in animals by chemical carcinogens are by definition benign tumors (Stoner, 1991; Maronpot, 1991); however, they do represent abnormal cell growth in the form of tumors and, hence, are significant biologically in that they are early steps in the pathway toward malignancy. It is not known if these tumors can progress to malignant tumors, because they are not as amenable to observation as are mouse skin tumors, where adenomas can be converted into adenocarcinomas at a frequency of approximately 8 to 10%. Similarly, it is not known with certainty whether pulmonary adenomas in humans progress to adenocarcinomas. It is clear, however, that lung tumors in mice are not equivalent to bronchogenic carcinomas in humans. However, because a shift is occurring, in that fewer squamous cell bronchogenic carcinomas and more peripheral adenocarcinomas are occurring in humans, the induction of peripheral adenomas and their progression to peripheral adenocarcinomas in mice may be a useful area for further mechanistic insight.

The study of Hassett et al. (1985) was reviewed extensively by scientists from EPA and the National Institutes of Environmental Health Sciences in 1985 and 1986 (Tilton, 1986). The consensus of these extensive reviews was that (1) the tumor yields in O_3-exposed mice were not statistically significantly different from the control animals, (2) any effects were marginally different from control values, and (3) the strain A mouse has a high spontaneous incidence of tumors, making it difficult to interpret the effects of O_3. Chemical induction of tumors in this assay system did not correlate well with the 2-year NCI carcinogenesis bioassay results. In addition, because Hassett et al. (1985) did not demonstrate a concentration-response effect in animals exposed to O_3, the consensus among the reviewers was that one could not conclude from these experiments that O_3 was a significant carcinogen or tumor promoter, and that rigorous inhalation carcinogenesis bioassays needed to be carried out with O_3-exposed animals to address this issue properly.

Last et al. (1987) also studied whether O_3 exposure could influence the yield of urethane-induced lung tumors in A/J and Swiss-Webster mice. Urethane treatment consisted of a single ip injection (1,000 mg/kg) 1 day before O_3 exposure began. In Swiss Webster mice, exposure to 0.4 or 0.8 ppm O_3 alone not only did not increase the tumor yield but actually decreased the yield of urethane-induced lung tumors per mouse, although the decrease was not statistically significant. In A/J mice, exposure to 0.4 ppm O_3 did not increase the lung tumor yield, but exposure to 0.8 ppm O_3 caused a threefold increase in tumor incidence and a 4.2-fold increase in lung tumor multiplicity. Exposure of urethane-treated mice to 0.4 or 0.8 ppm O_3 decreased lung tumor multiplicity but had no effect on tumor incidence. These differences in the strain A mouse were significant. The authors concluded that O_3 was not a tumor promoter or tumor-enhancing agent.

Ichinose and Sagai (1992) studied the ability of O_3 to interact with *N-bis*(2-hydroxypropyl) nitrosamine (BHPN) in the induction of lung tumors in Wistar rats. A single ip injection of BHPN (0.5 g/kg) did not cause any tumors in the rats. Rats were exposed for 10 h/day for 13 mo to a pattern of O_3 consisting of a sine curve from 0 to

0.1 ppm, with a mean concentration of 0.05 ppm. The 13-mo O_3 exposure started the day after BHPN injection, and the rats were examined 11 mo postexposure. No tumors were observed in the O_3-alone or control groups. When rats were exposed to 0.5 g/kg BHPN plus 0.05 ppm O_3, the lung tumor incidence increased to 8.3% (3/36), but this increase was not statistically significant. The tumors observed in this study cannot be stated definitively to have been induced by the treatment agent.

The data available on O_3 exposure and lung carcinogenesis up until 1988 have been reviewed by Witschi (1988). The chemical reactivity of O_3 and, in particular, its radiomimetic activity (ability to mimic radiation effects, such as causing cell-cycle arrest, chromosome breakage, etc.) also make O_3 a potential risk factor for human lung cancer. Nevertheless, as of 1988, there were no experimental studies conclusively linking lifelong exposure to O_3 with lung tumor induction in any animal species, nor was there conclusive epidemiological evidence to associate O_3 exposure with the development of lung cancer in humans. As of 1988, the only data implicating O_3 as a possible tumorigenic agent were from studies carried out in mice, where the tumors are adenomas derived from Type 2 alveolar cells or from Clara cells (reviewed in Witschi, 1988). In the A and Swiss-Webster mouse strains used to assay the carcinogenicity of O_3, the spontaneous incidence of lung tumors is very high. Hence, results of carcinogenicity experiments conducted on O_3 to date that utilize tumor incidence as an endpoint are not strongly positive, due to this high background. In strain A mice, in which the spontaneous multiplicity is usually less than one tumor per mouse, the tumor multiplicity is considered by many investigators to be a sensitive indicator of a carcinogenic effect. However, even using this indicator, the increase in tumor multiplicity after O_3 exposure is small, raising questions about the biological significance of the effects. In addition, the assay for inhalation carcinogenesis in strain A mice was not fully validated at this time. Whereas Hassett et al. (1985) concluded that O_3 increased the number of pulmonary adenomas in strain A mice, Witschi (1988) concluded that O_3 was not implicated unequivocally as a carcinogen in strain A/J mice, that no classical carcinogen bioassays had been conducted on O_3, and that a definitive judgment could not be made on the carcinogenicity of O_3.

A review (Witschi, 1991) of the available data on the carcinogenicity of O_3 and oxygen in mouse lungs indicated that oxidants can enhance or inhibit mouse lung tumorigenesis, depending on the experimental protocol employed, and the carcinogenicity of O_3 in mouse lung had not been established unequivocally. Exposure of strain A mice to O_3 induces hyperplasia of Type 2 alveolar cells, leading to expansion of the target cell population. It was speculated that this might have resulted in spontaneous transformation of these cells (Witschi, 1991). In lungs of animals treated with a carcinogen such as urethane and then exposed to O_3 before or after carcinogen administration, it was speculated that O_3 may cause cell proliferation and result in fixation of DNA damage (Witschi, 1991). The addition of O_3 after carcinogen exposure leads to a decreased tumor incidence compared to treatment with carcinogen alone; the reasons for this decrease with late O_3 exposure are not clear.

The effects of treating male Syrian Golden hamsters with dimethylnitrosamine (DEN) (20 mg/kg given subcutaneously twice per week) during the course of a 6-mo exposure to 0.8 ppm O_3 were studied by Witschi et al. (1993a; see Table 6-18). The rationale for this study was to test the hypothesis that O_3 acts in a manner similar to hyperoxia in enhancing neuroendocrine lung tumors in this animal model. After exposure ceased, the animals were maintained in air for 1 mo. Ozone exposure did not increase the incidence of lung, bronchus, trachea, or nasal cavity tumors in the DEN-treated hamsters. There was a 50% decrease in the

percent of animals with lung tumors in the DEN-plus-O_3-exposed animals compared to the DEN-plus-air-exposed animals, but this was not statistically significant. Ozone did not affect the incidence of DEN-induced liver tumors. The authors concluded that O_3 did not increase the number of DEN-induced respiratory tumors in hamsters and that O_3 exposure might have inhibited or delayed tumor development. Although reduction in tumor incidence caused by O_3 was not significant in this study alone, overall analysis of these data with other data from a 4-mo exposure of similar design (Witschi et al., 1993b) yielded significant results.

A definitive study of the carcinogenicity of O_3 and its ability to act as a co-carcinogen or tumor promoter was conducted by the U. S. National Toxicology Program (NTP) (National Toxicology Program, 1994; Boorman et al., 1994). Animals were exposed to air or O_3 for 6 h/day, 5 days/week for the number of weeks described below. Male and female F344/N rats were exposed to 0.12, 0.5, and 1.0 ppm O_3 for 104 weeks and to 0.5 and 1.0 ppm for 124 weeks ("lifetime" of the animals). Similar protocols were used for $B6C3F_1$ male and female mice, with the exception that the "2-year" study was 105 weeks and the lifetime study was 130 weeks. This study did not find any evidence of carcinogenic activity in male or female rats. There was a negative trend for mammary gland neoplasms in the female rats in the 2-year study, an effect that was not seen in the lifetime study. The NTP found "equivocal evidence"[1] of carcinogenic activity in O_3-exposed male mice and "some evidence" of carcinogenic activity in female mice exposed to O_3. In co-carcinogenesis experiments, male rats were treated with a known pulmonary carcinogen, 4-(*N*-methyl-*N*-nitrosomino)-1-(3-pyridyl)-1-butanone (NNK) (0.1 and 1.0 mg/kg, subcutaneous injection 3 times a week for first 20 weeks) and exposed to 0.5 ppm O_3 for 6 h/day, 5 days/week for 105 weeks. The NTP found "no evidence" that O_3 enhanced the incidence of NNK-induced pulmonary neoplasms. Table 6-19 shows the tumor incidences in mice. In the discussion to follow, all tumors described were at lung alveolar/bronchiolar sites. There was a decrease in the number of hepatocellular adenomas or carcinomas in female mice exposed to 1.0 ppm O_3 for 2 years and for hepatocellular carcinomas in the lifetime study. There was no statistically significant increase in tumors at any site other than the lung.

In male mice exposed to O_3 for 2 years, there were no statistically significant increases in the mice with alveolar/bronchiolar carcinomas or a combination of adenomas or carcinomas; at 0.5 ppm O_3, there was a small, twofold increase in the incidence of adenomas. In the lifetime studies of male mice, the incidence of mice with carcinomas increased 1.9-fold at 0.5 ppm O_3 and 2.3-fold at 1.0 ppm. The incidence of adenomas in

[1]The NTP evaluates the strength of the evidence for conclusions regarding each carcinogenicity study, under the conditions of that particular study. There are five categories: two for positive results ("clear evidence" and "some evidence"), one for uncertain findings ("equivocal evidence"), one for no observable effects ("no evidence"), and one for experiments that cannot be judged because of major flaws ("inadequate study") (National Toxicology Program, 1994). This approach is very different from the weight-of-evidence approach used by EPA for cancer classification because the EPA approach considers all the available studies.

Table 6-19. Alveolar/Bronchiolar Tumor Incidence in B6C3F$_1$ Mice in the National Toxicology Program's Chronic Ozone Study

	Males			Females		
ppm	Adenomas[a]	Carcinomas[a]	Both[a]	Adenomas[a]	Carcinomas[a]	Both[a]
2-year exposure						
0.0	6/50	8/50	14/50	4/50	2/50	6/50
0.12	9/50 (p = 0.3)	4/50 (p = 0.15)	13/50 (p = 0.44)	5/50 (p = 0.55)	2/50 (p = 0.65)[b]	7/50 (p = 0.57)
0.5	12/50 (p = 0.06)	8/50 (p = 0.45)	18/50 (p =0.12)	5/49 (p = 0.52)	5/49 (p = 0.26)	9/49 (p = 0.33)
1.0	11/50 (p = 0.11)	10/50 (p = 0.27)	19/50 (p = 0.10)	8/50 (p = 0.24)	8/50 (p = 0.053)	16/50 (p = 0.02)
Lifetime exposure						
0.0	8/49	8/49	16/49	3/50	3/50	6/50
0.5	8/49 (p = 0.61)[b]	15/49 (p = 0.05)	22/49 (p = 0.14)	3/49 (p = 0.63)	5/49 (p = 0.33)	8/49 (p = 0.34)
1.0	9/50 (p = 0.47)	18/50 (p = 0.007)	21/50 (p = 0.15)	11/50 (p = 0.02)	2/50 (p = 0.50)[b]	12/50 (p = 0.10)
Combined						
0.0	14/99	16/99	30/99	7/100	5/100	12/100
0.5	20/99 (p = 0.16)	23/99 (p = 0.08)	40/99 (p = 0.06)	8/98 (p = 0.48)	10/98 (p = 0.14)	17/98 (p = 0.20)
1.0	20/100 (p = 0.14)	28/100 (p = 0.009)	40/100 (p = 0.04)	19/100 (p = 0.01)	10/100 (p = 0.14)	28/100 (p = 0.004)

[a]Number of animals with neoplasm/number of animals necropsied (p [probability] value, logistic regression test).
[b]Lower incidence.

Source: National Toxicology Program (1994).

male mice did not change significantly. In female mice exposed for 2 years, there were no statistically significant changes at 0.12 or 0.5 ppm O_3. However, at 1.0 ppm O_3, there was a fourfold increase in the frequency of female mice with carcinoma and a 2.7-fold increase in combined adenomas plus carcinomas. In female mice exposed for their lifetimes to O_3, 0.5 ppm caused no significant effects. At 1.0 ppm O_3, there was a 3.7-fold increase in the incidence of mice bearing pulmonary adenomas, a nonsignificant change in the frequency of mice with carcinomas, and a twofold ($p = 0.1$) increase in the incidence of combined adenomas and carcinomas.

When the results of the 2-year and lifetime O_3 carcinogenesis studies were combined and analyzed, for male mice, there was no statistically significant increase in the incidence of animals bearing adenomas, and, for carcinomas, there was a marginally significant increase at 0.5 ppm O_3 (1.4-fold increase, $p = 0.08$) and a significant, 1.7-fold increase at 1.0 ppm. The incidence of male mice bearing adenomas or carcinomas showed a marginally significant increase (1.3-fold, $p = 0.06$) at 0.5 ppm O_3 and a 1.3-fold increase at 1.0 ppm ($p = 0.045$). In the combined analysis of the 2-year and lifetime exposure of female mice, there were no statistically significant changes at 0.5 ppm O_3. At 1.0 ppm O_3, there was a 2.7-fold increase in the percent of mice bearing adenomas and a 2.3-fold increase in the frequency of mice with adenomas or carcinomas. There was no statistically significant increase in the carcinoma incidence.

The overall conclusions of the authors of the NTP O_3 inhalation carcinogenesis study were (1) there was no increased pulmonary tumor incidence in male or female F344/N rats exposed to 0.12, 0.5, or 1.0 ppm O_3; (2) male F344/N rats treated with the tobacco carcinogen NNK and exposed to 0.5 ppm O_3 did not have an increase in the pulmonary tumor incidence above that caused by NNK alone; (3) O_3 caused a slightly increased incidence of alveolar/bronchiolar adenoma or carcinoma that yielded equivocal evidence of carcinogenicity

of O_3 in male B6C3F$_1$ mice; and (4) O_3 increased the incidence of alveolar/bronchiolar adenoma or carcinoma in female B6C3F$_1$ mice, yielding some evidence of carcinogenic activity of O_3 in female mice.

Generally, in mice, adenomas appear to progress into carcinomas with time, and, thus, the incidence of mice having both adenomas and carcinomas is probably the more useful indicator of effects. The incidence of tumor-bearing mice was elevated significantly only in female mice exposed for 2 years to 1.0 ppm O_3. When both the 2-year and lifetime exposure studies were combined, there was a increased incidence of tumors in the males at 0.5 and 1.0 ppm O_3 and in females at 1.0 ppm. The NTP designated the data for male mice as equivocal for carcinogenesis because the combined tumor incidence in the 2-year study was within the historical range, and the combined incidence for the lifetime study was not significant, even though the carcinoma incidence was significant in the lifetime study. The evaluation of female mice resulted in NTP's finding of "some evidence of carcinogenic activity" because the combined pulmonary adenoma/carcinoma incidence was significantly increased and outside the range of the historical control tumor rates. When the lifetime and 2-year studies were combined, there were 28/100 adenomas plus carcinomas in the 1.0-ppm O_3 exposure group versus 12/100 in the controls ($p = 0.004$).

In summary, the strongest data on carcinogenicity come from the NTP study, which was ambiguous in male mice and positive only in female mice at high concentrations of O_3 (i.e., 1.0 ppm). This may represent a toxic or irritant effect, giving a nonspecific type of tumor due to mitogenesis. The carcinogenicity data are weak or equivocal in male mice, negative in F344/N male and female rats, and negative for co-carcinogenesis in male rats. Therefore, the potential for animal carcinogenicity is uncertain at the present time.

6.2.6.7 Possible Effects of Ozone on Injected Tumor Cells That Lodge in the Lung and Form Lung Colonies

To date, no rigorous studies have been conducted to examine the effects of O_3 on true lung tumors that would have metastasized. No studies have been conducted in which lung tumor cells detach themselves from primary lung or other tumors growing in organs and invade adjacent tissue, blood vessels, or lymphatics. A few studies have been conducted in which tumor cells are injected intravenously into animals and then lodge in the lung, forming lung colonies (Table 6-20). It must be stressed, however, that this experimental model is not an adequate model for lung tumor cell metastasis.

Kobayashi et al. (1987) showed that exposure of C3H/He mice for 1 or 14 (but not other) days to 0.1 ppm O_3 after mice were injected in the tail vein with the fibrosarcoma cell line (NR-FS) increased the number of metastatic lung tumors. Animals were exposed to O_3 for 14 days, then fibrosarcoma cells were injected into the tail vein of the animals, and pulmonary metastases were scored 14 days later. One day of exposure to 0.8 ppm O_3 gave the maximal enhancement of pulmonary metastases. This enhancement of pulmonary metastasis was concentration-dependent, in the range from 0.4 to 0.8 ppm O_3 from 1 to 14 days, but increases were small. This effect may arise in two ways: (1) by damage to the microvasculature and (2) by the differential sensitivity of various tumor cells to O_3 cytotoxicity (reviewed by Witschi, 1988). Richters (1988) reported that exposure of mice to 0.15 or 0.30 ppm O_3 for 60 days did not increase colonization of the lungs of mice injected iv with B16 melanoma cells.

Table 6-20. Effects of Inhaled Ozone on the Ability of Injected Tumor Cells To Colonize the Lungs of Mice[a]

Ozone Concentration ppm	Ozone Concentration g/m^3	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effects	Reference
0.15 0.3	294 588	60 days	Mouse, M (C57B/6J) 5 weeks old	No increase in lung metastases from iv-injected B16 melanoma cells.	Richters (1988)
0.1 0.2 0.4 0.8	196 392 784 1,568	1-14 days	Mouse, M (C3H/He) 8-12 weeks old	Pulmonary metastases from iv-injected NR-FS fibrosarcoma cells. After 1 and 14 days of 0.1 ppm, 1.3-fold increases. After 5 and 7 days of 0.2 ppm, 1.3 and 2.3-fold increases. After 1 and 5 days of 0.4 ppm, 2.3- and 2.2-fold increases. After 1 day of 0.8 ppm, 4.6-fold increase.	Kobayashi et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Age at start of exposure.

6.2.6.8 Summary and Conclusions

In summary, there are some weakly positive data and some negative data on the genotoxicity of O_3 (summarized in Table 6-21). Ozone at very high concentrations (5 to 20 ppm) causes DNA strand breakage in plasmid DNA (Hamelin, 1985). Ozone is, at most, weakly mutagenic in some assays and negative in others. Ozone is not mutagenic in *Tradescantia* or *N. tabacam* at concentrations of 0.1 to 0.3 ppm (Gichner et al., 1992); is weakly mutagenic in *E. coli* at 50 ppm, and *S. cerevisiae* at 50 ppm (L'Herault and Chung, 1984; Dubeau and Chung, 1982); and is nonmutagenic in three strains of *Salmonella* and, at most, marginally mutagenic in *Salmonella* strain TA102 at concentrations of 0.024, 0.039, and 0.39 ppm (Victorin and Stahlberg, 1988a,b; Dillon et al., 1992). Despite extensive studies by Dillon et al. (1992), the mutagenicity of O_3 in *Salmonella* TA102 is not conclusive because convincing concentration-dependent mutagenic effects have not yet been demonstrated, possibly due to the strong cytotoxicity of this compound. Ozone causes cytogenetic damage in cultured cells in vitro (e.g., Hamelin et al., 1977a,b; Dubeau and Chung, 1979, 1982), but no effects or small and conflicting effects when animals are exposed in vivo (Zelac et al., 1971a,b; Tice et al., 1978). Cell transformation studies have shown positive results on exposure of cells to O_3, but these studies were conducted with a fluid barrier above the cells that may have resulted in artifacts compared to an in vivo exposure (Borek et al., 1986, 1989a,b).

The in vitro studies are mechanistically interesting, but there are difficulties in the design of many of these studies. First, the concentrations used in these in vitro studies were typically orders of magnitude greater than those found in ambient air. Second, extrapolation of in vitro exposure concentrations to human exposure dose requires special methods that were not used in these studies. Third, direct exposure of isolated cells to O_3 is somewhat

Table 6-21. Summary of Data on the Genotoxicity of Ozone[a]

Assay System in Which Ozone Was Tested	Result[b]	Comments
Mutation to histidine prototrophy in *Salmonella* TA100	−	Small effects obtained, less than twofold; concentration-response effect was not shown at 0.024, 0.039, and 0.39 ppm O_3.
Mutation to histidine prototrophy in *Salmonella* TA102	+/−	Small effects obtained, and there was no direct exposure-response at 0.024, 0.039, and 0.39 ppm O_3.
Mutation to streptomycin resistance in *Eshcherichia coli*	+	Only 50 ppm O_3 was tested.
Mutation in *Saccharomyces cerevisiae*	+	Ozone caused mutation and recombination at 50 ppm, but this was a weak response compared to known strong mutagens (20- to 200-fold less mutagenic than UV light, X rays, and MNNG).
Mutation in *Nicotiana tabacam* in a leaf-color reversion assay	−	0.1 to 0.3 ppm O_3 was tested.
Mutation in *Tradescantia* in a stamen-hair assay	−	0.1 to 0.3 ppm O_3 was tested.
Chromosomal breakage in cultured mammalian cells	+	8 ppm O_3 in human KB cells, and 0.1 to 1.0 ppm O_3 in V79 cells.
Chromosomal breakage in animals	+/−	Results are, at best, weak and controversial; results in this assay are considered ambiguous and not definitively positive at present. Ozone was tested at 0.2, 0.43, 7.3, and 7.9 ppm.
Morphological transformation in C3H/10T1/2 mouse embryo cells and in Syrian hamster embryo cells	+	Experiments need to be conducted without or with only minimal amounts of fluid bathing the cells. Concentrations giving positive results are high (5 and 6 ppm O_3).
Induction of preneoplastic variants in rat tracheal epithelial cells	−	Both in vitro and in vivo exposures give negative or, at most, only twofold increases in cells exposed to 0.14, 0.6, 0.7, 1.2, or 10 ppm O_3.

Table 6-21 (cont'd). Summary of Data on the Genotoxicity of Ozone[a]

Assay System in Which Ozone Was Tested	Result[b]	Comments
Lung tumor induction in whole animals		
(a) Strain A/J mice, Swiss-Webster mice, Syrian Golden hamsters, Wistar rats	+/□	Positive results marginal, not statistically significant; experiments not designed to determine whether a concentration-response exists. Ozone was tested at 0.05, 0.31, 0.4, 0.5, 0.8, and 1.0 ppm.
(b) National Toxicology Program Studies—Male and female F344/N rats	□	No increased incidence of pulmonary adenomas or carcinomas in rats exposed to 0.12, 0.5, or 1.0 ppm O_3 for 2 years or with 0.5 or 1.0 ppm O_3 for animals' lifetimes.
Male B6C3F$_1$ mice (2-year study)	+/□	No effect at 0.12 ppm O_3, slight increases in the total pulmonary neoplasms at 0.5 and 1.0 ppm O_3, but they were not statistically significant.
Male B6C3F$_1$ (Lifetime studies)	+/□	Alveolar/bronchiolar carcinoma incidence increased twofold at 0.5 ppm O_3 ($p = 0.05$) and 1.0 ppm O_3 ($p = 0.007$); no increases in total pulmonary neoplasms.
Female B6C3F$_1$ mice (2-year study)	+	Fourfold increase in alveolar/bronchiolar carcinoma at 1.0 ppm O_3 ($p = 0.053$).
Female B6C3F$_1$ (Lifetime studies)	+	Fourfold increase in alveolar/bronchiolar adenomas and carcinomas at 1.0 ppm O_3 ($p = 0.02$).
	+	Threefold increase in alveolar/bronchiolar adenomas at 1.0 ppm O_3 ($p = 0.02$).
	+	Twofold increase in bronchiolar/alveolar adenomas and carcinomas at 1.0 ppm O_3, but not statistically significant.

[a]See Appendix A for abbreviations and acronyms.
[b]□ = no effect; + = effect.

artifactual because it bypasses all the host defenses that would normally be functioning to protect the individual from the inhaled dose. Direct, in vitro O_3 exposure of isolated cells in tissue culture medium also results in chemical reactions between O_3 and culture media to generate chemical species that may not be produced in vivo. Therefore, for these reasons, the relevance and predictive value of in vitro studies to human health are questionable The most relevant data on the genotoxicity of O_3 should therefore be obtained from in vivo studies.

The earlier studies in whole animal carcinogenesis bioassays must be considered ambiguous at this time (Witschi, 1988, 1991). The NTP study utilized an inhalation model, assayed the carcinogenicity of O_3 in male and female F344/N rats and $B6C3F_1$ mice, and also tested whether O_3 could enhance the tumorigenicity of the tobacco-specific pulmonary carcinogen NNK (National Toxicology Program, 1994; Boorman et al., 1994). This study clearly showed that O_3 was not carcinogenic in female and male rats at 0.12, 0.5, and 1.0 ppm O_3 (6 h/day, 5 days/week for 2 years) or at 0.5 and 1.0 ppm O_3 (6 h/day, 5 days/week, lifetime). Exposure to 0.5 ppm O_3 did not enhance the carcinogenicity of NNK in male rats, leading to the conclusion that O_3 does not act as a co-carcinogen or tumor promoter in these animals. In the male mice, O_3 had equivocal effects at 0.5 and 1.0 ppm O_3 in the 2-year and lifetime inhalation studies. In the female mice, there was some evidence for the carcinogenicity of O_3 at 1.0 ppm (2.7-fold increase in total pulmonary neoplasms [$p = 0.02$] in the 2-year study; and twofold increase in total pulmonary neoplasms [$p = 0.1$] in the lifetime study; and 2.3-fold increase in total pulmonary neoplasms when the 2-year and lifetime study were combined [$p = 0.004$]).

Therefore, the earlier negative animal carcinogenesis studies, the negative carcinogenicity results in inhalation carcinogenesis studies in F344/N male and female rats, the ambiguous data in male $B6C3F_1$ mice, and the weak carcinogenicity of O_3 in female $B6C3F_1$ mice indicate that O_3 is carcinogenic only in female $B6C3F_1$ mice at high concentrations (1.0 ppm). The weak carcinogenicity of O_3 in female mice, the weak/ambiguous results in male mice, and the negative results in male and female F344/N rats point to, at best, a weak carcinogenicity of O_3 at very high concentrations.

6.3 Systemic Effects of Ozone

6.3.1 Introduction

Ozone has long been known to cause effects in organs and tissues outside the respiratory tract. The mechanisms are not known, but it is quite unlikely that O_3 itself enters the circulation (Pryor, 1992). Another possibility is that transported reaction products cause distant effects. Some effects may be secondary to effects on the lung (e.g., aversive behaviors that may result from lung irritation). The relatively few systemic studies reported since the last O_3 criteria document (U.S. Environmental Protection Agency, 1986) are discussed below. Some classes of effects (i.e., reproduction/development, endocrine system) were studied earlier and, hence, are cited briefly here in the introduction.

No reproductive toxicity studies of O_3 were found. Only two developmental studies provided sufficient details in the report to determine the exposures used. The only effect observed by Kavlock et al. (1979) in pregnant rats exposed to 0.44 to 1.97 ppm O_3 for the entire period of organogenesis or the three stages of gestation was an increased resorption of fetuses in rats exposed to 1.49 ppm in midgestation; no terata were found. A follow-up study revealed that pups from dams exposed to 1 ppm O_3 during mid- or late gestation showed lower

body weights 6 days after birth (Kavlock et al., 1980). A higher concentration (1.5 ppm) delivered during late gestation permanently runted 14% of the male pups.

Studies on the effects of O_3 on the endocrine system date to 1959. Generally, the body of work indicates that O_3 can affect the pituitary-thyroid-adrenal axis (U.S. Environmental Protection Agency, 1986). For example, a 1-day exposure to 1 ppm O_3 decreased serum levels of thyroid-stimulating hormone, thyroid hormones, and protein-bound iodine; prolactin levels increased (Clemons and Garcia, 1980a,b). Structural changes occurring in the parathyroid glands after a 4- to 8-h exposure to 0.75 ppm O_3 included hyperplasia of chief cells, but circulating hormone levels were not measured (Atwal and Wilson, 1974).

6.3.2 Central Nervous System and Behavioral Effects

Reports of headache, dizziness, and irritation of the nose, throat, and chest are common complaints that are associated with O_3 exposure in humans (see Chapter 7). Laboratory animal studies have been performed that demonstrate behavioral effects over a wide range of O_3 concentrations (0.08 to 1.0 ppm) and suggest that these behavioral changes may be analogous to the symptoms reported in humans. Although these behavioral changes may be indicative of O_3-induced symptoms, they are not indicative of neurotoxicity. Most of the studies prior to 1986 indicated that behavior could be suppressed with O_3 exposure. For example, Murphy et al. (1964) and Tepper et al. (1982) showed that running-wheel behavior was suppressed, and Peterson and Andrews (1963) and Tepper et al. (1983) showed that mice would alter their behavior to avoid O_3 exposure. Furthermore, Weiss et al. (1981) showed that bar-pressing responses for food reinforcement were suppressed, but greater O_3 concentrations were required to decrease this behavior than the concentrations needed to decrease running-wheel behavior.

Since 1986, several reports have extended the previous findings (Table 6-22). Tepper et al. (1985) compared the effects of a 6-h exposure to O_3 on the suppression of running-wheel behavior in rats and mice. The study indicated that the lowest effective concentration was about 0.12 ppm O_3 in the rat and about 0.2 ppm in the mouse. It also was observed that, with exposure to 0.5 ppm, recovery from O_3 required at least 3 h. In a follow-up study, Tepper et al. (1985) required mice to make a response that turned off the brief delivery (60 s) of O_3 at concentrations between 0.25 to 16 ppm. Mice learned to terminate O_3 exposures at 0.5 ppm. With each of three determinations of the concentration-response curve, mice got better at terminating O_3 exposure, rather than exhibiting an adaptation to exposure. The authors suggest that mice may have learned to use the odor of O_3 as a conditioned stimulus to initiate termination of exposure instead of responding directly to the irritant properties of O_3.

Because free-access wheel-running behavior was suppressed at 0.12 ppm O_3 (Tepper et al., 1982), and lever pressing for food reinforcement was reduced only at 0.5 ppm (Weiss et al., 1981), a series of experiments was performed to evaluate the behavioral determinants of the O_3 response (Tepper and Weiss, 1986). Food deprivation and response contingencies (having to perform a certain response to get a reward) were found to be relatively unimportant determinants of behavior because rats that had to run rather than press a lever to obtain food reinforcement showed behavioral suppression of running at 0.12 ppm. However, in another experiment, suppression of lever pressing was shown to be

Table 6-22. Effects of Ozone on Behavior[a]

Ozone Concentration ppm	Ozone Concentration μg/m³	Exposure Duration	Behavioral Conditions	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.08 0.12 0.25 0.5	157 980	6 h	Free-access wheel running	Rat, M (Long-Evans) 10 weeks old Mouse, M (Swiss-Webster) 5 weeks old	Mice less responsive than rats. Reduction in free-access wheel running at approximately 0.12 ppm in rats and 0.2 ppm in mice. Recovery from exposure to 0.5 ppm did not occur by 5 h postexposure in either mice or rats.	Tepper et al. (1985)
0.08 0.12 0.25 0.5	157 980	6 h	Wheel running for food	Rat, M (Long-Evans) 300 g	0.12 ppm O_3 decreased wheel running for food reinforcement.	Tepper and Weiss (1986)
0.08 0.12 0.25 0.5	157 980	6 h	Lever pressing for access to the running wheel	Rat, M (Long-Evans) 300 g	0.12 ppm O_3 decreased bar press for access to the running wheel. Two of the four animals were affected at 0.08 ppm.	Tepper and Weiss (1986)
0.12-1.5	235-2,940	6 h	Nose poke response for food	Rat, M (Long-Evans) 275 g	0.5 ppm O_3 decreased nose poking for food reinforcement. Effects were enhanced postexposure.	Tepper and Weiss (1986)
0.1-0.8	196-1,568	7 days continuous	Drinking, eating	Mice, M (ICR) 8-26 weeks old	Drinking, food consumption, and body weight initially decreased, but adapted with continued exposure, starting at 0.2 ppm.	Umezu et al. (1993)
0.25-16	490-31,360	60 s maximum	Nose poking terminated O_3 exposure	Mouse, M (Swiss-Webster) 30 g	At 0.5 ppm, mice learned to terminate O_3 exposure.	Tepper and Wood (1985)
0.4 1.2	784 2,352	13 days continuous	Home cage behavior	Mice	During first hour, rearing, grooming, sniffing, and social interactions increased, crossings and wall climbing decreased. These behaviors did not adapt with continued exposure.	Musi et al. (1994)
0.5 2.0	980 3,920	3 h	Lever pressing to avoid electric shock	Rat (Wistar) 300 g	Suppression of lever pressing began after 45 min of 2.0-ppm exposure and after 90 min of 0.5- or 1.0-ppm exposures.	Ichikawa et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

equally sensitive to O_3 exposure when pressing a lever allowed rats to have access to a running wheel. The authors concluded that increased physical activity, either used as the response to obtain reward, or as the reward, was an important behavioral variable in determining sensitivity to O_3 exposure. Ichikawa et al. (1988) demonstrated that behavior (lever pressing) maintained by the avoidance of electric shock, was even less sensitive to O_3 exposure (3 h, 1.0 ppm) than behaviors maintained by food reinforcement, as described above. Furthermore, the animals recovered quickly after O_3 exposure was terminated (60 to 120 min).

In mice exposed to O_3 continuously for 13 days (0.4 to 1.2 ppm), both increases and decreases in measured behaviors were observed (Musi et al., 1994). During the first hour of exposure to 0.8 or 1.2 but not 0.4 ppm O_3, increases in rearing, grooming, sniffing, and social interactions were observed, but locomotion and bar holding declined. With continued exposure (measurements on Days 3, 7, and 10), grooming and rearing were still increased but crossings and wall climbing remained depressed. The affected behaviors did not show adaptation. However, drinking, food consumption, and body weight were initially depressed, but abated with continued exposure, a finding previously reported in mice at O_3 concentrations as low as 0.2 ppm (Umezu et al., 1993).

In summary, the behavioral data indicate that transient changes in behavior occur in rodent models that are dependent on a complex interaction of factors such as (1) the type of behavior being measured, with some behaviors increased and others suppressed; (2) the factors motivating that behavior (differences in reinforcement); and (3) the sensitivity of the particular behavior (e.g., active behaviors are more affected than more sedentary behaviors).

6.3.3 Cardiovascular Effects

Several reports have demonstrated that O_3 exposure causes dramatic effects to the cardiovascular system in the rat (Table 6-23). Uchiyama et al. (1986) initially reported that heart rate (HR) and mean arterial blood pressure (MAP) were decreased by 53 and 29%, respectively, during a 3-h exposure to 1.0 ppm O_3. Arrhythmias, including atrioventricular block and premature atrial contractions, also were observed frequently. The effects appeared to be age- but not sex-dependent, with 11-week-old rats showing a greater response than did 8- or 4-week-old rats. Yokoyama et al. (1989b) showed that recovery from the effects of the 3-h, 1.0-ppm O_3 exposure was not complete by 5 h and that, with three consecutive daily exposures, both the HR and MAP responses were attenuated. Further investigations by the same group of authors (Uchiyama and Yokoyama, 1989) showed that, with exposures to 0.5 ppm O_3 for 6 h, HR and MAP decreased by 32 and 18%, respectively. A 4-week continuous exposure to 0.2 ppm initially resulted in a 12% decrease in HR, but this response was attenuated on Day 2 and was almost eliminated by Day 3. No further effects were observed during the rest of the 4-week exposure period. When these same animals were subsequently challenged with 0.8 ppm O_3 for 1.5 h, they also had an attenuated response when compared to rats that were O_3 naive. Additionally, some rats were instilled intratracheally with elastase to create an animal model of emphysema. This pretreatment, however, did not affect the outcome of either the HR or MAP responses to O_3 in any of the experiments, except in the 0.8-ppm challenge experiment. In this experiment, elastase-treated, O_3-exposed rats challenged with O_3 had a similar response to O_3 challenge as did O_3-naive rats, suggesting that the elastase treatment affected the ability of the rats to develop an adaptive lung response. In contrast, Tepper et al. (1990) did not observe an alteration in

Table 6-23. Effects of Ozone on the Cardiovascular System[a]

Ozone Concentration ppm	Ozone Concentration g/m	Exposure Duration and Conditions	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.1 0.2	196 392	5 days continuous	Rat, M (Wistar) 8 weeks old	0.1 ppm O_3 caused bradyarrhythmia up to 3 days of exposure; broadycardia occurred at 0.2 ppm during first 2 days of exposure. No effects on sleep-wakefulness patterns.	Arito et al. (1990)
0.2 0.5 1.0	392 980 1,960	4 weeks continuous 6 h 3 h	Rat, M (Wistar) 13 weeks old	At 0.2 ppm a 12% decrease in HR; response attenuated by 3 days. At 0.5 ppm, HR and MAP decreased by 32 and 18%, respectively.	Uchiyama and Yokoyama (1989)
0.25 1.0	490 1,960	2 h 18-20 C 30-32 C	Rat, M (F344) 13-16 weeks old	0.37 ppm O_3 caused bradycardia and bradyarrhythmia, ambient temperature of 30-32 C blocked response.	Watkinson et al. (1993)
0.5 1.0	980 1,960	6 h 3 h	Rat, M (Wistar) 10 weeks old	0.5 and 1.0 ppm O_3 caused bradycardia and bradyarrhythmia. 1.0-ppm response was partially blocked by atropine.	Arito et al. (1992)
0.5 1.0	980 1,960	6 h 3 h, 3 days	Rat, M (Wistar) 10-11 weeks old	1.0 ppm O_3 caused bradycardia, bradyarrhythmia, and decreased MAP. The response to 1.0 ppm lasted > 5 h postexposure and was attenuated with 3 consecutive daily exposures.	Yokoyama et al. (1989b)
1.0	1,960	3 h	Rat, M, F (Wistar) 4, 8, and 11 weeks old	1.0 ppm O_3 caused bradycardia, bradyarrhythmia, and decreased MAP. Older animals (11 weeks) were more affected than younger ones. No sex-related differences were noted.	Uchiyama et al. (1986)
1.0	1,960	135 min	Rat, M (F344) 90 days old	Ventilation stimulated with CO_2. No effect on mean blood pressure.	Tepper et al. (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

blood pressure of rats exposed to 1.0 ppm O_3 for 135 min, even though their ventilation was increased by CO_2.

Arito et al. (1990) demonstrated bradycardic responses at 0.2 ppm O_3 during the first 2 days of a continuous 5-day exposure; bradyarrhythmia occurred during the first 3 days of a 0.1-ppm exposure. Simultaneously, these authors measured the sleep/wakefulness of the rats during exposure and found that more bradyarrhythmias occurred during wakefulness than during slow-wave or paradoxical sleep. Sleep/wakefulness patterns were not altered by this O_3 exposure. At high O_3 concentrations (1 ppm for 3 h), wakefulness and paradoxical sleep were suppressed, the amplitude of the electroencephalogram (EEG) was lowered, and slow-wave sleep was increased (Arito et al., 1992). These EEG changes appear to be temporally associated with the decrease in behavioral activity previously discussed (Tepper et al., 1982). Atropine sulfate blocked the suppression of wakefulness and bradycardia in a concentration-related manner and decreased slow-wave sleep, suggesting that some of the O_3 effects are parasympathetically mediated. The effects of O_3 on paradoxical sleep and the EEG amplitude were not affected by atropine administration. Watkinson et al. (1993) extended these findings by showing that the core temperature of rats was also reduced when HR fell at O_3 exposure concentrations between 0.37 and 1.0 ppm (2 h). Increasing ambient temperature to 30 to 32 °C attenuated the 1.0 ppm O_3-induced reduction in HR and core temperature.

In an attempt to synthesize the results from these studies, Watkinson and Gordon (1993) questioned the relevance of these parameters in the rat as compared to the human. Rats have different thermoregulatory responses than humans and typically respond to toxic insult by lowering core temperature. This response has been shown to increase survival value (Watkinson et al., 1989). Similar changes in core temperature and HR have not been reported in humans. This may be because of the large, and thus stable, thermal mass of humans, or, alternatively, these effects have not been observed because they were not measured, and because most O_3 exposure experiments are done using exercise, which may mask these responses. In support of this latter idea, Coleridge et al. (1993) reported that stimulation of bronchial C-fibers produces bradycardia. Ozone preferentially stimulates bronchial C-fibers and, as a result, induces bradycardia and tachypnea in the anesthetized, open-chest dog model. Furthermore, the tachypnea produced by O_3 exposure is inhibited by atropine administration (the effect on HR was not reported).

6.3.4 Hematological and Serum Chemistry Effects

Hematological effects reported in laboratory animals and humans after inhalation of O_3 indicate that the gas or, more likely, some reaction product can cross the blood-gas barrier. The effects of in vivo O_3 exposure in animals were summarized in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). The hematologic parameters most frequently used to evaluate O_3 toxicity were morphologic and biochemical effects on erythrocytes (RBCs). These studies reported alterations in RBC morphology, increased RBC fragility, increased hemolysis, and decreased survival. The biochemical studies reported variable results, depending on the O_3 exposure concentration and the RBC enzyme under investigation.

More recent studies have stressed serum effects of O_3 exposure (Table 6-24). Exposure of rats for 2 h to 0.1 ppm O_3 increased plasma creatinine kinase activity, whereas no such effect was observed when exposure was to 0.05 and 0.25 ppm O_3 (Veninga and

Table 6-24. Hematology and Serum Chemistry Effects[a]

Ozone Concentration ppm	Ozone Concentration g/m³	Exposure Duration	Species, Sex (Strain) Age[b]	Observed Effect(s)	Reference
0.05 0.1 0.25	100 200 500	2 h	Rat, M (Wistar) 200 g	Increased plasma creatine kinase activity at 0.1 but not 0.05 and 0.25 ppm.	Veninga and Fidler (1986)
0.1 0.2 0.4 0.6	196 392 784 1,176	3 h	Rabbit, F (NZW) 2.5-3.5 years old	No change in plasma retinol, ascorbic acid, and -tocopherol concentrations.	Canada et al. (1987)
0.4	784	Continuous for 14 days	Rat, M (Wistar) 20 weeks	Decrease in serum retinol concentration.	Takahashi et al. (1990)
0.8	1,568	18 h	Rat, M (F344)	Decrease in plasma lactic dehydrogenase isoenzyme activity.	Nachtman et al. (1988)
1.0	1,960	1 h	Guinea Pig, M (Hartley) 250-300 g	Increases in plasma concentrations of TXB_2, 6-keto-PGF_{1}, and PGE_1.	Miller et al. (1987)
1.0	1,960	1 h	Guinea Pig, M (Hartley) 250-300 g	Increases in plasma concentrations of TXB_2, 6-keto-PGF_{1}, and PGE_1.	Miller et al. (1988)
1.0	1,960	23 h/day for 2 weeks	Rat, M (CD) 400 ± 25 g	Heat-inactivated plasma increases DNA synthesis by lung fibroblasts and pneumocytes.	Tanswell et al. (1989, 1990) Tanswell (1989)
1.0	1,960	4 h	Mouse, M (CD-1) 8 weeks old	Inhibition of RBC deformability.	Morgan et al. (1988)

[a]See Appendix A for abbreviations and acronyms.
[b]Age or body weight at start of exposure.

Fidler, 1986). Decreased serum retinol concentrations were observed following continuous exposure of rats for 14 days to 0.4 ppm O_3 (Takahashi et al., 1990), but no changes in plasma retinol, ascorbic acid, and α-tocopherol were observed following exposure of rabbits for 3 h to O_3 ranging from 0.1 to 0.6 ppm (Canada et al., 1987). In similar studies, a decrease in plasma lactic dehydrogenase isoenzyme activity also was observed following exposure of rats for 18 h to 0.8 ppm O_3 (Nachtman et al., 1988).

Miller et al. (1987, 1988) investigated the effect of a 1-h exposure of guinea pigs to 1.0 ppm O_3 on plasma eicosanoid levels and observed increases in TXB_2, 6-keto-$PGF_{1\alpha}$, and PGE_1. These data suggest that some of the systemic effects of O_3 exposure, such as impairment of peritoneal AM phagocytosis (Canning et al., 1991), may be mediated by the immunosuppressive effects of the prostanoids (Oropeza-Rendon et al., 1979). Heat-inactivated plasma from rats exposed for 23 h/day for 2 weeks to 1.0 ppm O_3 also increases DNA synthesis by lung fibroblasts (Tanswell et al., 1989) and lung pneumocytes (Tanswell et al., 1990).

6.3.5 Other Systemic Effects

Previous studies suggest that O_3 has effects on the xenobiotic metabolism of the liver (U.S. Environmental Protection Agency, 1986). This effect has been observed in mice, rats, and hamsters as a prolongation of pentobarbital sleeping time (Graham et al., 1981). The effect appears to be sex dependent, with females having greater responses than males. Canada and Calabrese (1985) performed a similar experiment in both young (3- to 4-mo-old) and older (2-year-old) rabbits exposed for 3.75 h/day to 0.3 ppm O_3 for 5 consecutive days. They observed significant prolongation of the elimination of theophylline in older rabbits, but not in young rabbits, and the effect was more pronounced in females than in males. In a follow-up study, Canada et al. (1986) could not demonstrate increased pentobarbital sleeping in young (2.5-mo-old) mice or rats of comparable age to the study by Graham et al. (1981). However, effects were observed in older (18-mo-old) female mice and rats. Two other studies (Heng et al., 1987; Zidenberg-Cherr et al., 1991) from the same group of investigators indicate that liver antioxidant enzymes (Cu/Zn- and Mn-SOD and GSHPx) are decreased commensurate with the increase in these enzymes that is observed in the lung.

6.3.6 Summary

Several reports recently have appeared that extend previous observations in laboratory animals that indicate that ambient levels of O_3 can affect animal behavior. These effects are interpreted as analogous to O_3-induced symptoms in humans, rather than as evidence of neurotoxicity. The behavioral changes are transient but may persist several hours after acute exposure. Different types of behaviors appear to be variably sensitive to O_3 exposure, with active behaviors showing suppression at lower O_3 concentrations than do more sedentary behaviors or behaviors maintained by electric shock (Ichikawa et al., 1988; Tepper et al., 1985; Tepper and Weiss, 1986). For example, a 6-h exposure of rats to 0.12 ppm suppressed running-wheel behavior (Tepper et al., 1985). Furthermore, animals will respond to terminate a 1-min exposure to 0.5 ppm O_3, thus directly implicating the irritant properties of O_3 (Tepper and Wood, 1985). It appears that with additional training, animals can learn to terminate exposure using conditioned stimuli rather than relying directly on the aversive properties of O_3 (Tepper et al., 1985).

Ozone has been found to decrease HR, MAP, and core temperature profoundly in rats (Watkinson et al., 1993; Arito et al., 1990; Uchiyama and Yokoyama, 1989). During exposure, arrhythmias frequently occur. After a 3-h exposure to 1.0 ppm O_3, these effects appear to occur more in adult rats (11 weeks old) than in younger animals (4 and 8 weeks old), especially when the rats were awake (as measured by EEG) during the exposure (Uchiyama et al., 1986). The lowest exposures causing bradycardia in rats was 0.2 ppm for 48 h; 0.1 ppm for 24 h caused bradyarrhythmia (Arito et al., 1990). Similar effects have not been observed in humans or other species. In part, this may be because they have not been systemically examined or that human studies have been carried out during concurrent exercise, which may mask these effects. More likely, these effects represent species differences related to the magnitude and localization of reflex responses and differences in thermal mass.

6.4 Interactions of Ozone with Other Co-occurring Pollutants

6.4.1 Introduction

Most of the toxicological data for O_3 are derived from studies using O_3 alone. However, it is also important to evaluate responses to inhalation of typical pollutant combinations because ambient exposures involve mixtures. Such mixtures provide a basis for toxicological interactions, whereby combinations of chemicals may behave differently than would be expected from consideration of the action of each separate constituent. This section discusses toxicological studies of pollutant mixtures in which O_3 is one component. Discussions of many of these studies addressing the effect of O_3 alone on various organs or systems appear elsewhere in this chapter.

Evaluating the role of O_3 in observed responses to inhaled mixtures is not easy. In spite of the myriad of interpretative difficulties, it is essential to attempt to understand the potential for interactions because O_3 does not exist alone. One of the problems involves definitions of terms, and the study of toxicant interaction is complicated by the dilemma of attempting to characterize the effects from exposure to two or more chemicals. Most studies have employed a statistical definition, but this merely provides a description, and tells nothing about the mechanism of any interaction. But, in many cases, the mechanism of action of the individual components may not be understood fully, and information concerning the types of interactions may provide useful beginnings for studying mechanisms of action of the mixture components. Furthermore, any conclusion of interaction is highly dependent on the specific type of model used. Because the purpose of this chapter is to provide a toxicologic background for effects of O_3 in terms of public health significance, interaction will be defined as a departure from the additivity model (i.e., interaction is considered to occur when the response to a mixture is significantly different from the sum of the responses to the individual components). A less than additive interaction is antagonism, whereas synergism is an interaction that is more than additive. A subclassification of synergism, termed potentiation, is often used to describe an interaction in which response to a mixture is greater than the sum of the responses to the individual components, but where only one component produced a response different from control when administered alone. In many instances, however, potentiation and synergism have been used interchangeably. Although some synergistic interactions actually may serve to stimulate repair processes, or otherwise reduce the harmful

effects of O_3, and some antagonistic interactions eventually may increase the risk of disease development, synergism, as currently used, generally implies greater risk, and antagonism implies lesser risk. However, such assumptions eventually may be proven to be invalid in some instances. Also, interactions with the large number of natural air pollutants, such as microbes, spores, and dusts, that can produce considerable responses alone are not included in this section.

In most cases, the interaction of O_3 with other pollutants has been studied using mixtures that contained only one other copollutant (i.e., simple or binary mixtures). In such studies, the role played by each pollutant in eliciting measured responses can be elucidated with the appropriate experimental design, but most of the database involves exposures to the mixture and O_3 only, with no exposure to the copollutant alone. Although the O_3 concentration may have been varied among exposure groups or was present in one group and not in another (so its relative influence could be assessed to some extent), it cannot be determined in such cases whether the response to the mixture involved actual interaction or was merely additive.

The ambient atmosphere in most environments is generally a mixture of a number of pollutants, and assessing effects of such multicomponent atmospheres may serve to provide some indication of biological responses under conditions that better mimic ambient exposure. However, very few studies have used realistic combinations of pollutant concentrations when assessing interaction.

The ability to discern the contribution of O_3 to observed responses becomes even more difficult when such complex mixtures are studied. Even when binary mixtures are used, they often do not mimic the ambient pattern (e.g., NO_2 levels peak before O_3 levels do) or ambient concentrations (as absolute values or as ratios). Rarely are concentration-response mixture studies performed. This raises the possibility that an unrealistic experimental design may lead to masking the effect of a copollutant or to identifying a response that may not occur in the real world.

Another problem in assessing responses to mixtures involves the statistical basis for the conclusion of significant interaction. For example, a number of studies determined interaction by comparison of the response from exposure to only one component of the mixture with that from exposure to the complete mixture. On the other hand, some studies used statistical approaches specifically designed to indicate interactions. As another example, it may be relatively straightforward to study interactions when one exposure concentration of each of two pollutants is used, but it becomes much more difficult when there are multiple concentrations used, and even more difficult still when more than two pollutants are involved. Because variable criteria for conclusions of interaction have been used, the available database is one in which the statistical significance for determination of interaction varies in terms of its robustness.

6.4.2 Simple (Binary) Mixtures Containing Ozone

Tables 6-25 and 6-26 outline studies performed since publication of the last O_3 criteria document (U.S. Environmental Protection Agency, 1986) in which experimental animals were exposed to atmospheres containing O_3 with only one other copollutant. These tables provide the experimental details for the discussion that follows.

Table 6-25. Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.05 0.04 0.05 0.4	98 75 98 752	O_3 NO_2 O_3 NO_2	NO_2: 24 h/day; O_3: Intermittent during hours 9-19/day following sine curve from 0-0.1 ppm (0.05 avg); total duration: 5-22 mo	Rat, M (Wistar) 7 weeks old	Lung protein content; lung lipid peroxides; antioxidant enzymes (G6PD, 6PGD, GR, GST, GSHPx, SOD)	0 PE	Protein: no change; peroxide: increase between 5 and 9 mo, return to control at >9 mo (greater effect with 0.4 ppm NO_2); enzymes: no change.	Protein: none (no effect of O_3 or NO_2 alone); peroxide: synergism (no change with O_3 or NO_2 alone for 9 mo); enzyme: none (no effect of O_3 or NO_2 alone).	Sagai and Ichinose (1991)
0.05 0.4	98 752	O_3 NO_2	O_3: Concentration ranged from 0 to 0.1 with sine curve over 9-19 h (0.05 avg); NO_2: 24 h/day; both 13 mo, 11 mo recovery	Rat, M (Wistar) 6 weeks old	Development of lung tumors from exposure (ingestion) of carcinogen, *N-bis*(2-hydroxypropyl) nitrosamine prior to O_3 and NO_2	0 PE	Increased tumor incidence (compared to air-exposed control).	Suggested synergism: no increase with O_3 alone (NO_2 alone not done).	Ichinose and Sagai (1992)
0.1 0.5 1.2 4.0 0.1 0.3 1.2 2.5 0.05 0.1 0.5 1.0 0.05 0.1 0.05 0.1	196 980 2,256 7,520 196 588 2,256 4,700 98 196 940 1,880 98 196 94 188	O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak) O_3 (baseline) O_3 (peak) NO_2 (baseline) NO_2 (peak)	15 day, continuous exposure to basal level; peaks: 1 h, twice daily, 5 days/week beginning after 64 h of continuous exposure	Mouse, F (CD-1) 4-6 weeks old	Bacterial infectivity (to *Streptococcus zooepidemicus* given after pollutant exposure)	Bacterial challenge given 0 or 18 h PE	No effect at low level; increased mortality at other levels.	Synergism: at 0.05 O_3 + 0.5 (1.0) NO_2, 0.1 O_3 + 1.2 (2.5) NO_2; marginal synergism at 0.1 O_3 + 1.2 (4) NO_2. Both O_3 and NO_2 increased mortality at two highest levels; only NO_2 increased at two lowest levels.	Graham et al. (1987)
0.1 0.3	196 564	O_3 NO_2	NO_2: 24 h/day; O_3: 8 h/day; 1, 3, 6, 18 mo	Rat, M (F344) 5 weeks old	Histopathology (LM, TEM)	0 PE	Connective tissue edema, Type 2 cell hypertrophy and enlarged lamellar bodies.	Changes more marked than with O_3 alone, NO_2 affected response to O_3 (no quantitation performed).	Terada et al. (1986)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] µg/m	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.15 0.35	294 564	O_3 NO_2	7 h/day, 5 days/week for 12 weeks	Mouse, M (C57Bl/6J) 5 weeks	Colonization of lung by melanoma cells (injected after pollutant exposure)	Inject melanoma cells 0 PE and sacrifice 3 weeks PE	Increase in number of colonies/lung (compared to air control).	Not specified: no change with O_3, but previous study showed effect with NO_2.	Richters (1988)
0.2 4.0	392 7,520	O_3 NO_2	Continuous 1-2 mo	Rat, M (Wistar) 22 weeks old	Pulmonary xenobiotic metabolism, lung protein (homogenate)	0 PE	No effect on protein content, increase in selected enzymes.	Suggested antagonism for xenobiotic enzymes: O_3 induced increase in selected enzymes is lowered by addition of NO_2.	Takahashi and Miura (1989)
0.2 3.6 0.4 7.2 0.6 10.8 0.8 14.4	392 6,768 784 13,536 1,176 20,304 1,568 27,072	O_3 NO_2 O_3 NO_2 O_3 NO_2 O_3 NO_2	24 h/day for 3 days 12 h/day for 3 days 8 h/day for 3 days 6 h/day for 3 days	Rat, M (S-D) 10-12 weeks old	Protein (lavage), lavaged cells (epithelial PMN)	0 PE	Increased protein and cells.	Synergism: protein at 0.6 and 0.8 ppm O_3 mixtures, lavaged cells at 0.4-0.8 ppm O_3; others additive.	Gelzleichter et al. (1992a)
0.2 3.6 0.4 7.2 0.6 10.8	392 6,768 784 13,536 1,176 20,304	O_3 NO_2 O_3 NO_2 O_3 NO_2	6 h/day for 3 days	Rat, M (S-D) 10-12 weeks old	Protein (lavage), lavaged cell counts (epithelial, PMN, AM), DNA content of cell pellet	0 PE	Increased protein and cells, depending on concentration.	Synergism: cell counts at 0.4 ppm O_3 mixture, protein additive.	Gelzleichter et al. (1992b)
0.2 3.6 0.4 7.2 0.6 10.8 0.8 14.4	392 6,768 784 13,536 1,176 20,304 1,568 27,072	O_3 NO_2 O_3 NO_2 O_3 NO_2 O_3 NO_2	24 h/day for 3 days 12 h/day for 3 days 8 h/day for 3 days 6 h/day for 3 days	Rat, M (S-D) 250-275 g	Airway labeling index	4 days PE	Increased index in peripheral airways (TBs opening into ADs) and large airways at three highest doses, increased alveolar index at 0.8 + 14.4 ppm only.	Synergism for peripheral airways at highest dose and large airways at three highest doses only.	Rajini et al. (1993)
0.3 1.2	588 2,256	O_3 NO_2	Continuous for 3 days	Rat, M (S-D) 3 mo old	Lung enzymes (G6PD, 6PGD, ICD, GSHPx, GR, DR, GDT, NADPH-CR)	0 PE	Increased activity.	Synergism: 6PGD, ICD, GR, SOD; additive: GP, DR; others: effect same as O_3 only.	Lee et al. (1990)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] μg/m	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.3 3.0	588 5,640	O_3 NO_2	2 h Nose-only	Rabbit, M (NZW) 4.5 mo old	Pulmonary eicosanoids (lavage) (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2, LTB_4)	0 or 24 h PE	Increases in PGE_2, $PGF_{2\alpha}$, and TXB_2 immediately PE (compared to air control).	Synergism: PGE_2, $PGF_{2\alpha}$; TXB_2 effect similar to O_3 alone.	Schlesinger et al. (1990)
0.3 3.0	588 5,640	O_3 NO_2	2 h/day for 14 days Nose-only	Rabbit, M (NZW) 4.5 mo old	Pulmonary eicosanoids (lavage) (PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2)	0 PE after 7 or 14 exposures, or 24 h PE after 14 exposures	Decrease in PGE_2 (compared to air control) after 7 and 14 days and 24 h PE; 6-keto-$PGF_{1\alpha}$ decreased 24 h postexposure.	None: effects additive or similar to NO_2 alone.	Schlesinger et al. (1991)
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Mouse, M (ICR); Hamster, M (Golden); Rat, M (Wistar); Guinea pig, M (Hartley) all 10 weeks old	Lung lipid peroxides, antioxidant content, phospholipids, and fatty acids	0 PE	Variable increases to no effect, depending on species.	Not determinable: compared mixture vs. air control, no measure of single pollutants performed.	Sagai et al. (1987)
0.4 0.4	784 752	O_3 NO_2	24 h/day for 2 weeks	Mouse, M (ICR); Hamster, M (Golden); Rat, M (Wistar); Guinea pig, M (Hartley) all 10 weeks old	Lung lipid peroxides, antioxidant enzymes, total protein (homogenate)	0 PE	Increases, which were species dependent.	Not determinable: compared mixtures vs. air control, no measure of single pollutants performed.	Ichinose et al. (1988)
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Rat, M (Wistar) 10 weeks old; Guinea pig, M (Hartley) 10 weeks old	Lipid peroxides, lung antioxidants, and antioxidant enzymes	0 PE	Increased peroxides in guinea pig but not rat; increased antioxidants in rat but not guinea pig; enzymes increased or decreased in guinea pigs, increased to no change in rat.	Synergism for some endpoints, additive to no interaction for others; species dependent.	Ichinose and Sagai (1989)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] µg/m	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.4 0.4	784 752	O_3 NO_2	Continuous for 2 weeks	Rat, M (Wistar) 10 weeks old; Guinea pig, M (Hartley) 10 weeks old	Lipid peroxides; lung antioxidants, and antioxidant enzymes	0 PE	Increased peroxides in guinea pig but not rat; increased antioxidants in rat but not guinea pig; enzymes increased or decreased in guinea pigs, increased to no change in rat.	Synergism for some endpoints, additive to no interaction for others; species dependent.	Ichinose and Sagai (1989)
0.45 4.8	882 7,520	O_3 NO_2	8 h/day for 7 days	Mouse, M (Swiss Webster) 2 mo old	Lung protein, DNA, sulfhydryl and nonsulfhydryl content; GR, GST, G6PD, 6PGD, ICD activities	0 PE	No change in protein or DNA; increase in activity of ICD, G6PD, 6PGD.	Synergism: enzyme activity.	Mustafa et al. (1985)
0.6 2.5 0.35 0.6	1,176 4,700 686 1,128	O_3 NO_2 O_3 NO_2	4 h (rest) 3 h (exercise)	Rat, M (S-D) 7 weeks old	Parenchymal histopathology	2 days PE	Increased focal lesions.	Synergism: ascribed to production of HNO_3 in exposure atmosphere.	Mautz et al. (1988)
0.6 2.5	1,176 4,700	O_3 NO_2	2 h (rest and exercise)	Rat, M (S-D) 47-52 days old	Epithelial permeability (tracheal, bronchoalveolar) (measured 1 and 24 h PE)	0, 1, 2 days PE	Rest: increased bronchoalveolar permeability at 1 and 24 h PE; exercise: increased bronchoalveolar permeability at 1 and 24 h PE (effects greater than with O_3 alone, no effect of NO_2).	Enhanced magnitude and duration of response (suggested potentiation).	Bhalla et al. (1987)
0.8 4.0	1,568 7,520	O_3 NO_2	Continuous for 3-56 days	Mouse, M (BALB/c) 8-10 weeks old	Antibody response to T-cell dependent and independent antigens in spleen	0 PE	Inconsistent pattern of increases and decreases of lung weight, thymus weight, or plaque formation.	Most responses similar to O_3 only; mixture affected some time points not affected by O_3 alone: implied nonadditive interaction, but specifics not determinable.	Fujimaki (1989)
0.8 14.4	1,568 27,072	O_3 NO_2	6 h/day for 3 days (concurrent) or sequential O_3 pre-NO_2; NO_2 pre-O_3; 6 h each	Rat, M (S-D) 10-12 weeks old	Protein (lavage); lavaged cell counts (epithelial, PMN, AM), DNA content of cell pellet	0 PE	Increased protein for concurrent or sequential, increase in cell counts for concurrent.	Synergism: protein and cell counts for concurrent, protein additive or antagonistic for sequential.	Gelzleichter et al. (1992b)

Table 6-25 (cont'd). Toxicological Interactions of Ozone and Nitrogen Dioxide[a]

Concentration[b] ppm	Concentration[b] µg/m	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.8 14.4	1,568 27,072	O_3 NO_2	6 h/day for 45-79 days	Rat, M (S-D) 10/12 weeks old	Various biochemical and histological endpoints	0 PE	Increased lung DNA, protein, collagen, elastin; some deaths with mixture only at 55 days; decreased hydroxypyridinium.	Suggested synergism for hydroxyproline and hydroxypyridinium.	Last et al. (1993b)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]Age or body weight at start of exposure.

Table 6-26. Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] μg/m	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.1	196 125	O_3 H_2SO_4 (0.3 μm)	2 h/day, 5 days/week for up to 1 year Nose-only	Rabbit, M (NZW) 4.5 mo old	Tracheobronchial mucociliary transport, bronchial tree epithelial secretory cell numbers	Mucociliary transport during exposure; secretory cells 3 days after 4, 8, and 12 mo of exposure	Normal to accelerated clearance, increase in secretory cell numbers at early time points (4-mo exposure).	Clearance: No interaction; secretory cell numbers: synergism at 4 mo, antagonism at 8 and 12 mo.	Schlesinger et al. (1992a)
0.1 0.3 0.6	196 588 1,176 50 75 125	O_3 O_3 O_3 H_2SO_4 (0.3 μm) H_2SO_4 (0.3 μm) H_2SO_4 (0.3 μm)	3 h Nose-only	Rabbit, M (NZW) 4.5 mo old	Lavage cell counts; lavage LDH, PGE_2, $PGF_{2\alpha}$; AM phagocytosis; superoxide production; TNF activity	0 PE	No effects on lavage cell counts or LDH, PGE_2, $PGF_{2\alpha}$ or increase or decrease in TNF and phagocytosis depending on exposure concentration; no change in superoxide.	Antagonism: Phagocytosis, at all combinations; antagonism: Superoxide at 0.1 and 0.3 ppm O_3 and 75 and 125 μg/m³ H_2SO_4; synergism: TNF at 125 μg/m³ H_2SO_4 and 0.3 and 0.6 ppm O_3.	Schlesinger et al. (1992b)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	6 h for 7 days (23.5 h/day)	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase (compared to air control).	Synergism at ≥100 μg/m³ H_2SO_4 and 0.2 ppm O_3 for 3 days.	Warren and Last (1987)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	23.5 h/day for 5-9 days	Rat, M (S-D) 250-300 g	Lung tissue protein	0 PE	Increase (compared to air control).	Synergism at 1,000 μg/m³ H_2SO_4 and 0.64 ppm O_3, ≥100 μg/m³ H_2SO_4 and 0.20 ppm O_3.	Warren and Last (1987)
0.12-0.64	235-1,254 40-1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase (compared to air control).	Synergism at ≥200 μg/m³ H_2SO_4 and 0.64 ppm O_3, ≥500 μg/m³ H_2SO_4 and 0.2 ppm O_3 (with suggestion at < 500 μg/m³ H_2SO_4).	Warren and Last (1987)
0.15	294 300	O_3 H_2SO_4 (0.09 μm)	1 h to H_2SO_4, 2 h rest, then 1 h to O_3 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Acid-induced decrease in DL_{CO} not affected by O_3.	None: O_3 did not alter acid effect.	Chen et al. (1991)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] μg/m³	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.15	294 84	O_3 H_2SO_4 (layered on ZnO)	1 h to H_2SO_4, 2 h rest, then 1 h to O_3 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Greater decrease in DL_{CO}, VC after O_3; no change in alveolar volume, TLC with mixture.	Suggested synergism (greater than additive) for DL_{CO}, but not VC.	Chen et al. (1991)
0.15	294 24	O_3 H_2SO_4 (layered on ZnO)	H_2SO_4 3 h/day for 7 days, O_3 on Day 9 Head-only (acid), whole-body (O_3)	Guinea pig, M (Hartley) 260-325 g	Pulmonary function	0 PE	Decrease in TLC, VC, DL_{CO} enhanced by O_3.	Suggested synergism (greater than additive).	Chen et al. (1991)
0.2	392 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase.	Possibly synergism: Effect different from O_3 alone.	Warren et al. (1988)
0.2	392 1,000	O_3 H_2SO_4	15 or 30 days	Rat, M (S-D) 250-300 g	Lung protein content	0 PE	Increase only at 15 days.	Suggested synergism.	Last (1991b)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Protein content (lavage)	0 PE	Increase.	None: Effect same as O_3 alone.	Warren et al. (1988)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Proximal acinar lesion volume	0 PE	Increase.	None: Effect same as O_3 alone.	Warren et al. (1988)
0.64	1,254 1,000	O_3 H_2SO_4	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein and free proline content	0 PE	Increase with H_2SO_4 and O_3 only.	Synergism.	Last et al. (1986)
0.8	1,568 1,200	O_3 H_2SO_4 (0.63 μm)	O_3 for 2 h, followed by H_2SO_4 for 1 h	Guinea pig, F, M (Hartley) 1.5-2 mo old	Airway constriction (measured by trapped gas volume)	0 PE	Increase compared to air.	No interaction: Effect same as O_3 alone; H_2SO_4 had no effect.	Silbaugh and Mauderly (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Rate of collagen synthesis	0 PE	Increase.	Synergism: Effect greater than O_3; sulfate had no effect.	Warren et al. (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 2 days	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase.	Synergism: Effect greater than O_3; sulfate had no effect.	Warren et al. (1986)
0.2	392 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 3 days	Rat, M (S-D) 250-300 g	Lavageable protein	0 PE	Increase.	No interaction: Effect same as O_3 alone.	Warren et al. (1986)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] μg/m³	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.2- 0.64	392- 1,254 5	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 250-300 g	Lung DNA content; tissue protein content; lavage LDH, acid phosphatase, N-acetyl-β-D-glucosaminidase	0 PE	No change in DNA, increase in tissue protein, increases in all enzyme levels.	No interaction: Effect on protein and enzymes same as O_3 alone.	Warren et al. (1986)
0.64	1,254 1,000	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein and free proline content	0 PE	No change.	None.	Last et al. (1986)
0.96	1,882 5,000	O_3 $(NH_4)_2SO_4$	23.5 h/day for 7 days	Rat, M (S-D) 225-275 g	Lung protein content, proline content, apparent collagen synthesis rate, fibroblast numbers in lesions, lesion volume	0 PE	Increase.	Synergism: Effect greater than O_3 alone; H_2SO_4 had no effect (previous study).	Last et al. (1986)
0.3 3.0	588 7,860	O_3 SO_2	5 h/day for 3 days Head-only	Sheep, F Adult (31 kg)	Tracheal mucus velocity	0 and 24 h PE	Decrease in velocity (compared to air control).	Not determinable: no measure of single pollutants performed.	Abraham et al. (1986)
0.15 0.1	294 250	O_3 HNO_3	4 h/day for 4 days Nose-only	Rat, M (F344) 250 g	Lavage cell population; protein (lavage); AM respiratory burst, LTC_4; elastase inhibitory capacity (lavage)	18 h PE	No change in any endpoint (compared to air control).	None.	Nadziejko et al. (1992)
0.6 0.4	1,176 1,000	O_3 HNO_3	4 h Nose-only	Rat, M (F344) 250 g	Lavage cell population; protein (lavage); AM respiratory burst, LTC_4; elastase inhibitory capacity (lavage)	18 h PE	Increased protein, PMN number, elastase inhibiting capacity; no effect on other endpoints (compared to air control).	Less than additive for protein, PMN number, elastase inhibitory capacity; no interaction for other endpoints.	Nadziejko et al. (1992)
0.4	784 380	O_3 HMSA[d] (0.32 μm)	4 h Nose-only	Rat, M (S-D) 7 weeks old	Breathing pattern, fatty acid composition of surfactant, nasal epithelium and parenchymal lesions, lavage protein (24-48 h PE)	Pulmonary function during exposure, others 23 h PE	Rapid breathing, increased protein, decreased fatty acid content, focal lesions with thickened alveolar septa and cellular infiltration in parenchyma.	None: Effect similar to O_3 alone.	Mautz et al. (1991)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] μg/m³	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.2 0.4 0.8 1.0	392 784 1,568 1,230	O_3 O_3 O_3 HCHO	22 h/day for 3 days	Rat, M (Wistar) 150-190 g	Nasal epithelial cell turnover, histopathology (LM)	Thymidine administered 2 h PE, sacrifice 4 h PE	Increased turnover (but some conditions produced decrease due to change in ventilation).	Synergism for turnover with 0.4 ppm O_3 and 1-3 ppm HCHO, depending on anatomical site (greater than the sum of individual responses to O_3 and HCHO); microscopic lesions similar to O_3 and/or HCHO alone.	Reuzel et al. (1990)
0.4 0.3 1.1 3.3	784 369 1,353 4,059	O_3 HCHO HCHO HCHO	22 h/day for 3 days						
0.6 10	1,176 12,300	O_3 HCHO	3 h (rest)	Rat, M (S-D) 7 weeks old	Nasal epithelial cell turnover, parenchymal histopathology	2 days PE	Increased focal parenchymal lesions with exercise, but no effect at rest; increased nasal cell turnover at rest or exercise.	Parenchyma: Synergism with exercise, antagonism at rest. Nasal: Synergism.	Mautz et al. (1988)
0.6 10	1,176 12,300	O_3 HCHO	3 h (exercise)						
1.0 1.0	1,960 1,960	O_3 Cigarette smoke O_3 Cigarette smoke	O_3: 0.5 h, then 5 puffs smoke (sequential) O_3: 1.5 h, then 10 puffs smoke (sequential) O_3: chamber, smoke: head-only	Guinea pig, F (Hartley) 350-400 g	Airway responsivity (to metacholine challenge), tracheal vascular permeability, 0-24 h PE	0, 5, or 24 h PE	Increased responsivity and permeability immediately PE at both doses (magnitude, but not duration of effect).	Suggested synergism: No effect of O_3 or smoke alone at low dose; high O_3 increased responsivity and permeability; high smoke increased responsivity.	Nishikawa et al. (1992)
0.8	1,568 2,000- 50,000	O_3 Silica (instilled)	Silica instilled on Day 1 followed by O_3 for 6 h/day, 5 days/week for 37 days beginning Day 4	Rat, M (S-D) 17 weeks old	Pulmonary fibrosis	24 h PE (last O)	No change in lung DNA, protein, or hydroxyproline content; increase in ratio of hydroxyproline to DNA, protein, or wet weight (compared to air control) at 50,000 μg.	None: No biological significance.	Shiotsuka et al. (1986)

Table 6-26 (cont'd). Toxicological Interactions to Binary Mixtures of Ozone with Acids and Other Pollutants[a]

Concentration[b] ppm	Concentration[b] g/m³	Pollutant	Exposure Duration	Species, Sex (Strain) Age[c]	Endpoints	Time of Endpoint Measurement	Response to Mixture	Interaction	Reference
0.8 1.5 -	1,568 2,940 10,000	O_3 O_3 Carbon black	4 h	Mouse, F (Swiss) 20-23 g	Cell counts in lavage; AM phagocytosis	20 h PE	Increase PMN counts compared to O_3 alone; greater depression of phagocytosis than O_3 alone.	At 0.8 ppm: No intereaction; at 1.5 ppm: Suggested synergism.	Jakab and Hemenway (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]Age or body weight at start of exposure.
[d]HMSA = hydroxymethanesulfonate.

6.4.2.1 Nitrogen Dioxide as Copollutant

The most commonly studied copollutant in binary mixtures with O_3 is NO_2. Studies discussed in the previous O_3 criteria document indicated that, although interaction may occur between these two pollutants, in general, O_3 often masked the effects of the NO_2 or accounted for most of the response. This is because, on a mole-to-mole basis, O_3 is considerably more toxic than NO_2, and the relative contribution of O_3 and NO_2 to pulmonary injury is driven by the exposure ratio of the two pollutants. Commonly studied endpoints for assessing effects of these mixtures were lung morphology, biochemistry, and resistance to bacterial infection.

To put the exposure concentrations of NO_2 into some perspective, short-term, 24-h averages are generally 0.17 ppm, and 1-h averages are generally 0.4 ppm in major metropolitan areas. However, hourly averages in most regions often exceed 0.2 ppm at least once during the year (Schlesinger, 1992).

An earlier study noted that the morphological response of the rat lung alveolar epithelium following 60 days of exposure to O_3/NO_2 mixtures (0.25 ppm O_3 + 2.5 ppm NO_2, or 0.9 ppm O_3 + 0.9 ppm NO_2) was due to the O_3 (Freeman et al., 1974). However, the duration of exposure may affect the contributory role of the copollutant. Thus, for example, Terada et al. (1986) exposed rats to O_3 alone, or to a mixture of 0.1 ppm O_3 + 0.3 ppm NO_2, with O_3 administered 8 h/day and NO_2 administered 24 h/day for up to 18 mo. Following 1 mo of exposure, observed lung lesions in the group exposed to the mixture were similar in severity to those noted with exposure to O_3 alone, but as the duration of exposure increased, the morphological changes in interstitial tissue appeared more marked in those animals exposed to the mixture. Edema of pulmonary connective tissue was more pronounced and alveolar Type 2 cells became swollen. Although this study was not quantitative, qualitative observations led the authors to conclude that the lesions were not due to O_3 alone but that NO_2 played some, albeit undefined, contributory role.

The effect of exposure duration on interaction was also noted in studies of Schlesinger et al. (1990, 1991). Rabbits exposed to 0.3 ppm O_3 + 3.0 ppm NO_2 for 2 h showed synergistic increases in certain BAL eicosanoids obtained immediately after exposure, whereas animals exposed to the same mixture for 2 h/day for 14 days showed no interaction for the same parameters.

A number of studies examined other biochemical responses to O_3/NO_2 mixtures (e.g., sulfhydryl metabolism and the activity of certain enzymes). Some of the studies discussed in the previous O_3 criteria document were found to involve synergism (e.g., Mustafa et al., 1984). More recent studies of lung biochemistry also suggest that O_3 and NO_2 interact synergistically. Ichinose and Sagai (1989) exposed rats and guinea pigs to 0.4 ppm O_3, 0.4 ppm NO_2, or a mixture of the two pollutants continuously for 2 weeks. No change in lung peroxide production was observed in rats, but the mixture synergistically increased peroxide levels in guinea pigs. The guinea pigs showed no change in lung antioxidant content following any exposure, whereas the mixture synergistically increased antioxidant levels in rat lung. The conclusion of a significant interaction was based on relative changes from air controls following exposure to the mixture, compared to changes following exposure to each pollutant alone, using the t-test. Synergism was defined as a change greater than the sum of the responses to individual pollutants; no specific test for interaction was performed.

Ichinose and Sagai (1989) also noted that levels of antioxidant enzymes in rat or guinea pig lungs were variously affected by exposure to the above mixture. For example, GST was decreased in both species exposed only to O_3, but the mixture produced a reduction of this

enzyme in guinea pigs and no change in rats. Thus, the occurrence of interaction was dependent on endpoint as well as species. This latter finding likely reflected interspecies differences in biochemical defenses against oxidant pollutants, given the results of a study by Sagai et al. (1987) with four animal species. This study suggested that observed species differences in lipid peroxide formation following exposure were related to the relative content of antioxidants and the specific composition of phospholipids and their fatty acids. The guinea pig was the most sensitive animal, and the hamster was the most resistant.

The effects of exposures to O_3/NO_2 mixtures on lung lipid peroxides and antioxidant activity have been examined in a number of other studies (Ichinose et al., 1988; Lee et al., 1990; Sagai and Ichinose, 1991), and the results generally confirm that noted above (i.e., such mixtures tend to produce synergistic interaction). However, there is also some evidence for antagonism. Takahashi and Miura (1989) examined effects on the pulmonary xenobiotic system of rats exposed for 1 or 2 mo to a mixture of 0.2 ppm O_3 + 4.0 ppm NO_2, as well as to each pollutant alone. Ozone induced an increase in lung cytochrome P-450 content, but the activity of these enzymes was reduced by the addition of NO_2 to the exposure atmosphere; that is, the mixture resulted in levels intermediate between those found with O_3 or NO_2 alone. However, the reduction in enzyme activity induced by NO_2 was restricted to those enzymes that had been increased by exposure to O_3 alone. The authors suggested that antagonism was due to the production of undefined secondary reaction products in the exposure atmosphere. A similar explanation was proposed to explain observed synergism of lung antioxidant activity in another study (Lee et al., 1990). Thus, the response to any secondary product likely depends on the endpoint examined, assuming that the same reaction products were formed in these two studies.

The role of exposure parameters in producing an interaction between simultaneously inhaled O_3 and NO_2 was examined by Gelzleichter et al. (1992a). Rats were exposed to various combinations of O_3 and NO_2 for various durations (6, 8, 12, and 24 h), such that the C × T products were identical for each of four exposure sets. As indicated in Table 6-24, as the exposure duration increased, the exposure concentration of each component of the mixture decreased. Lavaged protein levels and recovered cells were the endpoints. For each exposure combination, the additive response was predicted from the results of exposure to each pollutant alone, and then synergism was indicated when there was deviation from additivity. Responses to exposure to either O_3 or NO_2 alone for 6, 8, or 12 h showed that the product of C × T was a constant for the observed biological effects. However, less severe changes occurred when delivery was at the lowest dose rate (i.e., when the lowest concentration of each pollutant was delivered over the 24-h exposure duration). Exposure at higher dose rates (i.e., 6 to 12 h) increased the magnitude of the response. Thus, the degree of response to each pollutant alone was not a constant function of C × T throughout the entire range of dose rates, but was concentration driven, and was not identical at the highest and lowest rates. Responses following exposure to the mixture did not follow C × T, even over the range of dose rates in which C × T was constant following exposure to the pollutants individually. Thus, interaction, in this case, synergism, appeared to be concentration dependent, in that the response was disproportionately greater at the higher concentrations (higher dose rates) of the constituent pollutants in the mixture. The response following exposure to the mixtures appeared to be a function of peak concentration, rather than of cumulative dose. More recently, Rajini et al. (1993) noted that analysis of all kinetics following similar exposure to mixtures did not reflect a C × T relationship.

All of the studies described above involved simultaneous exposure to O_3 and NO_2. However, ambient exposure to these pollutants has temporal patterns, and exposure to one agent may alter the response to another, subsequently inhaled agent. The realism of these studies is somewhat dependent on their relationship to actual temporal patterns of pollutants in ambient air (i.e., whether one material is the precursor of the other, as is the case for O_3 and NO_2). As described in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), Fukase et al. (1978) exposed mice for 7 days to 3 to 15 ppm NO_2 for 3 h/day, followed by 1 ppm O_3 for 3 h/day, and noted an additive effect on the level of lung GSH.

Yokoyama et al. (1980) exposed rats to 5 ppm NO_2 or 1 ppm O_3 for 3 h/day, or to NO_2 for 3 h followed by O_3 for 3 h/day, for various total durations up to 30 days, and assessed lung mechanics in postmortem lungs, lung histology, and enzyme activity in subcellular fractions of lung tissue. The activity of phospholipase A2 in the mitochondrial fraction was increased in those animals exposed to O_3 only or to O_3 after NO_2, and the response in the latter was significantly greater than that in the former. A decrease in activity of lysolecithin acyltransferase in the supernatent fraction was found only in those animals exposed to both NO_2 and O_3. Pulmonary mechanics showed a change in pulmonary resistance (as a function of elastic recoil pressure) in the O_3- and NO_2/O_3-exposed animals. Histologically, the lungs of the animals exposed to both NO_2 and O_3 appeared similar to those exposed to O_3 alone; however, a slight degree of epithelial necrosis in medium bronchi, not found with either NO_2 or O_3 alone, was seen in the animals exposed to both pollutants. In addition, damage at the bronchoalveolar junction appeared to be somewhat more marked in animals exposed to both gases than in those exposed to O_3 alone. This study suggested that sequential exposures produced responses that, in most cases, did not differ greatly from those due to O_3 alone.

Aside from sequential exposures, simulation of ambient exposure scenarios involving NO_2 and O_3 has been performed by examining the effects of a continuous baseline exposure to one concentration of both pollutants, with superimposed short-term peaks to a higher level of one or both gases. The endpoint generally examined in this regard has been bacterial resistance. Studies reported in the previous criteria document (e.g., Ehrlich et al., 1979; Ehrlich, 1983) in which mice were exposed to O_3 under various scenarios of baseline concentrations of NO_2 on which were superimposed daily peak exposures to NO_2 or a combination of NO_2 and O_3 suggested that exposure with peaks can enhance response to pollutant mixtures, and that the sequence of peak exposures was important in producing reduced resistance to infection that was different from that due to exposure to the baseline concentration only.

As a comparison, toxicologic interactions for infectivity involving simultaneous exposure to NO_2 and O_3 discussed in the previous O_3 criteria document were found generally to be additive following acute exposures, with each pollutant contributing to the observed response when its concentration reached the threshold at which the gas would have affected bacterial resistance when administered alone (Goldstein et al., 1974). If the exposure level of either NO_2 or O_3 was below this threshold, then the response was due solely to the constituent inhaled at the more toxic concentration (Ehrlich et al., 1977).

More recently, Graham et al. (1987) examined resistance to respiratory infection (as measured by bacterial-induced mortality) in mice continuously exposed (for 15 days, 24 h/day) to baseline levels of an NO_2/O_3 mixture with two daily, 1-h peaks of the mixture at very high, high, intermediate, and low exposure concentrations (see Table 6-25 for concentrations). Animals were also exposed to the same baseline levels of either NO_2 or

O_3 onto which were superimposed two daily, 1-h peaks of the same single gas in concentrations as above. At the low concentration, only NO_2 increased mortality. At the intermediate exposure level, the mixture was synergistic; NO_2 alone increased mortality and O_3 had no effect. At the high exposure level, the combined exposure was again synergistic; exposure to each gas separately increased mortality. A similar effect was seen at the very high level, although the combined exposure just missed statistical significance for synergism. These results are consistent with those of the earlier studies reported in the previous O_3 criteria document and support the conclusion that response depends on the specific exposure pattern. The results of Graham et al. (1987) are also consistent with those from the earlier studies with simultaneous exposures.

The relationship between exposure and response is very complex and seems to depend on exposure duration, the ratio of O_3 and NO_2 concentrations, and other factors that may include the production of secondary reaction products within the exposure atmosphere. This complexity was highlighted by the study of Gelzleichter et al. (1992b), who examined effects of combined or sequential exposures of rats to mixtures of O_3 and NO_2 at various concentrations ranging from 0.2 to 0.8 ppm O_3 and 3.6 to 14.4 ppm NO_2. Sequential exposures consisted of 6 h of O_3 at night, followed by 6 h of NO_2 during the day, or vice versa; concurrent exposures were for 6 h/day for 3 days. Various endpoints were examined, and it was noted that sequential and concurrent exposures did not result in the same response. Thus, lavage protein levels were increased additively with sequential exposure (in any pollutant order) but were found to be greater than additive with concurrent exposure. An increase in the number of lavaged epithelial cells was additive for the O_3 night/NO_2 day sequence, antagonistic for the NO_2 night/O_3 day sequence, and additive for concurrent exposure. An increase in the number of lavaged PMNs was additive for both sequential conditions and was synergistic for concurrent exposure. It was concluded that production of synergism depended on the concentration of each pollutant within the mixture, and additivity would result for any endpoint when the concentration of each component of the mixture fell below a certain threshold level. However, these threshold concentrations were endpoint specific, with some endpoints being more sensitive than others; it was speculated that the least sensitive assays were based on changes that were reversible, whereas the most sensitive ones were irreversible. The authors also noted that the extent of chemical reaction within the O_3/NO_2 mixture atmosphere was related to the extent of toxicological interaction, suggesting that interaction was due to the production of some secondary reaction product, which, in this case, was suggested to be nitrogen pentoxide. This particular chemical also had been suggested in earlier studies to be responsible for interactions following exposure to NO_2/O_3 atmospheres (e.g., Diggle and Gage, 1955).

The concentrations at which synergism occurred in the study of Gelzleichter et al. (1992b) discussed above (0.4 ppm O_3 and 7.2 ppm NO_2) were higher than those that generally are found in ambient air. However, the threshold concentration for interaction was dependent on exposure dose rate, with higher rates leading to lower threshold concentrations for synergism.

Although interaction is clearly modulated by environmental exposure factors, such as concentration, duration of exposure, or specific exposure regime, host factors also may play a role. Mautz et al. (1988) examined the effect of exercise on rats exposed to mixtures of O_3 and NO_2. Exercise modified the toxic interactions of combined pollutants, resulting in synergistic interaction occurring at lower exposure concentrations of the constituent pollutants

than with exposure at rest. Thus, a similar magnitude of response, an increase in the extent of focal lesions in lung parenchyma, was noted 2 days following a 4-h exposure to 0.6 ppm O_3 + 2.5 ppm NO_2 at rest or with a shorter (3-h) exposure to lower concentrations, 0.35 ppm O_3 + 0.6 ppm NO_2, with exercise. In both cases, the response was different from that due to either pollutant given alone. Furthermore, a greater response was noted with a 3-h exposure to 0.6 ppm O_3 + 2.5 ppm NO_2 with exercise than to the same mixture for the same exposure duration at rest. Thus, exercise also increased the response at similar concentrations compared to rest. The effect of exercise was ascribed to an increase in delivered dose or dose rate, due to increased $\dot{V}_E$. The ability of exercise to enhance response to a pollutant mixture also was noted by Bhalla et al. (1987).

The study of Mautz et al. (1988) above also provided further evidence suggesting that chemical reactions within the exposure atmosphere may play some role in toxicologic interaction. In this case, nitric acid (HNO_3) vapor was noted at concentrations ranging from 0.02 to 0.73 ppm, depending on the concentrations of the primary constituents. As discussed below, acids have been found to interact with O_3. This study also found interaction to occur at a concentration of one of the components, NO_2, that had no effect when administered alone. Although this appears to contrast with the conclusions of Graham et al. (1987) above, the endpoints in these two studies were quite different.

Another aspect of pollutant interaction involves the ability of O_3/NO_2 mixtures to affect the course of other lung changes (e.g., malignant tumor colonization). Richters (1988) exposed mice to a mixture of 0.15 ppm O_3 + 0.35 ppm NO_2 for 7 h/day, 5 days/week for 12 weeks, following which the mice were injected (iv) with viable melanoma cells. The mice were sacrificed 3 weeks later, and the lungs were examined for melanoma colonies. Although exposure to O_3 alone produced no change in the percentage of animals with colonies or in the average number of colonies per lung (compared to air control), exposure to the mixture produced an increase in the latter, suggesting to the authors that the mixture faciliated cancer cell colonization. However, the exact role played by O_3 in the mixture is not clear because a previous study had indicated that NO_2 alone facilitates blood-borne cancer cell spread to the lungs (Richters and Kuraitis, 1981). Furthermore, the experimental model used is not generally accepted as representing metastatic mechanisms.

Ichinose and Sagai (1992) also examined the ability of an O_3/NO_2 mixture to promote primary lung tumor development. Rats were injected (ip) with BHPN and then were exposed for 13 mo to a mixture of 0.05 ppm O_3 + 0.4 ppm NO_2, O_3 alone, or to clean air (chamber control). Although the NO_2 exposure was continuous, the O_3 exposure was intermittent, with the concentration altered between 0 and 0.1 ppm following a sine curve from 9 to 19 h of each day (resulting in a daily mean concentration of 0.05 ppm). One other group of rats served as a room control, maintained in a clean room for 24 mo following injection of BHPN. After an 11-mo recovery period, all animals were autopsied. Compared to clean-air-exposed animals, lung tumor incidence was increased in mice exposed to the mixture; O_3 alone did not increase the tumor incidence. However, the authors noted that tumor incidence in the room control group was not different from that in the group exposed to the mixture, and suggested that the clean air (chamber control) group should be used as a control in interpreting the data from the pollutant-exposed animals. The enhanced incidence in mixture-exposed animals was suggested to be due to synergistic increases in lipid peroxidation, which was noted in other studies (see Table 6-25). A complication in interpreting this study is that a previous study (Sagai and Ichinose, 1991) had suggested tumor development in animals exposed to an

O_3 and NO_2 mixture without BHPN, although this latter study involved a longer exposure duration and somewhat higher pollutant concentrations.

6.4.2.2 Acidic Compounds as Copollutants

Binary mixtures containing acids comprise another type of commonly examined exposure atmosphere. Most of these mixtures included acidic sulfate aerosols as the copollutant. Peak (1-h) ambient levels of sulfuric acid (H_2SO_4) are estimated at 75 $\mu g/m^3$, with longer (12-h) averages about one-third of this concentration (Spengler et al., 1989).

Earlier studies that employed simultaneous single, repeated, or continuous exposures of various animal species to mixtures of acid sulfates and O_3 found responses for several endpoints, including tracheobronchial mucociliary clearance, alveolar clearance, pulmonary mechanics, and lung morphology, to be due solely to O_3 (U.S. Environmental Protection Agency, 1986; Cavender et al., 1977; Moore and Schwartz, 1981; Phalen et al., 1980; Juhos et al., 1978). However, synergism was noted for bacterial infectivity in mice (Grose et al., 1982), for response to antigen in mice (Osebold et al., 1980), and for effects on lung protein content and the rate of collagen synthesis in rats (Last et al., 1983, 1984a; Last and Cross, 1978). More recent studies, performed since publication of the previous O_3 criteria document, support the earlier finding of synergism between O_3 and acid sulfates on lung biochemistry, and provide possible explanations for underlying mechanisms.

Last et al. (1986) exposed rats for 7 days to O_3 alone (at 0.96 ppm) and to mixtures of O_3 with one of three aerosols, sodium chloride, sodium sulfate, or ammonium sulfate [$(NH_4)_2SO_4$] (all at 5 mg/m^3); only the $(NH_4)_2SO_4$ was acidic. Lung protein content, proline content, collagen synthesis rate, fibroblast numbers in parechymal lesions, and the volume of parenchymal lesions were examined following exposure. Mixtures of O_3 with sodium chloride or sodium sulfate produced changes that did not differ from those found with O_3 alone. On the other hand, mixtures of $(NH_4)_2SO_4$ with O_3 resulted in increases in all of the measured parameters, and the increases were greater in magnitude than those due to O_3 alone; synergism was concluded, although there has been some question concerning the statistical approach used (Last, 1991a). These results suggested that acidity was necessary for synergism of the aerosols with O_3. This conclusion was further supported by demonstrating that significant interaction of O_3 with H_2SO_4, which is much more acidic than $(NH_4)_2SO_4$, occurred at lower concentrations than was noted for mixtures of O_3 and $(NH_4)_2SO_4$ (Warren and Last, 1987); interaction was suggested with H_2SO_4 concentrations as low as 40 $\mu g/m^3$ (with 0.2 ppm O_3) for some lung biochemical endpoints. The studies above did not use any specific statistical test for interaction, and conclusions of interaction were based on findings of significant differences from responses following exposure to O_3 alone and the absence of detectable responses following exposure to H_2SO_4 aerosols at the same and higher concentrations.

Warren et al. (1986) found synergistic interaction with the above endpoints following 7 days of exposure to 0.2 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$. However, exposure for only 3 days produced responses that were not different from those noted with O_3 alone. This seems to indicate that the duration of exposure is a factor affecting the occurrence of any interaction. However, exposure duration may also affect the type of interaction. In a study by Schlesinger et al. (1992a) in which rabbits were exposed to a mixture of 0.1 ppm O_3 + 125 $\mu g/m^3$ H_2SO_4 for 2 h/day, 5 days/week, a synergistic increase in bronchial epithelial secretory cell number was noted after 4 mo of exposure, whereas antagonism was noted following 8 mo of continued exposure.

The mechanism underlying interaction between acid sulfates and O_3 is not known. Last et al. (1986) noted that similar sites of deposition for O_3 and acid aerosols favored synergism. A synergistic response of biochemical indices in rat lung with exposure to 1,000 $\mu g/m^3$ H_2SO_4 + 0.6 ppm O_3 was found when the acid droplet diameter was 0.5 μm, whereas no increase compared to the O_3-only response was noted when the droplet diameter was 0.02 μm. Apparently, the larger particles that deposited to a greater extent within the bronchoalveolar junction, the major target site for O_3, were most interactive.

Observed synergism between O_3 and acid sulfates in rats also was suggested to be due to a shift in the local microenvironmental Ph of the lung following deposition of acid, enhancing effects of O_3 by producing a change in the reactivity or residence time of reactants, such as free radicals, involved in O_3-induced tissue injury (Last et al., 1984a). If this were the only explanation, then the effects of O_3 should be enhanced consistently by the presence of acid in an exposure atmosphere. However, in the study of Schlesinger et al. (1992b) in which rabbits were exposed for 3 h to combinations of O_3 at 0.1, 0.3, and 0.6 ppm + H_2SO_4 (0.3 μm) at 50, 75, and 125 $\mu g/m^3$, antagonism was noted in the evaluation of stimulated production of superoxide anion by AMs harvested by lavage immediately after exposure to 0.1 or 0.3 ppm O_3 in combination with 75 or 125 $\mu g/m^3$ H_2SO_4 and also for AM phagocytic activity at all of the O_3/acid combinations. Mixtures of O_3 (0.6 ppm) and another acid, HNO_3 vapor (1,000 $\mu g/m^3$), also produced antagonism for certain aspects of the function of Ams harvested from acutely exposed rats (Nadziejko et al., 1992). Although the deposition sites of both acid and O_3 should be comparable in these two studies, perhaps the particular cellular endpoints examined are subject to this type of interaction.

Last (1989) observed an apparent all-or-none response in rats exposed to the acid sulfate/O_3 mixtures. That is, there was no concentration-response relationship between the concentration of acid in the mixture and the extent of change in various endpoints, compared to effects observed with O_3 alone. In the study of Schlesinger et al. (1992b), a similar phenomenon was noted, but, in this case, the concentration of O_3 in the mixture did not always influence the response compared to that seen with acid alone. Thus, exposure-concentration-response relationships noted with individual pollutants may not necessarily hold following exposure to their mixtures. This is consistent with the results of Gelzleichter et al. (1992a) for mixtures of O_3 and NO_2.

The above studies involved simultaneous exposures to O_3 and acidic pollutants, but some studies involving sequential exposures to O_3 and acid sulfate aerosols were described in the previous O_3 criteria document. For example, Gardner et al. (1977) found an additive increase in infectivity when mice were exposed to 0.1 ppm O_3 for 3 h prior to a 2-h exposure to 900 $\mu g/m^3$ H_2SO_4, whereas no difference from air control was noted when the acid was administered prior to O_3. Grose et al. (1980) noted a reduction in ciliary activity in isolated tracheal sections obtained from hamsters exposed to 0.1 ppm O_3 for 3 h, followed by exposure to 1,090 $\mu g/m^3$ H_2SO_4 for 2 h, that was less in magnitude than that found with exposure to acid alone; O_3 alone had no effect.

Silbaugh and Mauderly (1986) examined the ability of O_3 to increase susceptibility to a subsequent exposure to H_2SO_4 in terms of producing airway constriction. Guinea pigs were exposed to 0.8 ppm O_3 for 2 h followed by H_2SO_4 (12 mg/m^3 for 1 h). An increased volume of trapped gas in the lungs (the metric of constriction) was seen with both O_3 alone and with the mixture, but the response to the latter did not differ from that due to the former, and

acid alone had no effect. Thus, in this case, preexposure to O_3 did not affect response to a subsequent exposure to acid.

Chen et al. (1991) examined the reverse exposure scenario, whether exposure to H_2SO_4 affected subsequent response to O_3. Guinea pigs were exposed to H_2SO_4 or ultrafine zinc oxide (ZnO) particles coated with H_2SO_4. A 1-h exposure to 0.15 ppm O_3 following a 1-h exposure to acid (300 $\mu g/m^3$, 0.09 μm) did not alter the response seen with acid alone, a decline in DL_{CO}. However, when single (1-h) or multiple (3-h/day, 7-day) exposures to acid-coated ZnO (24 or 84 $\mu g/m^3$ equivalent H_2SO_4) were followed by a 1-h exposure to 0.15 ppm O_3, the effect on DL_{CO} appeared to be greater than additive, although no specific statistical test for interaction was performed. This study suggested that prior exposure to acid increased the susceptibility of the guinea pig to subsequent exposure to O_3, but it also showed that the manner in which the acid was delivered affected whether or not any interaction occurred. It is likely that the number of particles was greater in the ZnO-H_2SO_4 aerosol than in the H_2SO_4 aerosol, and the interaction may reflect this greater particle number.

6.4.2.3 Other Copollutants

Although the bulk of the database for binary mixtures of O_3 involves NO_2 or acids, a few studies examined responses to combinations of O_3 with other pollutants.

Reuzel et al. (1990) exposed rats to mixtures of O_3 (0.2, 0.4, or 0.8 ppm) + HCHO (0.3 to 3.0 ppm). Although exposure to the mixtures did not alter the nature or extent of histological lesions, (cilia loss and epithelial hyperplasia) compared to exposure to each pollutant alone, a site-specific synergistic increase in turnover of nasal epithelial cells was found with all concentrations of HCHO together with 0.4 ppm O_3. A lack of such response with 0.8 ppm O_3 was ascribed to an O_3-induced alteration in breathing pattern, which reduced the delivered dose. It was, however, noted that interaction occurred only when one constituent of the mixture was administered at cytotoxic concentrations, an exposure scenario that rarely occurs in ambient air. In any case, the authors concluded that because cell proliferation likely plays a role in carcinogenesis, and that if mixtures potentiate cell proliferation, then exposure to pollutant mixtures may increase cancer risk.

Mautz et al. (1988) exposed rats for 3 h, both at rest and with exercise, to a mixture of 0.6 ppm O_3 + 10 ppm HCHO. A synergistic increase in nasal epithelial cell turnover followed exposure with exercise, whereas exposure at rest resulted in no difference from that seen with HCHO alone. Likewise, exposure to the mixture with exercise resulted in an increase in the number of focal lesions in lung parenchyma compared to either O_3 or HCHO alone, but exposure at rest resulted in a lower incidence of lesions than seen with O_3 alone. This latter observation was ascribed to an effect of HCHO on breathing pattern, producing a change in inhaled dose of O_3 that did not occur with exercise.

Nishikawa et al. (1992) examined the effect of sequential exposure to cigarette smoke and O_3 in altering airway responsivity to inhaled bronchoconstrictor challenge and tracheal vascular permeability in guinea pigs. Animals were exposed to 1 ppm O_3 for 0.5 h, followed by 5 puffs of cigarette smoke, or to 1 ppm O_3 for 1.5 h, followed by 10 puffs. Exposure to O_3 and five puffs increased responsivity and vascular permeability immediately after exposure, whereas no effect on either endpoint was noted with either pollutant given alone. Exposure to O_3 and 10 puffs also increased responsivity and permeability, but to the same extent as did the lower concentration mixture or exposure to O_3 alone, whereas exposure to 10 puffs of smoke only increased responsivity. Thus, sequential exposure to O_3 and

cigarette smoke enhanced the magnitude of response compared to either pollutant alone, but the duration of response was not altered.

The potential role of O_3 in enhancing fibrotic lung disease by interaction with silica was examined by Shiotsuka et al. (1986), who exposed rats with developing silica-induced fibrosis to O_3 at 0.8 ppm for 6 h/day, 5 day/week for 37 exposure days. Silica had been instilled (2, 12, or 50 mg) on Day 1 of the study and exposure to O_3 began on Day 3 or 4 postinstillation. There was found to be no interaction between silica and O_3 in development of fibrosis, as assessed biochemically (lung content of hydroxyproline) or histopathologically. Although an increase was found in the ratio of hydroxyproline to total protein in the group exposed to the mixture and instilled with the highest amount of silica, this was not considered by the authors to be biologically significant.

6.4.3 Complex (Multicomponent) Mixtures Containing Ozone

Ambient pollution in most areas is a complex mix of more than two chemicals, and a number of studies have examined the effects of exposure to multicomponent atmospheres containing O_3. Some of these attempted to simulate photochemical reaction products occurring under actual atmospheric conditions. However, the results of these studies are often difficult to interpret due to chemical interactions between the components, as well as the resultant production of variable amounts of numerous secondary reaction products, and a lack of precise control over the ultimate composition of the exposure environment. In addition, the role of O_3 in the observed biological responses is often obscure.

One type of experimental multicomponent atmosphere that has been examined is ultraviolet-irradiated and nonirradiated automobile exhaust mixtures. Irradiation leads to the formation of photochemical reaction products that are biologically more active than those in nonirradiated mixtures. Such mixtures are characterized by total oxidant concentrations (expressed as O_3) in the range of 0.2 to 1.0 ppm. Although the effects described following exposure were not necessarily uniquely characteristic of O_3, and, although O_3 could have been responsible for some, or even most of them, in most cases, the biological effects have been difficult to associate with any one particular component. Effects of exhaust mixtures on different species have been discussed in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Pulmonary function changes were demonstrated in guinea pigs after short-term exposures to irradiated exhaust and in dogs after long-term exposure to both irradiated and nonirradiated exhaust mixtures.

Additional studies of complex mixtures have been performed since publication of the previous O_3 criteria document. Kleinman et al. (1985) exposed rats (Sprague-Dawley, male, 7 weeks old, nose-only) for 4 h to atmospheres designed to represent photochemical pollution and consisting of 0.6 ppm (1,180 $\mu g/m^3$) O_3 + 2.5 ppm (4,700 $\mu g/m^3$) NO_2 + 5.0 ppm (13,100 $\mu g/m^3$) sulfur dioxide (SO_2) + particles. The particulate phase consisted of 1,000 $\mu g/m^3$ of either H_2SO_4 or $(NH_4)_2SO_4$ laced with iron sulfate [$Fe_2(SO_4)_3$] and manganese sulfate ($MnSO_4$). The metallic salts act as catalysts for the conversion of sulfur IV into sulfur VI and for the incorporation of gases into the aerosol droplets. The respiratory region was examined for morphological effects. A confounding factor in these studies was the production of HNO_3 vapor in atmospheres that contained O_3 and NO_2, a phenomenon discussed previously, and nitrate in those that contained O_3 and $(NH_4)_2SO_4$, but not NO_2. Nevertheless, a significant enhancement of tissue damage was noted with exposure to atmospheres containing H_2SO_4 or secondarily produced HNO_3 compared to those containing $(NH_4)_2SO_4$, a less acidic

compound. In addition, there was some suggestion that the stronger acidic atmospheres resulted in a greater area of the parenchyma becoming involved in lesions, which were characterized by a thickening of alveolar walls, cellular infiltration in the interstitium, and an increase in free cells within alveolar spaces. An increased rate of nasal epithelial cell turnover was noted following exposure to atmospheres containing particulate acids compared with exposure to either O_3 alone or to a mixture of O_3 + NO_2. Furthermore, exercise seemed to potentiate the nasal and parenchymal responses to the complex mixtures containing strong acids (Kleinman et al., 1989), a finding similar to that with the previously discussed mixtures of O_3 and NO_2 or O_3 and HCHO.

Bhalla et al. (1987) examined the effects of a seven-component atmosphere (similar to that above) on epithelial permeability of rat lungs (Sprague-Dawley, male, 47 to 52 days old). The animals were exposed for 2 h (chambers, relative humidity [RH] = 85%) to the following: O_3 (0.6 ppm) + NO_2 (2.5 ppm) + SO_2 (5.0 ppm) + ferric oxide (Fe_2O_3) (241 $\mu g/m^3$) + $(NH_4)_2SO_4$ (308 to 364 $\mu g/m^3$) + $Fe_2(SO_4)_3$ (411 to 571 $\mu g/m^3$) + $MnSO_4$ (7 to 9 $\mu g/m^3$). The response to this mixture was compared to that following exposure to O_3 (0.6 ppm) + NO_2 (2.5 ppm), O_3 alone (0.6 or 0.8 ppm), or NO_2 alone (6 or 12 ppm). As above, the complex mixture was found to result in production of HNO_3, in this case at measured concentrations of 1,179 to 2,558 $\mu g/m^3$ (0.46 to 1.02 ppm); the O_3 + NO_2 atmosphere also resulted in some HNO_3 vapor formation. Epithelial permeability was found to increase immediately following exposure to O_3, O_3 + NO_2, or to the complex mixture. Although the magnitude of this change was similar following exposure to O_3 alone or in combination with other pollutants, there was increased persistence of effect after exposure to either the binary or complex mixture.

Prasad et al. (1988) used a similar multicomponent atmosphere and examined effects on AM surface receptors. Rats (Sprague-Dawley, male, 200 g) were exposed for 4 h/day, for 7 or 21 days to a mixture of O_3 (0.3 ppm) + NO_2 (1.2 ppm) + SO_2 (2.5 ppm) + $(NH_4)_2SO_4$ (270 $\mu g/m^3$) + $Fe_2(SO_4)_3$ (220 $\mu g/m^3$) + $MnSO_4$ (4 $\mu g/m^3$) + Fe_2O_3 (150 $\mu g/m^3$), or to O_3 alone. Both the mixture and O_3 alone resulted in a decrease in Fc receptor activity beginning immediately after the last exposure. Exposure to the complex atmosphere for 7 days resulted in a response similar to that seen with O_3 alone, but continued exposure to this mixture for up to 21 days resulted in an even greater reduction in receptor function compared to O_3 alone. However, as with most studies of complex mixtures, although the response to the mixture was different from that found with O_3, the role of other constituents was not clear. Phagocytic function of AMs was also examined following exposure to the mixture, but there were no O_3-only controls for comparison.

Mautz et al. (1985a) examined the effects of a complex mixture on pulmonary mechanics in exercising dogs. Exposures (nose-only) were for 200 min to a mixture of O_3 (0.45 to 0.7 ppm) + SO_2 (4.8 to 5.2 ppm) + H_2SO_4 (800 to 1,200 $\mu g/m^3$, 0.2 μm) + catalytic salts of $Fe_2(SO_4)_3$ and $MnSO_4$. A greater increase in resistance and decrease in compliance was found with the complex atmosphere than with O_3 alone, but the effect was ascribed to the presence of H_2SO_4. Although synergism was implied, it could not be concluded definitively because the mixture was not tested without O_3.

Mautz et al. (1991) further examined the ability of components of acidic fogs to alter the response to O_3. Rats (Sprague-Dawley, male, 7 weeks old, n = 12/group) were exposed for 4 h (nose-only; temperature = 22 to 23 °C, RH = 82 to 83%) to 0.4 ppm O_3 or to a mixture of 0.4 ppm O_3 + 670 $\mu g/m^3$ HNO_3 vapor + 610 $\mu g/m^3$ H_2SO_4 particles (0.32

m). Exposure to either O_3 or the mixture resulted in comparable changes: development of a rapid, shallow breathing pattern; a decrease in fatty acid composition of pulmonary surfactant; and focal parenchymal lesions with thickened alveolar septa and cellular infiltration. The lack of any modulation of the O_3-induced effects by acids prompted the authors to raise the question of the sensitivity of rats to inhaled acids. Although responses to any pollutant are somewhat species dependent, there is some evidence that rats are not the most sensitive species to acidic aerosols (U.S. Environmental Protection Agency, 1989). As discussed previously, the extent of interaction within any one species of animal is endpoint dependent, and it is likely that the sensitivity of various endpoints is species dependent. Thus, rats do show biochemical changes (e.g., in collagen metabolism) with exposure to fairly low levels of acidic aerosols in combination with O_3 (see Table 6-10), although these involved longer duration exposures. In any case, the underlying reasons for the lack of interaction in the complex-mixture study above remain unclear.

Kleinman et al. (1989) exposed rats (Sprague-Dawley, male, 7 weeks old, nose-only) to a mixture of O_3 (0.8 ppm) + SO_2 (5.0 ppm) + H_2SO_4 or $(NH_4)_2SO_4$ (1,000 g/m^3) at high RH (85%) and noted a delay in early clearance of inert particles from the lungs, compared to air-exposed controls. However, it is difficult to relate any effects to the O_3 because responses to O_3 alone were not examined.

The ideal complex mixture is one that actually exists in the ambient environment. Saldiva et al. (1992) exposed rats (Wistar, male, 2 mo old) for 6 mo to actual atmospheres of São Paulo, Brazil, with controls maintained for the same period of time in a clean, rural environment. The mean pollution levels over the exposure period were as follows: 0.011 ppm O_3, 1.25 ppm CO, 29.05 g/m^3 SO_2, and 35.18 g/m^3 particles. The animals exposed to the urban air showed evidence of bronchial secretory cell hyperplasia, ciliary structural changes, increased viscosity of mucus, and impaired mucociliary clearance. Although chronic exposure to air pollution may result in pulmonary dysfunction, the specific components producing the response could not be determined.

Inhalation exposures to air pollutants are, of course, the ideal way to assess interaction, but in vitro exposures may provide indications of potential interactions. Shiraishi and Bandow (1985) exposed Chinese hamster V79 cells for 2 h to photochemical reaction products produced from the reaction of propylene and NO_2 in a smog chamber. The resultant exposure atmospheres consisted of various proportions of propylene (0.07 to 0.16 ppm), NO_2 (0.22 to 0.28 ppm), O_3 (0.09 to 0.38), PAN (0.04 to 0.41 ppm), and HCHO (0.23 to 1.50 ppm). Exposures to NO_2 and O_3 alone also were performed. All of the complex mixtures resulted in an increased frequency of sister chromatid exchange and growth inhibition. The effects of the mixture were greater than those due to either O_3 or NO_2 alone for sister chromatid exchange, but growth inhibition was similar to that induced solely by O_3. The authors concluded that the observed effects were not due to any single compound within the mixture, but rather to various compounds producing multiple effects.

6.4.4 Summary

It is difficult to summarize the role that O_3 plays in response to exposure to binary mixtures, and it is even harder to determine its role in response to multicomponent atmospheres. One of the problems in understanding interactions is that, although the specific mechanisms of action of the individual pollutants within a mixture may be known, the exact bases for toxic interactions have not been elucidated clearly. There are, however, certain

generic mechanisms that may underlie pollutant interactions. One is physical, involving adsorption of one pollutant onto another and subsequent transport to more or less sensitive sites or to sites where one of the components of the mixture normally would not deposit in concentrated amounts. This, however, probably does not play a major role in O_3-related interactions. A second mechanism involves production of secondary products that may be more toxicologically active than the primary materials. This has been demonstrated or suggested in a number of studies as a basis for interaction between O_3 and NO_2. A third mechanism involves biological or chemical alterations at target sites that affect response to O_3 or the copollutant. This has been suggested to underlie interactions with mixtures of O_3 and acid sulfates. A related mechanism is an O_3- or copollutant-induced physiological change, such as alteration in ventilation pattern, resulting in changes in the penetration or deposition of one pollutant when another is present. This has been implicated in enhanced responses to various O_3-containing mixtures with exercise.

Evaluation of interactions between O_3 and copollutants is a complex procedure. Responses are dependent on a number of host and environmental factors, such that different studies using the same copollutants may show different types or magnitudes of interactions. The occurrence and nature of any interaction is dependent on the endpoint being examined and is also highly related to the specific conditions of each study, such as animal species, health status, exposure method, dose, exposure sequence, and the physicochemical characteristics of the copollutants. Because of this, it is difficult to compare studies, even those examining similar endpoints, that were performed under different exposure conditions. Thus, any description of interactions is really valid only for the specific conditions of the study in question and cannot be generalized to all conditions of exposure to a particular chemical mixture. Furthermore, it is generally not possible to extrapolate the effect of pollutant mixtures from studies on the effects of each component when given separately. In any case, what can be concluded from the database is that interactions of O_3-containing mixtures are generally synergistic (antagonism has been noted in a few studies), depending on the various factors noted above, and that O_3 may produce more significant biological responses as a component of a mixture than when inhaled alone. Furthermore, although most studies have shown that interaction occurs only at higher than ambient concentrations with acute exposure, some have demonstrated interaction at more environmentally relevant levels (e.g., 0.05 to 0.1 ppm O_3 with NO_2) with repeated exposures.

6.5 Summary and Conclusions

6.5.1 Introduction

In the past 30 years, thousands of research studies on the effects of O_3 in laboratory animals have been reported in the literature. This body of evidence presents a clear picture of the types of alterations O_3 can cause on respiratory tract host defense mechanisms, biochemistry, structure, and lung function. Less is known about carcinogenic potential and effects on organs distant from the lungs. These types of effects are observed in many animal species from mice to nonhuman primates, lending credence to the qualitative extrapolation of these effects to humans. The major issue is what levels, durations, and patterns of exposure are capable of causing these effects in humans. Extrapolation is discussed in Chapter 8. Suffice it to say here that the animal toxicological studies assist in interpreting observations

made in O_3-exposed humans and extend the knowledge of potential human hazards that never can be studied adequately in humans.

This summary and conclusion section deals exclusively with the effects of O_3, alone and in mixture. Other photochemical oxidants either have been evaluated elsewhere (NO_2 and HCHO; U.S. Environmental Protection Agency, 1993; Grindstaff et al., 1991) or in an earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986). This section is organized by molecular mechanisms of effects, respiratory tract effects, systemic effects, and effects of mixtures. Generally, it is an interpretative, factual summary of the array of effects observed in animals. Chapter 8 presents the current state of extrapolation of these effects to humans, and Chapter 9 integrates knowledge from animal toxicology, epidemiology, and human clinical studies.

Together, this chapter and the animal toxicological chapter in the 1986 document (U.S. Environmental Protection Agency, 1986), contain more than 1,000 references. Although all of them contribute to choosing and understanding the key issues to be summarized here, there obviously must be a highly selective choice made as to which references to include here. Generally, the papers discussed here were selected either because they represent the lowest effective concentration for an endpoint or they significantly influenced a particular conclusion.

6.5.2 Molecular Mechanisms of Effects

Molecular mechanisms (the manner in which chemical reactions of O_3 are translated into biological effects) are alluded to in different sections of this document. Studies that link O_3 chemistry with O_3 effect measurements would greatly strengthen the theoretical basis for understanding the biological effects of O_3. They also would allow examination of the similarity between animals and humans, thus strengthening interspecies extrapolations. Ozone has been shown to react directly with a variety of biomolecules that are present in both animals and humans. Most of the attention has been centered on polyunsaturated fatty acids and carbon-carbon double bonds, although reactions with sulfhydryl, amino, and some electron-rich compounds may be equally important. Free radicals may be involved, and antioxidant defenses appear to lessen the effect of these reactions. A "molecular target" for O_3 (the biomolecules most affected by reaction with O_3 or most crucial in mediating the observed responses) has not been identified for any of the endpoints studied. In fact, the target may be different for different endpoints.

An important concept in evaluating molecular targets was elucidated recently by Pryor (1992), who suggests (based on reaction and diffusion rate data) that the O_3 molecule does not penetrate through cell membranes or even the surfactant layer of the lung. Instead, a "reaction cascade" forms intermediates (organic or oxygen-free radicals, lipid hydroperoxides, aldehydes, hydrogen peroxide, etc.), which penetrate into the cells, causing the biological effects observed (Pryor et al., 1991). Confirmation of such O_3-induced free-radical autoxidation of lipids has been sought in vivo, but the indirect nature of the measurement methods produced equivocal results. More direct evidence has been obtained by Kennedy et al. (1992), who used electron spin-trapping methods to measure a concentration-related increase in radical adducts of the lipid fraction of lungs from O_3-exposed rats. Increased radical signals were detected after a 2-h exposure to $\geq$0.5 ppm O_3, but because the rats' respiration was stimulated by CO_2, the effective dose would be greater than it appears. Oxidized (oxygenated) biomolecules that result from reaction with O_3 also may mediate the

effects of O_3. Studies by Hatch et al. (1994) show that crude fractions of the lung lining layer become labeled with oxygen-18 after exposure to oxygen-18-labeled O_3. The label is concentrated in the airway lining layers, and the amount of oxygen-18 incorporation in this layer appears to be correlated with effects of O_3 (permeability and inflammation) in both rats and humans. These findings are consistent with the hypothesis that O_3 reacts with the lining of the lung, that the same types of interactions occur in both animals and humans, and that these reactions lead to similar effects.

6.5.3 Respiratory Tract Effects

6.5.3.1 Effects on Host Defenses

Several systems defend the respiratory tract of the host against infectious and neoplastic disease as well as nonviable inhaled particles; all of these systems can be affected by O_3. The mucociliary clearance system moves particles deposited on the mucous layer (either through deposition from the air stream or entry of cells or cellular debris from the alveoli) upwards and out of the lower respiratory tract. The nasal passages also have an effective clearance system. Concentrations as low as 0.15 ppm O_3 (8 h/day, 6 days) caused structural changes in the nasal respiratory epithelium (e.g., ciliated cell necrosis, shortened cilia) of monkeys (Harkema et al., 1987). Ciliated cells also are lost or damaged in the conducting airways of the lower respiratory tract after short exposures (e.g., 0.96 ppm O_3, 8 h, monkeys; Hyde et al., 1992). Mucous chemistry also is changed (McBride et al., 1991). Sufficient morphologic damage would be expected to have functional consequences. Acute exposures (0.6 ppm O_3, 2 h) slow mucociliary particle clearance in rabbits, but repeated exposures (up to 14 days) caused no effects. Alveolar clearance is slower and involves clearance of particles through interstitial pathways to the lymphatic system or movement of particle-laden AMs up to the bottom of the mucociliary escalator. Effects on alveolar clearance are concentration-dependent. A single 2-h exposure of rabbits to 0.1 ppm O_3 accelerated clearance up to 14 days postexposure, exposure to 0.6 ppm caused no effect, and a higher concentration (1.2 ppm) slowed alveolar clearance (Driscoll et al., 1986). Alveolar clearance of asbestos particles was slowed by a 6-week exposure to an urban pattern of O_3 (Pinkerton et al., 1989).

Alveolar macrophages are the first line of defense against microbes and nonviable particles that reach the pulmonary region of the lung. They phagocytize particles, kill microbes, and interact with lymphocytes in the development of an immune response. Thus, their proper functioning is critical. Alveolar macrophages from several species of animals exposed acutely to O_3 can exhibit decreased phagocytosis; decreased lysosomal enzyme activities and superoxide anion radical production, both of which function in killing bacteria; alterations in membrane morphology; chromosomal damage; decreased cytotoxicity to tumor cells; increased release of PGE_2 and $PGF_{2\alpha}$; and alterations in the number of AMs. Phagocytic changes are the most investigated. Exposure of rabbits to a level as low as 0.1 ppm O_3 (2 h/day) decreased nonspecific phagocytosis (of latex microspheres) after 2 or 6 but not 13 days of exposure (Driscoll et al., 1987). Recovery from the single 2-h exposure to 0.1 ppm O_3 was complete by 7 days postexposure. This pattern of response was confirmed in mice for Fc-receptor mediated phagocytosis (Gilmour et al., 1991; Canning et al., 1991).

The humoral- and cell-mediated immune system of the lung also is affected by O_3. Generally, T-cell-dependent immunity is more susceptible than B-cell-dependent immunity, but most immune functions examined have exhibited effects. However, because relatively few studies have been conducted, it currently is not possible to adequately interpret the impacts of

O_3-induced alterations on the immune system (e.g., decreases in mitogenic responses of T cells, alterations in T:B-cell ratios in the MLN). Only a few studies have attempted to correlate immunological changes and infectious disease outcome. Van Loveren et al. (1988) infected rats with Listeria, exposed them to O_3 for 1 week (0.26 to 1.02 ppm) and measured several endpoints. Ozone concentrations of 1.02 and 0.77 ppm, respectively, increased Listeria-induced mortality and severity of pathologic lesions in the lung and liver. They interpreted these findings as due to O_3-induced impaired clearance of the bacteria caused by decreased AM function and decreased cellular immunity (e.g., decreased delayed-type hypersensitivity and decreased T:B-cell ratios in MLN).

A reasonably large body of evidence indicates that the impact of O_3 on one or several host defense mechanisms leads to the inability of animals to fight bacterial infection and alters the course of viral infection. Antibacterial models are more commonly used. Mice exposed for 3 h to 0.4 ppm O_3 have decreased intrapulmonary killing of *S. zooepidemicus* (Gilmour et al., 1993a; Gilmour and Selgrade, 1993). Similar results have been obtained for *S. aureus* at a slightly higher concentration (Goldstein et al., 1971b). Correlations have been made between O_3 exposure and decreases in AM phagocytosis, decreases in bactericidal activity, growth of bacteria in the lungs, presence of bacteria in the blood, and mortality in mice (Coffin and Gardner, 1972; Gilmour and Selgrade, 1993). The lowest O_3 exposure causing increased streptococcal-induced mortality is 0.08 ppm for 3 h in mice (Coffin et al., 1967; Coffin and Gardner, 1972; Miller et al., 1978). However, prolonged intermittent exposure to 0.1 ppm O_3 for 15 weeks only slightly increased the mortality (Aranyi et al., 1983), and continuous exposure for 15 days to 0.1 ppm with two daily 1-h peaks (5 days/week) to either 0.3 or 0.5 ppm did not enhance mortality in the same model system (Graham et al., 1987). Prolonged exposure (1 to 2 weeks) also did not affect bactericidal activity to *S. aureus* (Gilmour et al., 1991).

Generally, short-term exposure to O_3 does not affect viral titers in the lungs of mice infected with influenza virus; however, reduced numbers of lung tissue T and B cells will reduce antibody titers to the virus, and mortality, lung pathology, and increased lung wet weight do occur (Selgrade et al., 1988; Jakab and Hmieleski, 1988). Ozone also enhances postinfluenzal alveolitis and structural changes that begin at 30 days postinfection (Jakab and Bassett, 1990). The complexity of the interaction of viral infection and O_3 exposure is further illustrated by Selgrade et al. (1988), who found that the effects of O_3 on influenza virus infection were dependent on the temporal relationship of O_3 exposure and day of infectious challenge. Also, interferon, which can be induced by viral infection, mitigates the O_3-induced lung lessons in mice, raising the possibility that certain stages of viral infection may have interactions with the lung that are different from other stages (Dziedzic and White, 1987b).

6.5.3.2 Effects on Inflammation and Permeability

The barrier function of the respiratory tract is disrupted by O_3, allowing cellular and fluid components from the blood to enter the lung and allowing certain types of substances in the lung to enter the blood. Markers of inflammation generally included increased proteins and PMNs in BAL. Concurrent with these events, but not necessarily interdependently, AMs liberate more arachidonic acid, which results in the production of biologically active LTs and PGs. Similar responses are observed in mice, rats, rabbits, guinea pigs, hamsters, and nonhuman primates. After acute exposure, the lowest effective concentration that increases BAL protein and number of PMNs is 0.12 ppm O_3 (mice, 24 h of exposure, BAL immediately

after exposure) (Kleeberger et al., 1993a). However, the increase in BAL protein typically is maximal roughly 16 to 24 h postexposure. In rats exposed to 0.8 ppm O_3 for 6 h and examined by lavage and morphometry several times postexposure, the increase in nasal PMNs occurs sooner and wanes about the time that these cells are increasing in number in the lungs (Hotchkiss et al., 1989a,b). It should be recognized that BAL can enable measurement of the protein and cells accessible by lavage, including the resident material (i.e., may include protein from O_3-induced cellular destruction) and the material entering from the tissue or circulation. Thus, interstitial inflammation, which has been observed in several species microscopically, is not detectable by BAL.

Several C × T studies have been conducted in mice using BAL protein as an endpoint. In two studies, there was combination of various Cs (0.1 to 2.04 ppm O_3) and Ts (1 to 12 h), resulting in a number of different C × T products (Rombout et al., 1989; Highfill et al., 1992). Both of these studies showed that the influence of T increased as C increased (i.e., there was no simple relationship of $C^a \times T^a$ = constant product; however, at the lowest C × T products, there was a more equivalent influence of C and T). Gelzleichter et al. (1992b) used a single C × T product composed of a variety of Cs and Ts for up to 3 days of exposure. The 24 h/day exposure group had less response than the other groups that responded equivalently. Effects of longer term exposure on permeability and inflammation are more complex to interpret (also see subsequent discussion on lung structure). Histological examination of rat lungs exposed to 0.5 ppm O_3 (2.25 h/day) showed more inflammatory cells in the alveoli after 5 days of exposure than after 1 day of exposure (Tepper et al., 1989). In contrast, the increase in BAL PMNs that occurred after Day 1 of exposure of rats had resolved by Day 4 (7 h/day) (Donaldson et al., 1993).

Some studies suggest that, although protein and PMN increases are observed concurrently, this may be more a function of experimental design than the actual biological sequence of events. For example, in rats depleted of PMNs with anti-PMN serum, O_3 did not increase BAL PMNs, but BAL protein still was increased (Pino et al., 1992b). Also Young and Bhalla (1992) observed an increase in tracheal protein earlier than increased tracheal PMNs. They interpreted this and other related results to suggest that the recruited PMNs may serve to sustain an increase in permeability.

6.5.3.3 Effects on Structure, Function, and Biochemistry

Theoretically, and in some cases empirically, lung structure, function, and biochemistry are linked. Correlations are not exact because of differences in available measurement methods (e.g., most lung function tests used do not measure sensitively the function of the smallest airways, where the "classical" O_3 lesion is observed) and some independence of effects (e.g., a transient change in breathing frequency would not be morphologically detectable). Also, most biochemical measurements are made of whole lung, rather than focal areas of damage, and only some enzyme activities measured would be expected to be correlated to structure or function (e.g., collagen metabolism, antioxidant metabolism).

After acute exposure to O_3, the most commonly observed effect in several species is tachypnea (increased f and decreased V_T) with little (if any) change in $\dot{V}_E$; the lowest exposure causing tachypnea was 0.2 ppm O_3 for 3 h in rats (Mautz and Bufalino, 1989). Other effects reported after acute exposure to □1 ppm include increased R_L and decreased Cdyn, TLC, VC, FRC, RV, FVC, DL_{CO}, and the multibreath N_2 slope (e.g., Fouke et al., 1991; Mautz et al.,

1985b; Miller et al., 1988). However, these changes are not observed in all studies, probably due to differences in animal species, measurement method, and exposure protocols. With rare exception, concentrations well in excess of 1 ppm O_3 are required to increase airway reactivity.

Two C × T studies of pulmonary function using acute exposure periods have been performed. Costa et al. (1989) found that FVC, DL_{CO}, and the multibreath N_2 slope decreased with increasing C × T products in rats and that the influence of T is greater at higher Cs. In guinea pigs, Nishikawa et al. (1990) observed that airway responsiveness to methacholine increased at higher C × T products (e.g., at 90 but not at 30 ppm · min); the authors concluded that T was an important factor in the O_3 response.

When rats were exposed for 5 days (2.25 h/day, with CO_2 to stimulate ventilation equivalent to light exercise in humans) to 0.35, 0.5, and 1.0 ppm O_3, the change in shape of the flow-volume curve occurred and tachypnea peaked on Days 1 and 2, but by Day 5, there was no difference from control (except at 1 ppm) (Tepper et al., 1989). This attenuation is similar to that observed in humans. However, in other, similar groups of animals, histological changes in the lung progressed, and BAL protein remained elevated. Other similarities between laboratory animals and humans in their pulmonary function responses to short-term O_3 exposure are explored in Chapter 8.

Ozone causes similar types of alterations in lung morphology in all laboratory animal species studied. The most affected cells are the ciliated epithelial cells of the airways and Type 1 cells in the gas exchange region. Within the nasal cavity, anterior portions of the respiratory and transitional epithelium are affected. Cilia are lost or damaged; some ciliated cells become necrotic, are lost, and are replaced with nonciliated cells. Mucus-secreting cells are affected.

The CAR (the junction of the conducting airways and the gas exchange region) is a primary target, possibly because it receives the greatest dose of O_3 delivered to the lower respiratory tract (see Chapter 8) and has Type 1 epithelial cells covering a large surface area. Even though there are significant interspecies differences in the structure of the CAR (e.g., primates, including humans, have RBs, which are rudimentary or absent in laboratory animals such as rats or mice), it is the target in all species studied. Exposure to O_3 causes loss of cilia or necrosis of the ciliated cells, leaving a bare basement membrane that is replaced by nonciliated bronchiolar cells, which may become hyperplastic after longer exposures. Mucous secreting cells can be affected, but not as significantly as ciliated cells. Type 1 cells also are damaged and can be sloughed from the surface; Type 2 cells, which are thicker, replace them. Sometimes, Type 2 cells differentiate into Type 1 cells. This epithelial remodeling is accompanied by an inflammatory response in the CAR, primarily consisting of an increase in number of PMNs in the earlier stages and an increase in number of AMs in later stages; interstitial edema occurs. With increased duration of exposure, alveolar septa in the CAR thicken due to increased matrix, basement membrane, collagen, and fibroblasts and a thickened alveolar epithelium.

These patterns of change have different relationships to duration of exposure, as illustrated by Dungworth (1989) (see Figure 6-3; Section 6.2.4.5). Inflammatory changes peak after a few days of O_3 exposure; are still observable, but to a much lesser degree, in tissue during months of exposure; and begin to return to control values after exposure ceases. In contrast, epithelial hyperplasia rapidly increases during about the first week of exposure, plateaus as exposure continues, and begins to decrease slowly when exposure stops. Interstitial

fibrosis requires months of exposure to be observed microscopically and increases slowly, but when exposure ceases, interstitial fibrosis still can persist or continue to increase. Numerous studies using several different species and experimental approaches support these findings. Only a few of the studies (primarily those using more sensitive morphometric measurements) are used here to illustrate key points and to show correlations with pulmonary function and lung biochemistry. Only rat and nonhuman primate studies are discussed because most investigations were conducted on them. At equivalent exposures, nonhuman primates appear to be more responsive than rats (Section 6.2.4).

Generally, short-term exposures to concentrations 0.2 ppm O_3 do not cause changes detectable by LM in the nasal cavities of rats or nonhuman primates, except for inflammation and an occasional delayed postexposure finding of mild hyperplasia. For example, Hotchkiss et al. (1989a) reported inflammation in the nasal epithelium of rats up to 66 h after a 6-h exposure to levels as low as 0.12 ppm O_3; there was no necrosis, loss of cilia, or hyperplasia even at 1.5 ppm. After 3 days (22 h/day) of exposure, 0.4 ppm caused loss of cilia and hyperplasia and metaplasia of the nasal epithelium of rats (Reuzel et al., 1990). Nonhuman primates appeared to be more responsive. Harkema et al. (1987) observed that exposure to 0.15 or 0.3 ppm O_3 for 6 or 90 days (8 h/day) caused necrosis of ciliated cells, shortened cilia, and increased mucous granule cells in the respiratory epithelium; alterations in cell numbers also were found in the transitional epithelium.

Within the CAR, a number of alterations occur. In rats and monkeys, ciliated and Type 1 cells become necrotic and are sloughed from the epithelium as early as the first 2 to 4 h of an exposure to about 0.5 ppm O_3 (Stephens et al., 1974a,b). Repair, as shown by increased DNA synthesis by nonciliated bronchiolar and Type 2 cells, begins by about 18 to 24 h of exposure (Evans et al., 1976a,b; Stephens et al., 1974a; Castleman et al., 1980), although cell damage continues (Castleman et al., 1980). The lesion is fully developed by about 3 days of continuous exposure, after which the rate of repair exceeds the rate of damage. The increase in antioxidant enzyme activities (e.g., succinate oxidase, G6PD, and 6PGD) parallels the increase in Type 2 cells, which are rich in these enzymes; the increase in the Type 2 cell population is probably responsible for these biochemical changes (Bassett et al., 1988a; U.S. Environmental Protection Agency, 1986).

Lesions in the CAR are one of the hallmarks of O_3 toxicity, having been well established. The study by Chang et al. (1992) provides examples of some of the patterns of cellular alterations. Chang et al. (1992) exposed rats to an urban pattern of O_3 (0.06 ppm background, 7 days/week on which were superimposed 9-h peaks [5 days/week] slowly rising to 0.25 ppm) for 78 weeks and made periodic examinations of the CAR TB and proximal alveoli by TEM morphometry during and after exposure. Type 1 cells had a larger volume at Week 13 and increased numbers at Weeks 13 and 78; there were no such changes at 17 weeks after exposure ceased. Type 2 cell volume per area of basement membrane increased immediately after Week 78 and was still increased 17 weeks after exposure ceased. Interstitial cells and matrix were increased after Weeks 1, 13, and 78, but returned to control by 17 weeks after exposure ceased. However, epithelial and endothelial basement membrane were thickened and accompanied by increased collagen fibers at the later examination times and 17 weeks after the 78-week exposure ended. In TBs, surface areas of ciliated and nonciliated cells decreased during exposure. Pulmonary function studies conducted in identically exposed groups of rats were consistent with the morphometric findings (Tepper et al., 1991). Generally, expiratory resistance was increased (suggesting central airway narrowing), but it

was only significantly different from control at 78 weeks. Tidal volume was reduced at all evaluation times. Overall, breathing frequency was reduced, but no single evaluation time was significant. Monkeys exposed to a higher concentration of O_3 (0.64 ppm, 1 year) also showed increased resistance and decreased flows, which were interpreted as central and peripheral airway narrowing; during a 3-mo postexposure period, decreases in static lung compliance persisted (Wegner, 1982).

Several studies have demonstrated distal airway remodeling. This bronchiolization of CAR alveoli is so named because bronchial epithelium replaces the Type 1 and 2 cells typical of ADs, resulting in the appearance of RBs in rats and increased volume fraction and volume of RBs in monkeys. This has been observed at exposures as low as 0.5 ppm O_3 (50 days) in rats (Moore and Schwartz, 1981) and as low as 0.25 ppm (8 h/day, 18 mo) in monkeys (Tyler et al., 1988). Inflammation occurs concurrently, perhaps indicating an influence on remodeling. In monkeys, such bronchiolization can persist 6 mo after the end of a 1-year (8 h/day) exposure to 0.64 ppm (Tyler et al., 1991b).

Exposure regimens can have unexpected impacts on experimental outcomes. Several investigations of combinations of O_3 "episodes" or O_3 "seasons" with clean-air periods have been examined. In the first of these, Last et al. (1984b) compared air control rats to two groups of rats exposed to 0.96 ppm. One group received a 90-day (8 h/day) exposure ("daily"); the other group had intermittent units of 5 days of O_3 (8 h/day) and 9 days of air, such that there were 35 O_3 exposure days over the 90-day period (episodic). Both groups had equivalent increases in lung collagen. Using a similar exposure regimen, Barr et al. (1990) found equivalent CAR remodeling and volumes of CAR lesions in both groups. In contrast, RB thickness increased in the daily group only, and the CAR interstitium increased in thickness only in the episodic group. Monkeys were studied more extensively after a daily (8 h/day) exposure to 0.25 ppm for 18 mo and a seasonal exposure only during the odd months of the 18-mo period (Tyler et al., 1988). Most morphometric measurements were similar between the two groups (e.g., both had respiratory bronchiolitis). However, only the daily group had an increased number of AMs in the lumen and interstitium. Only the seasonal group had increased lung collagen content; increased chest wall compliance, suggesting delayed lung maturation; and increased inspiratory capacity. This body of work indicates that under these types of exposure circumstances, the simple product of $C \times T$ does not predict the outcome. Indeed, half the O_3 (on a $C \times T$ basis) caused equivalent or more effects than a "full" O_3 exposure.

The complexity of understanding $C \times T$ relationships is further illustrated by Chang et al. (1991), who compared two different exposure regimens (one a square wave and the other an urban pattern) on the basis of $C \times T$ products. There was a linear relationship between $C \times T$ products and the increase in Type 1 cell volume in the CAR; a similar observation on Type 2 cell volumes was less robust. There was no such relationship for other morphometric endpoints in the same animals. Cell proliferation in the nasal epithelium does not increase linearly with increasing $C \times T$ but does increase linearly with increasing C (Henderson et al., 1993).

Long-term exposure also thickens CAR alveolar septa, due to an increase in inflammatory cells, fibroblasts, and amorphous extracellular matrix (Fujinaka et al., 1985; Barry et al., 1985; Zitnik et al., 1978). There is some morphological evidence of mild fibrosis (i.e., local increase in collagen) in CAR interalveolar septa (Last et al., 1979; Boorman et al., 1980; Chang et al., 1992; Pickrell et al., 1987b; Freeman et al., 1974; Moore and Schwartz,

1981). Biochemical evidence supports these findings, even though biochemical approaches would be expected to be less sensitive because the whole lung (rather than focal lesions) is examined. Last et al. (1979) directly demonstrated the correlation by observing increased collagen histologically and biochemically (collagen synthesis rate) in rats similarly exposed to 0.5 to 2.0 ppm O_3 for 7 to 21 days. The increase became greater with increasing concentration and duration of exposure. Similar correlations were observed at a higher concentration by Pickrell et al. (1987b). The increased collagen content can persist after exposure ceases (Chang et al., 1992; Hussain et al., 1976a,b; Last et al., 1984b), but some studies suggest that higher concentrations (>0.5 ppm) may be required for such persistence (Last and Greenberg, 1980; Pickrell et al., 1987b). Collagen cross-links were studied in monkeys exposed to 0.61 ppm O_3 for 1 year (8 h/day) (Reiser et al., 1987). Earlier examination of these same monkeys revealed that collagen content was increased (Last et al., 1984b). When specific collagen cross-links were measured, the increase in "abnormal" cross-links observed immediately after exposure remained in the lungs at 6 mo postexposure.

These morphologic/morphometric and biochemical findings of fibrotic changes are supported by some pulmonary function studies. For example, rats exposed for up to 78 weeks, using the same urban exposure protocol as Chang et al. (1992), exhibited reduced lung volume and hastened N_2 washout patterns, consistent with a "stiffer" lung (i.e., restrictive lung disease) (Costa et al., 1994).

The chronic O_3 study by the NTP and the Health Effects Institute (HEI) (Last et al., 1994; Szarek, 1994; Radharkrishnarmurthy, 1994; Parks and Roby, 1994; Harkema and Mauderly, 1994; Harkema et al., 1994; Chang et al., 1995; Pinkerton et al., 1995; Catalano et al., 1995a,b) further illustrates some of the complex interrelationships between lung structure, function, and biochemistry. All of these endpoints were evaluated in a collaborative project using rats exposed 6 h/day, 5 days/week for 20 mo to 0.12, 0.50, or 1.00 ppm O_3. Although lung biochemistry and structure were affected at the higher O_3 concentrations (0.50 ppm), there were no observed effects on pulmonary function. This is consistent with the relative sensitivity of the tests used and suggests that the observed effects were not sufficient to overcome the reserve function of the lung.

Combined analyses of the NTP/HEI collaborative studies showed that 0.50 and 1.00 ppm O_3 caused a variety of structural and biochemical effects; 0.12 ppm O_3 did not cause any major effects, although a few specific endpoints were altered. Hallmarks of chronic rhinitis (e.g., inflammation, mucous cell hyperplasia, decreased mucous flow) were observed in focal regions of the nasal cavity. Structural and biochemical changes included some, but not many hallmarks of airway disease. Typical O_3-induced changes (e.g., bronchiolarization, increased interstitial matrix) observed in the tracheobronchial region and in the CAR were characteristic of centriacinar fibrosis; however, diffuse pulmonary fibrosis was not observed.

An integrative, multiple endpoint analysis (Catalano et al., 1995a) utilizing median polish techniques produced composite variables for disease surrogates that were tested for trends across all three O_3 concentrations. Trends for centriacinar fibrosis, airway disease, and chronic rhinitis were examined for 10, 18, and 3 endpoints, respectively, from the individual NTP/HEI studies. A statistically significant trend was noted for the association between chronic rhinitis and increasing O_3 concentration. The differences between control and exposed rats were statistically significant at 0.50 and 1.00 ppm O_3. Marginally significant and significant trends were found for the association between centriacinar fibrosis or airway disease

and increasing O_3 concentration; however, no statistically significant differences were found between control and O_3-exposed rats.

As discussed above, long-term O_3 exposure can cause lung fibrotic changes; however, there is no evidence that O_3 causes emphysema, using the currently accepted morphological definition of human emphysema (U.S. Environmental Protection Agency, 1986).

6.5.3.4 Genotoxicity and Carcinogenicity of Ozone

A significant amount of research has been conducted to determine whether O_3 is genotoxic or carcinogenic. Many of the early experiments have flaws in experimental design or have used O_3 concentrations far above levels that could occur in ambient air. In evaluating the data, a number of conclusions can be made. In vitro exposure of naked plasmid DNA to very high O_3 concentrations results in single and double-strand breaks in the DNA, as confirmed by gel electrophoresis and electron microscopy studies (Hamelin, 1985). Testing of O_3 in various mutagenesis assays has led to marginal or small results in a number of assays and negative results in other assay systems. Ozone is not mutagenic in *Salmonella* strains TA98, TA100, TA104, and TA1535 and causes, at most, weak effects in strain TA102 that are not strictly concentration dependent (Dillon et al., 1992; Victorin and Stahlberg, 1988a,b). Extremely high concentrations of O_3 (50 ppm) caused mutation to streptomycin resistance in *E. coli* and caused various types of mutations in the yeast *S. cerevisiae*, but O_3 was a weak mutagen compared to known strong mutagens in the yeast system (L' Herault and Chung, 1984; Dubeau and Chung, 1982). Ozone was not mutagenic in the *N. tabacam* or *Tradescantia* mutation assay systems (Gichner et al., 1992). Hence, overall, the data on the mutagenicity of O_3 are mixed: negative in six assays, marginally positive in one assay, and weakly positive in two assays. The present data indicate that O_3 is, at most, a weak mutagen, but further data are needed in mammalian cell systems to draw definitive conclusions regarding this point. There are some data indicating that O_3 may cause chromosome breakage in cultured cells, but in vivo animal studies are conflicting (Zelac et al., 1971a,b; Tice et al., 1978). A human study with an appropriate experimental design was negative (McKenzie et al., 1977; McKenzie, 1982).

Regarding carcinogenicity, O_3 has been shown to induce morphological transformation in cultured C3H/10T1/2 mouse embryo cells and in SHE cells and to cause a synergistic morphological transformation in cells treated also with gamma radiation (Borek et al., 1986, 1989b). However, these results could be due to interactions of O_3 with the culture medium that generate chemical species different from those produced in vivo. Whole animal carcinogenesis assays performed in strain A mice have demonstrated marginal increases in tumor yield that were not statistically significant or concentration dependent (Hassett et al., 1985; Last et al., 1987). The NTP study demonstrated that O_3 was not a tumor promoter or a co-carcinogen when NNK-treated male F344/N rats were exposed for 2 years to 0.5 ppm O_3 (National Toxicology Program, 1994). In the NTP study, rats and mice were exposed to 0.12, 0.5, or 1.0 ppm O_3 for 6 h/day, 5 days/week for two years or a lifetime. This NTP study showed no evidence of carcinogenic activity in male or female F344/N rats, equivocal evidence of carcinogenic activity in male $B6C3F_1$ mice, and some evidence of carcinogenic activity in female $B6C3F_1$ mice at a high concentration (1.0 ppm). Hence, O_3 has been shown to be a weak pulmonary carcinogen only in female $B6C3F_1$ mice at toxic concentrations in one experiment.

At present, O_3 is shown to be nonmutagenic in some assay systems; at most, weakly mutagenic in a few assay systems; and clastogenic in vitro but not in vivo. Ozone can transform cells in vitro. Ozone does not cause concentration-dependent tumor induction that is statistically significant in hamsters, Wister male rats, F344/N male or female rats, male or female A/J mice, or Swiss-Webster male mice. There are ambiguous data for pulmonary carcinogenesis in male $B6C3F_1$ mice and weak carcinogenesis data in female $B6C3F_1$ mice from chronic exposure to 1.0 ppm O_3. Therefore, O_3 has been shown to be a carcinogen only in female $B6C3F_1$ mice in one experiment. Because a chronic exposure to 1 ppm O_3 was required to induce pulmonary tumors in female mice, it is possible that pulmonary toxicity, which occurs only at high O_3 concentrations (1.0 ppm) and does not occur at lower levels, contributed to the tumor development. Hence, the potential for animal carcinogenicity is uncertain at the present time.

6.5.3.5 Factors That Influence Ozone Exposure

Factors that increase the delivered dose of O_3, decrease biochemical defense mechanisms, or increase cellular sensitivity can increase the impact of a given O_3 exposure. The most commonly studied factors include exercise, age, and nutrition.

As discussed in Chapter 8, exercise increases the dose of O_3 delivered to the respiratory tract and alters the distribution of O_3. As would be expected, exercise during exposure enhances the effect of O_3. This has been demonstrated by Mautz et al. (1985b), who showed that exercising rats had more extensive lung lesions than rats exposed at rest. Similarly, Tepper et al. (1990, 1994) found that rats were more responsive to O_3 when coexposed to CO_2 to increase ventilation, simulating exercise.

A number of studies have been conducted to compare the effects of O_3 on various ages of mice and rats, from 1 day old to older adults. Interpretation of these studies is difficult because, prior to weaning, the huddling behavior of the neonates with their dams as well as the bedding material (present in some studies) may have affected the concentration of O_3 in the breathing zone and hence the subsequent delivered dose. Generally, in short-term exposure biochemical studies of antioxidant metabolism, there was a decrease or no change in enzyme activity in neonates. As age increased after weaning, the typical increase in antioxidant metabolism became greater with age (Elsayed et al., 1982; Tyson et al., 1982; Lunan et al., 1977; Mustafa et al., 1985). Stephens et al. (1978) found that morphological effects did not occur in animals exposed prior to weaning at 21 days of age. This may explain the results of Barry et al. (1985, 1988), who found no morphometric differences in the CAR and TB in rats that started a 42-day exposure at ages of 1 day and 42 days. In identically exposed rats, however, Raub et al. (1983) found more, though admittedly subtle, pulmonary function changes in the youngest group of animals. Yokoyama et al. (1984) did not detect any age-related differences in lung function of rats at 4, 7, and 10 weeks of age. Although O_3-induced increases in BAL protein and PMNs do not show age dependence, BAL prostaglandins increased sooner and more leukocytes were dead in younger (13-day-old) rats, compared to adults (e.g., 16 weeks old) (Gunnison et al., 1990, 1992a). Age (5 weeks versus 9 weeks) did not influence the O_3-induced decrease in lung bactericidal activity (Gilmour et al., 1993a).

The literature on O_3-exposed pregnant animals is extremely sparse. Exposure of rats (1 ppm O_3 6 h) on Day 17 of pregnancy or Days 3, 13, and 20 of lactation caused a greater increase in lung permeability and inflammation than that observed in nonpregnant rats (Gunnison et al., 1992b).

Numerous reports document that animals made vitamin E deficient are more susceptible to the biochemically detected effects of O_3 (e.g., lipid changes, antioxidant metabolism changes) (U.S. Environmental Protection Agency, 1986; Pryor, 1991). Generally, the research shows that, although vitamin E deficiency enhances susceptibility to lung biochemical changes, there is not a proportionate relationship between vitamin E supplementation (above normal levels) and protection from O_3. Also, vitamin E deficiency did not alter the impact of O_3 on lung structure (Chow et al., 1981). Vitamin C deficiency also has an influence. Guinea pigs deficient in vitamin C had a greater increase in BAL protein (compared to vitamin C-normal animals) when exposed acutely to 0.5 but not 1.0 ppm O_3 (Slade et al., 1989).

6.5.4 Systemic Effects

Theoretical analyses (Pryor, 1992) indicate that the O_3 molecule does not penetrate to the blood, yet there are numerous reports of systemic effects (i.e., effects on lymphocytes, erythrocytes, serum, central nervous system, parathyroid gland, circulatory system, and liver). Possibly one or several of the reaction products of O_3 (see Section 6.2.1) penetrates the lung tissue, or perhaps some systemic responses are secondary to pulmonary effects. Although a variety of clinical chemistry changes occur after O_3 exposure, they cannot be interpreted and will not be discussed here (see U.S. Environmental Protection Agency, 1986, and Section 6.3). Effects on systemic immunity are discussed in Section 6.5.3.1.

6.5.4.1 Central Nervous System and Behavioral Effects

Acute exposure to O_3 caused transient changes in behavior. The lowest exposure causing effects was 0.12 ppm O_3 for 6 h in rats; wheel-running activity decreased (Tepper et al., 1985; Tepper and Weiss, 1986). Because exercising animals were exposed in these studies (i.e., they received a higher dose of O_3), it is not surprising that higher O_3 concentrations (0.5 ppm, 6 h) are required to affect sedentary behavior (e.g., operant behaviors such as lever pressing for food reinforcement) (Weiss et al., 1981). Mice show aversive responses to O_3 (0.5 ppm, 60 s) by terminating O_3 exposure (Tepper et al., 1985). The lowest exposures causing effects are impacted by the type of reward. For example, O_3 had less effect on behaviors to avoid electric shock (Ichikawa et al., 1988) than on behaviors to obtain food or access exercise (Tepper et al., 1982, 1985; Weiss et al., 1981).

6.5.4.2 Cardiovascular Effects

In rats, O_3 can cause bradyarrhythmia at exposures as low as 0.1 ppm for 3 days; bradycardia, at exposures as low as 0.2 ppm for 2 days; and decreased mean arterial blood pressure, at exposures as low as 0.5 ppm for 6 h (Arito et al., 1990, 1992; Uchiyama and Yokoyama, 1989; Watkinson et al., 1993; Yokoyama et al., 1989b; Uchiyama et al., 1986). There is an interaction between some of these responses and thermoregulation in the rat. For example, when heart rate decreased, the core temperature of the exposed rats also decreased, and when exposures were conducted at higher ambient temperatures, there was no change in core temperature or heart rate (Watkinson et al., 1993). Such interactions add to the complexity of extrapolating this type of response to humans, and therefore, without more information, qualitative extrapolation would be highly speculative.

6.5.4.3 Reproductive and Developmental Effects

No reports of "classical" (e.g., 2-generation studies) reproductive assays with O_3 were found. Kavlock et al. (1979, 1980) performed several developmental toxicity experiments in rats. Pregnant rats exposed intermittently (8 h/day) to 0.44 to 1.97 ppm O_3 during early, mid-, or late gestation or during the entire period of organgensis (Days 6 to 15) had no significant teratogenic effects. Continuous exposure during mid-gestation increased the resorption of embryos. Postnatal growth and behavioral development also were investigated. There was no effect on neonatal mortality (up to 1.5 ppm). Pups from dams exposed continuously to 1 ppm during mid- or late gestation weighed less 6 days after birth. Pups from pregnant rats exposed continuously to 1 ppm during late gestation had delays in behavioral development (e.g., righting, eye opening).

6.5.4.4 Other Systemic Effects

A number of investigations have shown the effects of O_3 on the pituitary-thyroid-adrenal axis, as evidenced by changes in circulating hormones and morphological changes in the thyroid and parathyroid glands (U.S. Environmental Protection Agency, 1986). No more recent studies could be found.

Several approaches have been used to study the effects of O_3 on the liver: increase in sleeping time following the injection of drugs (e.g., pentobarbital) metabolized by the liver, drug pharmacokinetics, and changes in liver enzymes. The lowest exposure causing increased sleeping time from pentobarbital was 0.1 ppm O_3 for at least 15 or 16 days (3 h/day) in female mice (Graham et al., 1981). In three species of animals, only females were affected (Graham et al., 1981). Pentobarbital pharmacokinetics was marginally ($p = 0.06$) slowed in mice exposed to 1 ppm O_3 for 3 h (Graham et al., 1985); theophylline clearance was slowed in older rabbits exposed to 0.3 ppm O_3 for 5 days (3.75 h/day) (Canada and Calabrese, 1985). Ozone has caused both increases, decreases, and no changes in liver xenobiotic metabolism, depending on the exposure and enzyme being measured (U.S. Environmental Protection Agency, 1986).

6.5.5 Effects of Mixtures

Humans in the real world are exposed to complex mixtures of gases and particles. Sufficient evidence exists to know that the health outcome is dependent on the mixture, but the relative role (or even the exact identity) of the "major" components is not known. Because of this, it is crucial to evaluate the health effects of O_3 in light of epidemiological, human clinical, and animal toxicological studies. For the purposes of this document, an interaction is considered to occur when the response to the mixture is statistically significantly higher (synergism) or lower (antagonism) than the sum of the individual pollutants. Most animal toxicological studies of O_3 interactions have been conducted with binary mixtures (predominantly NO_2 and H_2SO_4). The rarer reports on complex mixtures are interesting, but less helpful because often the studies did not include a group exposed only to O_3, and therefore knowledge of the role of O_3 is confounded. Thus, only the binary mixture studies will be summarized here. This research has demonstrated that exposure to O_3 in combination with another chemical can result in antagonism, additivity, or synergism, depending on the animal species, exposure regimen, and endpoint studied. Interpretation is further complicated by the fact that most studies used exposure regimens unlike the real world in terms of ratios of pollutant concentrations, "natural" sequencing of exposure patterns, and other factors. For example, when O_3 and NO_2 exposures were sequential (in any order), there was an additive

increase in BAL protein, as compared to a synergistic increase when the exposures were concurrent (Gelzleichter et al., 1992a).

A range of interactions has been shown with O_3 and NO_2 combinations. For example, a 2-week exposure to an O_3-NO_2 mixture (0.4 ppm of both) synergistically increased antioxidants in the lungs of rats but not guinea pigs, peroxide levels were synergistically increased in guinea pigs but not rats, and GST activity was decreased in guinea pigs and unchanged in rats (Ichinose and Sagai, 1989). Most of the interaction studies using lung biochemical endpoints display synergism. A rare exception was the antagonism to the increase in lung cytochrome P-450 content caused by 0.2 ppm O_3 (1 to 2 mo) when the rats were coexposed to 4 ppm NO_2 (Takahashi and Miura, 1989). Combinations of various acute exposure durations and of O_3 and NO_2 concentrations did not follow a $C \times T$ relationship for increased lung permeability, but were synergistic at higher $C \times T$ products (Gelzleichter et al., 1992b). For pulmonary host defenses against bacterial infection, the interaction is dependent on the exposure pattern. Graham et al. (1987) showed that a 15-day exposure of mice to mixtures of O_3 and NO_2, each having a baseline level with two daily 1-h peaks of the pollutant, resulted in synergism only when exposure to either gas alone caused an increase in bacterial-induced mortality.

Both synergistic and antagonistic interactions have been found with combinations of O_3 and acidic sulfates. Warren et al. (1986) reported that with 3 days of exposure to 0.2 ppm O_3 + 5 mg/m^3 $(NH_4)_2SO_4$, O_3 alone was responsible for increasing BAL protein, collagen synthesis rate, and other parameters, but, by 7 days of exposure, synergism occurred. When rabbits were exposed for 4 mo (2 h/day, 5 days/week) to 0.1 ppm O_3 + 125 μg/m^3 H_2SO_4, there was a synergistic increase in epithelial secretory cell number, whereas 8 mo of exposure resulted in antagonism (Schlesinger et al., 1992a). Antagonism also was observed for effects or certain AM functions after acute exposures to O_3-H_2SO_4 mixtures (Schlesinger et al., 1992b). Sequential exposures to O_3 and H_2SO_4 also have been examined. Exposure to O_3 did not influence the subsequent effects of H_2SO_4 on bronchoconstriction in guinea pigs (Silbaugh and Mauderly, 1986). Gardner et al. (1977) found an additive increase in bacterial infectivity when mice were exposed acutely to 0.1 ppm O_3 before (but not after) H_2SO_4.

In summary, the animal toxicological studies clearly demonstrate the major complexities and potential importance of interactions, but do not provide a scientific basis for predicting the results of interactions under untested ambient exposure scenarios.

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7

Human Health Effects of Ozone and Related Photochemical Oxidants

7.1 Introduction

In the previous chapter, results of ozone (O_3) studies in laboratory animals were presented in order to understand the wide range of potential effects that might occur in exposed human populations and to expand the understanding of the mechanisms of O_3 toxicity and the basic exposure-response relationships for O_3. The concept of quantitatively extrapolating results from laboratory animals to humans is further explored in Chapter 8. Whenever possible, however, risk assessment of pollutants should be based on direct evidence of their health effects in human populations. Information on human health responses to O_3 can be obtained through controlled human exposure studies on volunteer subjects or through field and epidemiological studies of populations that are exposed to ambient air containing O_3. Controlled human studies typically use fixed concentrations of O_3 under carefully regulated environmental conditions, whereas realistic O_3 exposure conditions occur in field and epidemiology studies, but are more variable. The primary purpose of all these studies, however, is to obtain exposure-response data for O_3. This chapter will summarize the results of controlled human, field, and epidemiologic studies on the health effects of exposure to O_3 that have been published or accepted for publication in the peer-reviewed literature. Further evaluation of the most important key information from this chapter, as it relates to the rest of the document, will be provided in Chapter 9, where the overall database on O_3 health effects is integrated and summarized.

Most of the scientific information selected for review and comment in this chapter comes from the literature published since completion of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Some of these newer studies were briefly reviewed in the supplement to that document (U.S. Environmental Protection Agency, 1992), but more thorough evaluation of these studies is included here. In order to give a broader overview of the known human health effects of O_3, the older literature is summarized, and specific studies whose data were judged to be significant because of their usefulness in deriving the current National Ambient Air Quality Standards (NAAQS) are discussed briefly. The reader is, however, referred to the more extensive discussion of these key studies in the previous document. Other, older studies also are briefly discussed in this chapter if they are (1) open to reinterpretation because of newer data or (2) potentially useful as criteria for the O_3 NAAQS reevaluation. To further aid in the development of this chapter, summary tables of the relevant O_3 literature are included for each of the major subsections. In summarizing the

human health effects literature, changes from control are described if they were statistically significant at a probability (p) value less than 0.05. A specific p value is provided, however, if it aids understanding of the data, particularly trends toward significance, or if major effects need to be emphasized. Where appropriate, critique of a statistical procedure also is mentioned.

7.2 Controlled Human Exposure Studies

7.2.1 Pulmonary Function Effects of One- to Three-Hour Ozone Exposures

7.2.1.1 Healthy Subjects

Introduction

The pulmonary responses observed in healthy human subjects exposed to ambient O_3 concentrations consist of decreased inspiratory capacity; mild bronchoconstriction; rapid, shallow breathing pattern during exercise; and subjective symptoms of cough and pain on deep inspiration. In addition, O_3 has been shown to result in airway hyperresponsiveness as demonstrated by an increased physiological response to a nonspecific stimulus. The decrease in inspiratory capacity results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC) and, in combination with bronchoconstriction, contributes to a decrease in the forced expiratory volume in 1 s (FEV_1). However, it is important to stress that in many of the studies reporting the effects of ambient ranges of O_3 concentrations (i.e., <0.3 ppm), the observed decrements in FEV_1, to a large extent, reflect decrements in FVC of a similar magnitude (i.e., a decreased inspiratory capacity) and, to a lesser extent, increases in central and peripheral airway resistance (R_{aw}).

The majority of controlled human studies have been concerned with the effects of various O_3 concentrations in healthy subjects performing continuous exercise (CE) or intermittent exercise (IE) for variable periods of time. These studies have two weaknesses: (1) the failure to detect short-term effects with long-term consequences and (2) their use of small numbers that are not generally representative of the general population. Controlled human exposure studies of this type have provided the strongest and most quantifiable concentration-response data on the health effects of O_3. As a result of these studies, a large body of data regarding the interaction of O_3 concentration (C), minute ventilation ($\dot{V}_E$), and duration of exposure (T) is available. The most salient observations from these studies are (1) O_3 concentration is more important than either $\dot{V}_E$ or T in determining pulmonary responses and (2) normal, healthy subjects exposed to O_3 concentrations $\geq$0.12 ppm (the level of the current NAAQS) develop significant reversible, transient decrements in pulmonary function if $\dot{V}_E$ or T are increased sufficiently. There is typically a large intersubject variability in physiologic and symptomatic responses to O_3; however, with most individuals these responses tend to be reproducible. The relationship among response variables such as spirometry, resistance measurements, symptoms, and nonspecific bronchial responsiveness is yet to be fully determined, but the generally weak associations suggest that several response mechanisms may be operant. In addition, a growing number of studies are beginning to provide insight into the relationship between regional dosimetry (see Chapter 8), mechanisms of pulmonary responses elicited by acute O_3 exposure, and tissue level events within the airways. This type of information promises to provide further insight into the health effects relevance of O_3-induced pulmonary responses in determining which individuals are at greatest risk from ambient O_3 exposure.

In this section, the effects of acute (single 1- to 3-h) O_3 exposures on pulmonary function in healthy subjects are examined by reviewing studies that investigate (1) the O_3 exposure-response relationship; (2) intersubject variability, individual sensitivity, and the association between responses; and (3) mechanisms of pulmonary function responses and the relationship between tissue-level events and functional responses. Unless otherwise stated, the term "significant" is used in this section to denote statistical significance at $p < 0.05$. Recent, single O_3 exposure studies of greater than 3 h duration are reviewed in Section 7.2.2. These single-exposure, longer duration studies are beginning to provide important insights into the $C \times T \times \dot{V}_E$ interaction related to a significant pulmonary response. Key studies of less than 3 h duration that have contributed to the exposure-response database and other studies that have contributed to a better understanding of O_3-induced pulmonary responses in healthy individuals are summarized in Table 7-1. Table 7-1 summarizes studies reviewed in the previous air quality criteria document (U.S. Environmental Protection Agency, 1986), as well as studies published since completion of this earlier document. Not reviewed in this section are studies that examine changes in airway responsiveness induced by O_3 inhalation (see Section 7.2.3). All of the studies discussed here used appropriate controls and therefore, for simplicity, the text will not indicate for each study that subjects were also exposed under similar conditions to filtered air (FA [reported at 0 ppm O_3]).

The Ozone Concentration-Response Relationship

At-Rest Exposures. No new studies examining the acute effects of a single exposure to O_3 concentrations below 1 ppm in resting humans have been published since the 1986 U.S. Environmental Protection Agency (EPA) criteria document (U.S. Environmental Protection Agency, 1986). Seven studies (Young et al., 1964; Bates et al., 1972; Silverman et al., 1976; Folinsbee et al., 1978; Horvath et al., 1979; Kagawa and Tsuru, 1979; König et al., 1980) examining 2-h, at-rest exposures were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986) involving 91 healthy subjects (74 males, 17 females) exposed to O_3 concentrations ranging from 0.1 to 1.0 ppm. The lowest concentration at which significant reductions in FVC and FEV_1 were reported was 0.5 ppm (Folinsbee et al., 1978; Horvath et al., 1979). Reports of increases in R_{aw} are inconsistent in resting human subjects exposed to O_3 concentrations below 1.0 ppm.

Exposure with Exercise. Bates et al. (1972) and Hazucha et al. (1973) were the first investigators to examine the effect on pulmonary function responses of increasing ventilation via exercise during O_3 inhalation. The IE protocol used consisted of the subjects alternating rest and light exercise on a cycle ergometer at a rate sufficient to double resting $\dot{V}_E$ for 15 min during a period of 2 h.

Hazucha et al. (1973) observed significant decreases in forced expiratory endpoints at 0.37 ppm O_3 ($p < 0.05$) and 0.75 ppm O_3 ($p < 0.001$), with subjects exposed to 0.75 ppm having the greatest decrements. After exposures, all subjects complained to varying degrees of substernal soreness, chest tightness, and cough. The important findings from these early studies were that the exercise-induced increase in $\dot{V}_E$ accentuated the observed pulmonary response at any given O_3 concentration and lowered the minimum O_3 concentration at which significant pulmonary responses were observed. Subsequently, the interaction between O_3 concentration and $\dot{V}_E$ was examined by using similar IE protocols in which both

Table 7-1. Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Adult Subjects at Rest							
0.25 0.50 0.75	490 980 1,470	2 h	NA	8 M 5 F	Young, healthy adults, 21 to 22 years old	FVC decreased with 0.50- and 0.75-ppm O_3 exposure compared with FA; 4% nonsignificant decrease in mean $\dot{V}O_{2max}$ following 0.75 ppm O_3 compared with FA exposure.	Horvath et al. (1979)
0.37 0.50 0.75	726 980 1,470	2 h	NA	20 M 8 F	Young, healthy adults, 19 to 29 years old	Decrease in FEV_1, $V_{25\%VC}$, and $V_{50\%VC}$ with 0.75 ppm O_3 exposure compared to FA.	Silverman et al. (1976)
0.50	980	2 h	NA	40 M	Young, healthy adults, 18 to 28 years old	Decrease in forced expiratory volume and flow.	Folinsbee et al. (1978)
Healthy Exercising Adult Subjects							
0.08 0.10 0.12 0.14 0.16	157 196 235 274 314	2 h IE (4 × 15 min at $\dot{V}_E$ = 68 L/min)	Tdb = 32 °C RH = 38%	24 M	Young, healthy adults, 18 to 33 years old	No significant changes in pulmonary function measurements.	Linn et al. (1986)
0.10 0.15 0.20 0.25	196 294 392 490	2 h IE (4 × 14 min treadmill at mean $\dot{V}_E$ = 70.2 L/min)	Tdb = 22 °C RH = 50%	20 M	Young, healthy NS, 25.3 ± 4.1 (SD) years old	FVC, FEV_1, $FEF_{25-75\%}$, SG_{aw}, IC, and TLC all decreased with (1) increasing O_3 concentration, and (2) increasing time of exposure; threshold for response was above 0.10 ppm but below 0.15 ppm O_3.	Kulle et al. (1985)
0.12 0.18 0.24	235 353 470	1 h competitive simulation exposures at mean $\dot{V}_E$ = 87 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	10 highly trained competitive cyclists, 19 to 29 years old	Decrease in FVC and FEV_1 for 0.18- and 0.24-ppm O_3 exposure compared with FA exposure; decrease in exercise time for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm O_3, respectively.	Schelegle and Adams (1986)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 65 L/min])	Tdb = 22 °C RH = 40%	20 M 22 M 20 M 21 M 20 M 29 M	Young, healthy adults, 18 to 30 years old	Significant decrease in FVC, FEV_1, and $FEF_{25-75\%}$ at 0.12 ppm O_3; decrease in V_T and increase in f and SR_{aw} at 0.24 ppm O_3.	McDonnell et al. (1983)

[a] O_3 concentration and level of $\dot{V}_E$ were varied.

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics[c]	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2 × 2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA]). Exposure separated by 48 ± 30 days and 301 ± 77 days	Tdb = 22 C RH = 40%	8 M 8 M 5 M 5 M 6 M	Young, healthy adults, 18 to 30 years old	Pulmonary function variables SR_{aw} and $\dot{V}_E$ were not significantly different in repeat exposures, indicating that the response to 0.18 ppm O_3 or higher is reproducible.	McDonnell et al. (1985b)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2 × 2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA])	Tdb = 22 C RH = 40%	290 M	Young, healthy adults, 18 to 32 years old	O_3 concentration and age predicted FEV_1 decrements; it was concluded that age is a significant predictor of response (older subjects being less responsive to O_3).	McDonnell et al. (1993)
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.5 h IE (4 × 15 min treadmill exercise [$\dot{V}_E$ = 25 L/min/m^2 BSA])	Tdb = 22 C RH = 40%	17 WM/15 BM/15 WF/ 15BF 15 WM/15 BM/15 WF/ 16BF 15 WM/17 BM/17 WF/ 15BF 16 WM/15 BM/17 WF/ 16BF 15 WM/15 BM/15 WF/ 15BF 15 WM/15 BM/15 WF/ 15BF	Young, healthy whites and blacks, 18 to 35 years old	Decreases in FEV_1 for all levels of O_3 as compared with FA; increase in SR_{aw} with 0.18 ppm O_3 and greater compared with FA; black men and women had larger FEV_1 decrements than white men, and black men had larger FEV_1 decrements than white women.	Seal et al. (1993)
0.12 0.20	235 392	1 h CE (mean $\dot{V}_E$ = 89 L/min)	Tdb = 31 C	15 M 2 F	Highly trained competitive cyclists, 19 to 30 years old	Decrease in $\dot{V}_{Emax}$, VO_{2max}, V_{Tmax}, work load, ride time, FVC, and FEV_1 with 0.20 ppm O_3 exposure during maximal exercise conditions, but not significant with 0.12 ppm O_3 exposure, as compared to FA exposure.	Gong et al. (1986)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.16 0.24 0.32	314 470 627	1 h CE (mean $\dot{V}_E$ = 57 L/min)	Tdb = 32 °C RH = 42 to 46%	42 M 8 F	Competitive bicyclists, 26.4 ± 6.9 (SD) years old	Small decrements in FEV_1 at 0.16 ppm with larger decrements at 0.24 ppm O_3.	Avol et al. (1984)
0.20	392	4 h IE (4 × 50 min cycle ergometry or treadmill running [$\dot{V}_E$ = 40 L/min])	Tdb = 20 °C RH = 50%	11 M 3 F (FA exposure); 9 M 3 F (O_3 exposure)	Adult, healthy NS, 19 to 41 years old	Decrease in FVC, FEV_1, V_T, and SR_{aw} and increase in f with O_3 exposure compared with FA; total cell count and LDH increased in isolated left main bronchus lavage and inflammatory cell influx occurred with O_3 exposure compared to FA exposure.	Aris et al. (1993a)
0.20 0.30	392 588	30 to 80 min CE cycle ergometry ($\dot{V}_E$ = 33 or 66 L/min)	Tdb = 20 to 24 °C RH = 40 to 60%	8 M	Aerobically fit, 22 to 46 years old	O_3 effective dose was significantly related to pulmonary function decrements and exercise ventilatory pattern changes; multiple regression analysis showed that O_3 concentration accounted for the majority of the pulmonary function variance.	Adams et al. (1981)
0.20 0.35	392 686	1 h CE or competitive simulation (mean $\dot{V}_E$ = 77.5 L/min)	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Well-trained distance runners, 19 to 31 years old	Decrease in FVC, FEV_1, and $FEF_{25-75\%}$ with 0.20 and 0.35 ppm O_3 exposure compared with FA; V_T decreased and f increased with continuous 50-min O_3 exposures; three subjects unable to complete continuous and competitive protocols at 0.35 ppm O_3.	Adams and Schelegle (1983)
0.21	412	1 h CE (75% $\dot{V}O_{2max}$)	Tdb = 19 to 21 °C RH = 60 to 70%	6 M 1 F	Well-trained cyclists, 18 to 27 years old	Decrease in FVC, FEV_1, $FEF_{25-75\%}$, and MVV with 0.21 ppm O_3 compared with FA exposure.	Folinsbee et al. (1984)
0.21	412	1 h CE cycle ergometry (mean $\dot{V}_E$ = 80 L/min)	Tdb = 22.5 °C RH = 58.8%	14 M 1 F	Highly fit endurance cyclists, 16 to 34 years old	No significant differences in the effects of albuterol on metabolic data, pulmonary function, airway reactivity, and exercise performance vs. placebo; decrease in $\dot{V}_{Emax}$ during O_3 conditions.	Gong et al. (1988)
0.25	490	1 h CE (mean $\dot{V}_E$ = 63 L/min)	Tdb = 20 °C RH = 70%	19 M 7 F	Active nonathletes	FVC, FEV_1, and MVV all decreased with 0.25 ppm O_3 exposure compared with FA.	Folinsbee et al. (1986)
0.25	490	1 h CE cycle ergometer ($\dot{V}_E$ = 30 L/min/m² BSA)	NA	5 M 2 F	Young, healthy NS, 22 to 30 years old	12.4% decrease in FEV_1. Significant elevation of substance P and 8-epi-$PGF_{2\alpha}$ in segmental airway washing, but not bronchoalveolar lavage fluid.	Hazbun et al. (1993)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.30	588	1 h CE cycle ergometry (mean $\dot{V}_E$ = 60 L/min)	NA	5 M	Normal	Decrease in FVC and FEV and increase in SR 1 h post-O exposure; increase in percent PMNs at 1, 6, and 24 h post-O_3 exposure compared with FA in first aliquot "bronchial" sample. PMNs peaked at 6 h post-O_3 in "bronchial" sample. Percent PMNs elevated at 6 and 24 h post-O_3 in pooled aliquots.	Schelegle et al. (1991)
0.30	588	1 h CE cycle ergometry ($\dot{V}_E$ = 60 L/min) and 2 h IE cycle ergometry ($\dot{V}_E$ = 45 to 47 L/min)	Tdb = 21 to 25 °C RH = 45 to 60%	12 M	Moderately fit, young and healthy	Decrease in FEV_1 equivalent for all protocols.	McKittrick and Adams (1995)
0.35	686	1 h CE cycle ergometry (mean $\dot{V}_E$ = 60 L/min)	Tdb = 21 to 25 °C RH = 45 to 60%	14 M	Moderately fit, young, healthy adults, 18 to 34 years old	Significant decreases in FVC and FEV_1 with O_3 exposure compared to FA exposure; FVC and FEV_1 decreases with O_3 exposure were attenuated significantly with indomethacin compared to no drug and placebo; SR_{aw} increases were not affected by indomethacin.	Schelegle et al. (1987)
0.37 0.50 0.75	726 980 1,470	2 h IE cycle ergometry ($\dot{V}_E$ = 2.5 × rest)		20 M 8 F	Young, healthy adults, 19 to 29 years old	Decrease in FVC with 0.50 ppm and FEV_1 with 0.50 and 0.75 ppm O_3 compared to FA; decrease in $V_{25\%VC}$ with 0.37 and 0.75 ppm and $V_{50\%VC}$ with 0.37, 0.50, and 0.75 ppm O_3 exposure compared to FA.	Silverman et al. (1976)
0.40	784	2 h IE treadmill exercise ($\dot{V}_E$ = 50 to 75 L/min)	Tdb = 22 °C RH = 40%	8 M	Young, healthy NS, 18 to 27 years old	Decreases in FVC, FEV_1, V_T, and TLC and increases in SR_{aw} and f with O_3 exposure compared with FA. Atropine pretreatment abolished O_3-induced increase in SR_{aw} and attenuated FEV_1 and $FEF_{25-75\%}$ response.	Beckett et al. (1985)
0.40	784	1 h CE treadmill exercise; ($\dot{V}_E$ = 20 L/min/m² BSA)	NA	20 M	Young, healthy NS	V_T fell by 25%, and O_3 uptake efficiency in the lower respiratory tract fell by 9% during O_3 exposure.	Gerrity et al. (1994)
0.40	784	2 h IE (4 × 15 min heavy treadmill exercise [$\dot{V}_E$ = 35 L/min/m² BSA])	NA	11 M	Young, healthy NS, 18 to 35 years old	No correlation between pulmonary function and inflammatory endpoints measured in BAL fluid obtained 18 h after exposure; increase in percentage of PMNs, total protein, albumin, IgG, and neutrophil elastase; decrease in percentage of macrophages with O_3 exposure compared to FA exposure.	Koren et al. (1989a)

Table 7-1 (cont'd). Controlled Exposure of Healthy Human Subjects to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Healthy Exercising Adult Subjects (cont'd)							
0.40	784	2 h IE (4 × 15 min heavy treadmill exercise [$\dot{V}_E$ = 35 L/min/m^2 BSA])	Tdb = 22 °C RH = 40%	10 M	Young, healthy NS, 18 to 35 years old	PMN, PGE_2, and IL-6 were higher in BAL fluid obtained 1 h post-O_3 exposure than 18 h; fibronectin and urokinase-type plasminogen activator were higher 18 h post-O_3 exposure than 1 h.	Koren et al. (1991)
0.40	784	2 h IE (4 × 15 min bicycle ergometry [$\dot{V}_E$ = 30 L/min/m^2 BSA])	Tdb = 22 °C RH = 50%	13 M	NS, 18 to 31 years old	Indomethacin pretreatment and O_3 exposure resulted in a significantly smaller decrease in FVC and FEV_1 than O_3 exposure alone; airway hyperresponsiveness was not significantly affected by indomethacin pretreatment.	Ying et al. (1990)
0.40	784	1 h CE (treadmill exercise; $\dot{V}_E$ = 20 L/min/m^2 BSA)	Tdb = 22 °C RH = 40%	22 M	Young, healthy NS, 18 to 35 years old	Significant decreases in FVC, FEV_1, FEV_1/FVC, and $FEF_{25-75\%}$. The half-width of an expired aerosol bolus was significantly increased, suggesting an ozone-induced change in small airway function.	Keefe et al. (1991)
0.40 0.60	784 1,176	2 h IE (4 × 15 min cycle ergometry at 100 W for males and 83 W for females)	Tdb = 71.5 °C RH = 55%	7 M 3 F	Healthy NS, 23 to 41 years old	Increase in airway responsiveness to methacholine challenge, in mean percentage of neutrophils, and in $PGF_{2\alpha}$, TXB_2, and PGE_2 concentrations measured in BAL fluid 3 h after 0.40- and 0.60-ppm O_3 exposure compared with FA exposure.	Seltzer et al. (1986)
0.50	980	2 h IE (4 × 15 min treadmill exercise; $\dot{V}_E$ = 40 L/min)	Tdb = 21 °C RH = 40%	18 M	Healthy, young adults, 20 to 30 years old	Decrease in VC, V_T, and maximal transpulmonary pressure, and increase in SR_{aw} and f with O_3 exposure compared to FA exposure; lidocaine inhalation partially reversed the decrease in VC.	Hazucha et al. (1989)
0.75	1,470	2 h IE (4 × 15 min light [50 W] cycle ergometry)	NA	13 M	4 light S, 9 NS, 19 to 30 years old	Decrease in FVC, FEV_1, ERV, IC, and $FEF_{50\%}$ after 1 h exposure to 0.75 ppm O_3; decrease in $\dot{V}O_{2max}$, V_{Tmax}, $\dot{V}_{Emax}$, maximal workload, and heart rate following 0.75-ppm O_3 exposure compared with FA.	Folinsbee et al. (1977)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by rest and exercise; within groups listed from lowest to highest O_3 concentration.

Silverman et al. (1976) and Folinsbee et al. (1975) exposed a group of 20 males and 8 females to 0.37, 0.50, or 0.75 ppm for 2 h while resting or exercising intermittently. The IE protocol used alternated 15 min of rest with 15 min of exercise, sufficient to increase the $\dot{V}_E$ value at rest by a factor of 2.5. The submaximal exercise responses of the subjects were tested postexposure using a three-stage cycle ergometer test, with loads adjusted to 45, 60, and 75% of maximum oxygen uptake ($\dot{V}O_{2max}$) (Folinsbee et al., 1975). Pulmonary function responses were related to the total inhaled dose or the "effective dose" of O_3 calculated as the product of $C \times T \times \dot{V}_E$. Neither submaximal exercise oxygen uptake ($\dot{V}O_2$) nor $\dot{V}_E$ were affected significantly by any level of O_3 exposure; however, a significant increase in respiratory frequency (f) and a significant decrease in tidal volume (V_T) at the 75% $\dot{V}O_{2max}$ workload were observed. The relationship between the effective dose of O_3 and the mean percent change in selected measures of lung function was analyzed using linear regression. Forced vital capacity, maximum expiratory flow at 25 and 50% of FVC ($\dot{V}_{max25\%}$ and $\dot{V}_{max5\ \%}$, respectively), and FEV_1 were found to have a significant linear correlation with the effective dose. The description of the relationship between O_3 pulmonary function decrements and effective dose was apparently improved by the use of a second-order polynomial model in which effective dose was used as the independent variable.

Although the investigations of Silverman et al. (1976) and others (Bates et al., 1972; Hackney et al., 1975; Hazucha et al., 1973) clearly demonstrate the potentiating effects of exercise on O_3 responses, the level of exercise used in these studies was low, requiring increases in $\dot{V}_E$ of only 2 to 2.5 times resting, a level of exercise lower than that of a subject walking at 5.5 km/h (DeLucia and Adams, 1977). In order to address this concern, DeLucia and Adams (1977) exposed six healthy nonsmoking male subjects on 12 separate occasions to FA and 0.15 and 0.30 ppm O_3 for 1 h, while at rest and while exercising continuously at workloads that required 25, 45, and 65% of the subjects' $\dot{V}O_{2max}$. They observed a significant time-dependent increase in f during the 65% $\dot{V}O_{2max}$, 0.30-ppm O_3 exposure, and, immediately following this same exposure, there was a significant decrease in FEV_1 and forced expiratory flow at 25 to 75% of FVC ($FEF_{25\ 75\%}$).

These initial studies, which clearly demonstrated the potentiating effects of exercise on human responses to acute O_3 exposure, provided the impetus for a series of studies (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985; Linn et al., 1986) designed to define more precisely O_3 exposure-response relationships. These investigations utilized both IE (Folinsbee et al., 1978; McDonnell et al., 1983) and CE (Adams et al., 1981) of varying intensity. Folinsbee et al. (1978) exposed four groups of 10 subjects each to FA, 0.1, 0.3, and 0.5 ppm O_3 for 2 h. One group was exposed while at rest, and the other three groups were exposed while performing IE at levels requiring a ventilation of 30, 50, or 70 L/min. These combinations of ventilation and O_3 concentration ($C \times T \times \dot{V}_E$) resulted in a range of total inhaled effective dose of 0.00 to 4.41 mg O_3. Adams et al. (1981) exposed eight trained male subjects to FA, 0.2, 0.3, and 0.4 ppm O_3 while they exercised continuously at two different workloads (35 and 62% of $\dot{V}O_{2max}$) for durations ranging from 30 to 80 min. Each subject completed all 18 protocols with at least 3 days between each. The findings from these two studies confirmed that significant pulmonary responses occurred at 0.3 ppm when subjects exercised at moderately heavy workloads. It was further demonstrated, by multiple regression analysis, that the O_3 effective dose was a better predictor of response than O_3 concentration, $\dot{V}_E$, or duration of exposure, alone. Multiple regression analysis also revealed that the majority of variance for pulmonary function responses was accounted for by

O_3 concentration, followed by $\dot{V}_E$. In the Adams et al. (1981) study, in which both workload and duration of exposure were varied, duration of exposure was observed to be the poorest predictor of response for all parameters analyzed. However, the minor impact of changes in exposure duration could have been an artifact of the limited combinations of ventilation and durations of exposure used by these investigators.

McDonnell et al. (1983) conducted a study with the primary purpose of discerning the lowest concentration of O_3 at which group mean decrements in pulmonary function occur in heavily exercising healthy men. In order to determine a concentration-response relationship, six groups of subjects (n = 20 to 29) were exposed to either an FA control or one of five O_3 concentrations (0.12, 0.18, 0.24, 0.30, or 0.40 ppm) at a $\dot{V}_E$ of 67 L/min and exposure duration of 2.5 h (15-min rest, 15-min exercise). These investigators observed small significant changes in FVC, FEV_1, $FEF_{25\ 75\%}$, and cough at 0.12 ppm O_3 and concentration-dependent responses in all variables measured (FVC, FEV_1, $FEF_{25\ 75\%}$, specific airway resistance [SR_{aw}], f, V_T, and subjective symptoms) at O_3 concentrations >0.24 ppm.

Kulle et al. (1985) also conducted a similar study on healthy, nonsmoking men performing IE at a $\dot{V}_E$ of 70 L/min for an exposure duration of 2 h, (16-min rest, 14-min exercise). Twenty subjects were exposed to an FA control or one of four O_3 concentrations (0.10, 0.15, 0.20, or 0.25 ppm). These investigators observed a significant C × T interaction at 0.15 ppm O_3 for FVC, FEV_1, $FEF_{25\ 75\%}$, and in all variables measured (FVC, FEV_1, $FEF_{25\ 75\%}$, SR_{aw}, f, V_T, and subjective symptoms) at O_3 concentrations greater than 0.15 ppm.

Linn et al. (1986) exposed 24 healthy, well-conditioned male subjects (18 to 33 years of age) for 2 h to 0.00, 0.08, 0.10, 0.12, 0.14, or 0.16 ppm O_3, using an IE protocol (15-min rest, 15-min exercise; $\dot{V}_E$ = 68 L/min) combined with an ambient heat stress (32 °C and 38% relative humidity [RH]). They observed no statistically significant changes in forced expiratory endpoints and symptoms after exposure to O_3 concentrations from 0.08 to 0.14 ppm. These authors observed a small (−2.3%) but significant ($p < 0.05$) reduction in FEV_1, which was not associated with symptoms of respiratory discomfort, following the 2-h 0.16-ppm O_3 exposure.

More recently, Seal et al. (1993) examined whether gender or race differences exist in responsiveness to O_3. The authors exposed 372 white and black, males and females (n > 90 in each gender-race group) once for 2.33 h to 0.0, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 using an IE protocol (15-min rest, 15-min exercise; $\dot{V}_E$ = 25 L/min/m^2 body surface area [BSA]). Statistical analysis (nonparametric two-factor analysis of variance) of the percent changes from baseline for FEV_1, SR_{aw}, and cough responses demonstrated no significant differences in responsiveness to O_3 between the gender-race groups studied. Changes in FEV_1, SR_{aw}, and cough were first noted at 0.12, 0.18, and 0.18 ppm O_3, respectively, for the group as a whole. It is difficult to compare the results from this study with other studies that have examined the O_3 concentration-response relationship in healthy adult males because the authors did not present a separate analysis of male responses. For further evaluation of the influence of gender and race on O_3 responsiveness, see Section 7.2.1.3.

The observation of significant decrements in pulmonary function in heavily exercising healthy subjects at O_3 concentrations of 0.2 ppm and lower has been confirmed by numerous investigators (Adams and Schelegle, 1983; Avol et al., 1984; Folinsbee et al., 1984; Gong et al., 1986) who utilized 1-h continuous heavy exercise exposure protocols. Adams and Schelegle (1983) and Folinsbee et al. (1984) observed significant decrements in FVC and FEV_1

in well-trained subjects exposed to 0.2 ppm O_3 while exercising with a $\dot{V}_E$ of approximately 80 L/min. Avol et al. (1984) observed small but significant decrements in FVC and FEV_1 in a group of 50 competitive cyclists (42 males, 8 females) exposed to 0.16 ppm O_3 while exercising with a $\dot{V}_E$ of 57 L/min in combination with added heat stress (32 °C). Similarly, Gong et al. (1986) observed modest but significant decrements in FVC and FEV_1 in a group of 17 top-caliber endurance cyclists exposed to 0.12 ppm O_3 while exercising at approximately 70% of their $\dot{V}O_{2max}$ (mean $\dot{V}_E$ = 89 L/min) with an added heat stress (32 °C). In addition to the above studies that used continuous exercise, Schelegle and Adams (1986) observed significant reductions in FVC and FEV_1 and increased symptoms of respiratory discomfort following exposure to 0.18 but not 0.12 ppm O_3 in a group of competitive endurance athletes exposed while performing a competitive simulation consisting of a 30-min warm-up followed by a 30-min competitive bout (mean $\dot{V}_E$ over entire protocol = 87 L/min).

The studies reviewed above demonstrate that in healthy young adults performing moderate to severe IE and CE of 1 to 3 h duration, an O_3 concentration of 0.12 to 0.18 ppm is required to elicit statistically significant decrements in pulmonary function and subjective respiratory symptoms.

Retrospective analysis by Hazucha (1987) confirmed the previously reported (Adams et al., 1981; Folinsbee et al., 1978) dominant role that O_3 concentration plays in determining O_3-induced responses. Hazucha (1987) analyzed data from studies that utilized IE protocols of 2 h in duration. While controlling for ventilation, this investigator found that the data best fit a model that was a quadratic function of O_3 concentration. Based on this analysis, Hazucha (1987) also concluded that an O_3 concentration below which no pulmonary function response would be elicited could not be defined.

The studies reviewed in this subsection used different patterns (i.e., CE or IE) of exercise during their exposure protocols. An important question to ask is to what extent are the results of these studies comparable when total inhaled doses are the same but exercise pattern differs. A recent study by McKittrick and Adams (1995) addresses this question. These investigators exposed 12 aerobically trained men to 0.30 ppm O_3 (three protocols) and FA (three protocols) on six occasions. These protocols consisted of a 1 h CE (O_3 and FA) and two 2-h IE (2 × O_3 and 2 × FA) protocols delivered in random sequence separated by a minimum of 3 days. Lung function FEV_1 decrements of 17.6, 17.0, and 17.9% were obtained for the 1-h CE and the two 2-h IE, 0.3-ppm O_3 protocols, respectively. These values were significantly different from the FA values, but were not significantly different from each other. The O_3 CE protocols resulted in greater postexposure values for subjective symptoms than obtained with either of the O_3 IE protocols. However, the overall symptom severity during the last minute of exercise for the two IE protocols was not significantly different from the CE postexposure value. McKittrick and Adams (1995) concluded that when the total inhaled dose of O_3 is equivalent at a given O_3 concentration, there is no difference between pulmonary function responses induced by CE and IE protocols of 2-h or less duration, although subjective symptoms are reduced slightly during the last rest period of IE.

Intersubject Variability, Individual Sensitivity, and the Association Between Responses

Bates et al. (1972) noted that variation in sensitivity and response was evident for various symptoms and pulmonary functions assessed following O_3 exposure. This observation of large intersubject variability in response to O_3 also has been reported by numerous other investigators (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985) and is illustrated by data from Kulle et al. (1985) plotted in Figure 7-1. The description of the factors that contribute to intersubject variability is important for the understanding of individual responses, mechanisms of response, and health risks associated with acute exposures. The effect of this large intersubject variability on the ability to predict individual responsiveness to O_3 was recently demonstrated by McDonnell et al. (1993). These investigators analyzed the data of 290 white male subjects (18 to 32 years of age) who inhaled either 0.00, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 for 2 h while performing an IE protocol ($\dot{V}_E$ = 35 L/min/m^2 BSA) to identify personal characteristics (i.e., age, height, baseline pulmonary functions, presence of allergies, and past smoking history) that might predict individual differences in FEV_1 response. Of the personal characteristics studied, only age contributed significantly to intersubject responsiveness (younger subjects were more responsive), accounting for 4% of the observed variance. Interestingly, O_3 concentration accounted for only 31% of the variance, clearly demonstrating the importance of as yet undefined individual characteristics that determine responsiveness to O_3.

McDonnell et al. (1985b) examined the reproducibility of individual responses to O_3 exposure in healthy human subjects exposed twice, with from 21 to 385 days separating exposures (mean = 88 days). This investigation was conducted in order to determine whether the observed intersubject variability is due primarily to real differences in O_3 responsiveness among subjects, or whether it can be accounted for by other sources of variability. The authors examined FVC, FEV_1, $FEF_{25\ 75\%}$, SR_{aw}, cough, shortness of breath (SB), pain on deep inspiration (PDI), V_T, and f responses induced by O_3 exposure to concentrations ranging from 0.12 to 0.40 ppm. Reproducibility was assessed using the intraclass correlation coefficient (R), which incorporates into a single measure all the information contained in the correlation coefficient, slope, and intercept obtained in linear regression analysis. Similar to the more routinely used correlation coefficient, R is equal to one when two identical measurements occur in the same subject; and the "worst" possible coefficient is equal to 1/(n − 1), which approaches zero for a large n. The ranking of most to least reproducible for the responses studied was FVC (R = 0.92), FEV_1 (R = 0.91), $FEF_{25\ 75\%}$ (R = 0.83), cough (R = 0.77), SB (R = 0.60), SR_{aw} (R = 0.54), PDI (R = 0.37), f (R = −0.20), and V_T (R = −0.03). The value of R was significantly different from zero for FVC, FEV_1, $FEF_{25\ 75\%}$, cough, SB, and SR_{aw}. McDonnell et al. (1985b) concluded that the reported large intersubject variability in magnitude of response was due to large differences in the intrinsic responsiveness of individual subjects to O_3 exposure. However, the factors that contribute to this large intersubject variability remain undefined.

The examination of intersubject variability is complicated by a poor association between the various O_3 responses. In their study investigating O_3 exposure-response relationships, McDonnell et al. (1983) observed very low correlation between changes in SR_{aw} and FVC (r = −0.16) for 135 subjects exposed to O_3 concentrations ranging from 0.12 to 0.40 ppm for 2.5 h.

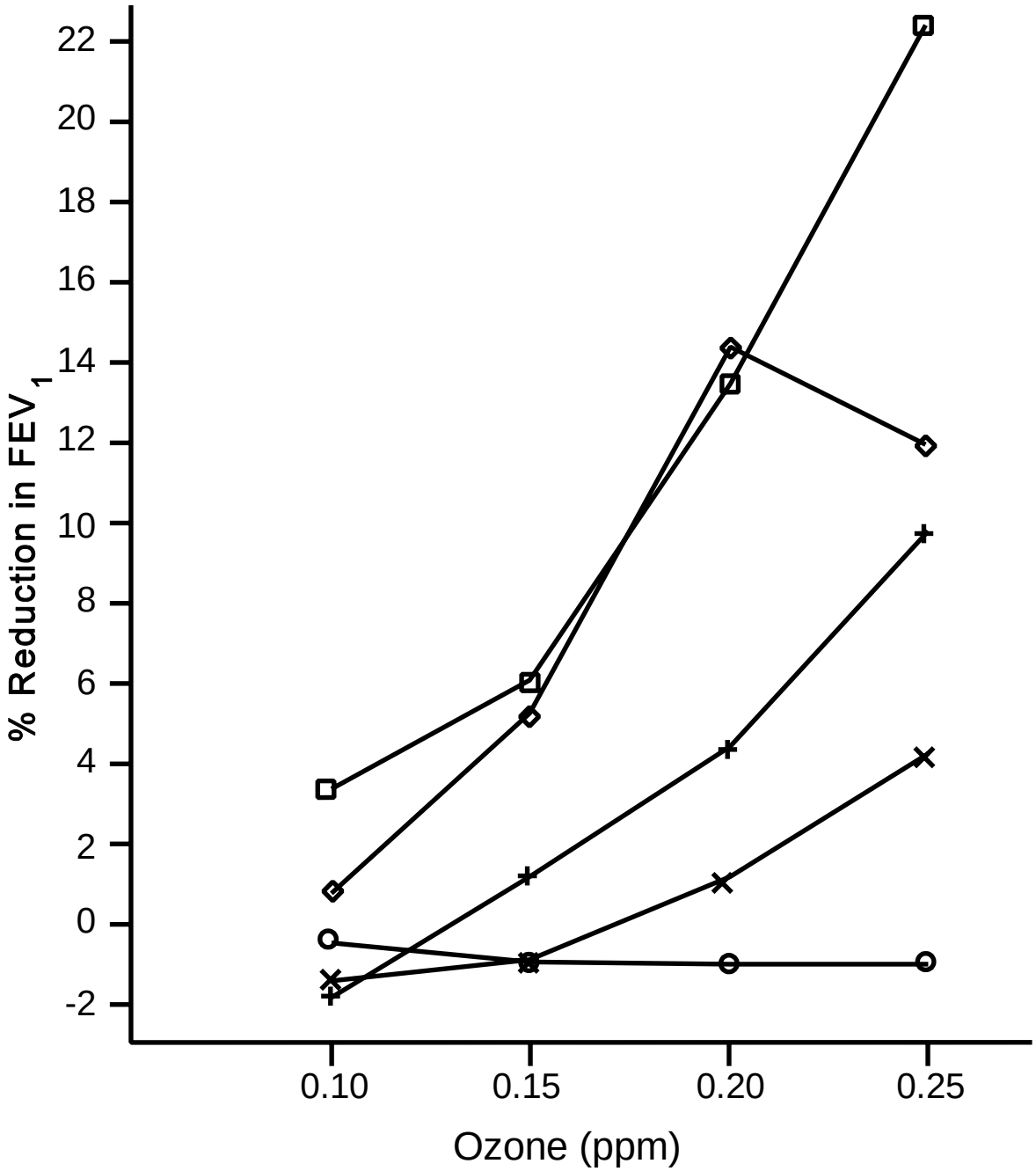

Figure 7-1. Individual concentration-response curves for five separate subjects exposed to 0.10, 0.15, 0.20, and 0.25 ppm ozone (O_3) for 2 h with moderate intermittent exercise. Illustrates the wide variability in responsiveness to O_3 from individual to individual.

Source: Kulle et al. (1985).

Mechanisms of Acute Pulmonary Responses

The pulmonary responses observed during and following acute exposure to O_3 at concentrations between 0.1 and 0.5 ppm in normal healthy human subjects include decreases in TLC, IC, FVC, FEV_1, $FEF_{25\ 75\%}$, and V_T; and increases in SR_{aw}, f, and airway responsiveness. Ozone exposure also has been shown to result in the symptoms of cough, PDI, SB, throat irritation, and wheezing. When viewed as a whole, changes in these specific parameters can be categorized into four general responses: alterations in (1) lung volumes, (2) airway caliber, (3) bronchomotor responsiveness, and (4) symptoms. The absence of consistent associations among the various responses from individual to individual suggests that the functional responses observed are the result of multiple interactions within the respiratory tract. These interactions may be the result of O_3 action on the biochemical, anatomical, and physiological systems of the respiratory tract. In turn, these factors determine O_3 dose distribution and the resulting cellular and reflex responses.

Bates et al. (1972) observed that the most significant decrement in pulmonary function was the reduction in the transpulmonary pressure at maximal inspiratory volume without a concomitant decrease in static compliance. This would suggest an inhibition of maximal inspiratory effort after O_3 exposure that may result in reductions in IC. These authors speculated that this inhibition is an early result of stimulation of rapidly adapting pulmonary stretch receptors, or "irritant receptors", located in the major bronchi. Since 1972, when this hypothesis was first published, numerous studies have examined the underlying mechanisms leading to the functional responses observed in human subjects. These mechanistic studies have used both animal models and human subjects. This discussion of mechanisms will focus on studies that used human subjects but also will cover those animal studies that have direct relevance to O_3-induced functional responses.

The acute inhalation of ambient concentrations of O_3 by healthy human subjects has been shown to result in a concentration-dependent increase in R_{aw} (Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985; Seal et al., 1993). This O_3-induced increase in R_{aw} has been shown to be poorly correlated with changes in forced expiratory endpoints (McDonnell et al., 1983). Ozone-induced increases in R_{aw} have a rapid onset (Beckett et al., 1985) compared with the gradual development of decrements in forced expiratory endpoints (Kulle et al., 1985). Ozone-induced increases in R_{aw} also appear to be greater in atopic subjects as a group (Kreit et al., 1989; McDonnell et al., 1987), although this does not appear to be the case for O_3-induced decrements in FVC and symptoms. Taken together, these observations suggest that different pathways lead to O_3-induced decrements in IC and to O_3-induced increments in R_{aw}.

Increases in R_{aw} induced by O_3 have been shown to be blocked by atropine sulfate pretreatment in human subjects (Beckett et al., 1985; Adams, 1986). This inhibition suggests that the release of acetylcholine from parasympathetic postganglionic fibers that innervate airway smooth muscle plays a role in this response. However, the observation that a 2-h, 0.6 ppm O_3 inhalation also results in a hyperresponsiveness to methacholine, a cholinergic agent (Holtzman et al., 1979), suggests the possibility that acute O_3 exposure also can increase the sensitivity of airway smooth muscle to acetylcholine independent of a reflex mechanism involving cholinergic postganglionic nerves. The role that an increase in airway smooth muscle sensitivity to the endogenous release of acetylcholine might play in O_3-induced increases in R_{aw} has not been studied.

Analyses by Colucci (1983) have suggested that the increase in R_{aw} is not as large as would be expected when O_3 exposure is combined with moderate to heavy exercise. However, the observation that circulating epinephrine levels increase as a function of the relative workload in exercising human subjects (Galbo, 1983; Warren and Dalton, 1983) suggests that stimulation of airway smooth muscle beta-adrenoreceptors may counteract airway smooth muscle contraction induced by O_3 exposure. The observations by Beckett et al. (1985) that the beta-agonists abolish O_3-induced bronchoconstriction is consistent with this possibility.

Another question to be addressed with regard to O_3-induced increases in R_{aw} is where along the airway (central versus peripheral airways) is the increase in resistance produced? Studies of acutely and subchronically exposed animals have demonstrated tissue damage in the centriacinar region (Castleman et al., 1977; Mellick et al., 1977), as well as increases in peripheral resistance and reactivity (Gertner et al., 1983a,b,c; Beckett et al., 1988). Keefe et al. (1991) examined the possibility of an effect on small airways using an inhaled aerosol bolus dispersion technique in 22 healthy, nonsmoking male subjects exposed to 0.4 ppm O_3 for 1 h using a CE protocol ($\dot{V}_E$ = 20 L/min/m^2 BSA). The bolus dispersion technique is not dependent on vital capacity maneuvers and compares the profile of a bolus of small (0.5- to 1.0-μm) aerosol particles injected into the inspired airstream (at a fixed lung volume) with the profile of the bolus during expiration. Dispersion of the bolus during expiration can be affected by increases in turbulence within the airway, the development of asymmetries in ventilation due to unequal regional time constants within the lung, and an increase in aerosol deposition in the small airways. Keefe et al. (1991) observed that O_3 exposure in their subjects resulted in a significant increase in dispersion of an aerosol bolus (without an increased aerosol deposition) that was not correlated with changes in SR_{aw}. These findings suggest that exposure to 0.4 ppm O_3 under the conditions of this experiment results in changes in small airway function that are not detectable by more conventional techniques.

Ozone-induced alterations in ventilatory pattern have been observed in exercising dogs (Lee et al., 1979) and humans (Adams et al., 1981; Folinsbee et al., 1978; McDonnell et al., 1983; Kulle et al., 1985). In exercising humans, O_3 exposure has been shown to result in a decrease in V_T and an increase in f in the absence of any change in $\dot{V}_E$. A rapid, shallow breathing pattern is consistent with the maintenance of an appropriate ventilation with a reduced VT. Reduction of VT is probably related to the reduction of IC and is anecdotally related to reduction in breathing discomfort caused by PDI.

Lee et al. (1979), who produced a reversible vagotomy by cooling the vagus nerves to 0 °C, abolished the rapid, shallow breathing induced by O_3 inhalation in conscious dogs. More recently, Schelegle et al. (1993) have shown in anesthetized dogs exposed to O_3 that cooling the cervical vagus nerves to 7 °C did not abolish the observed O_3-induced rapid, shallow breathing pattern and bronchoconstriction, but cooling the vagus nerves to 0 °C did abolish both the rapid, shallow breathing and the bronchoconstriction. These findings suggest that O_3 stimulates nonmyelinated C fiber afferents arising from the lung, whose conduction is not totally blocked at 7 °C but is blocked totally at 0 °C. This conclusion is consistent with the findings of Coleridge et al. (1993) that bronchial C fibers are the only receptors that are stimulated directly during O_3 inhalation in anesthetized dogs. If similar bronchial C fibers were stimulated or sensitized in humans exposed to O_3, this could explain the O_3-induced rapid, shallow breathing observed during exercise, as well as the subjective symptoms associated with taking a deep inspiration.

Hazucha et al. (1989) exposed 11 healthy normal volunteers to FA and 0.5 ppm O_3 for 2 h while they were performing moderate IE. Ozone exposure induced a significant decrement in FVC, which was associated with a marked fall in IC without an increase in residual volume. Spraying of the upper airway with lidocaine aerosol in these subjects was immediately followed by return of FVC toward control values. Hazucha et al. (1989) concluded that O_3 inhalation stimulates lidocaine-sensitive tracheal and laryngeal airway receptors, which leads to an involuntary inhibition of full inspiration, a reduction in FVC, and a concomitant decrease in maximal expiratory flow rates in humans.

The airway afferents blocked by lidocaine in the Hazucha et al. (1989) investigation remain undefined. However, it seems likely that the lung afferents involved are the same ones that result in O_3-induced rapid, shallow breathing in dogs (i.e., bronchial C fibers). When stimulated with exogenous chemicals in animal experiments, bronchial C fibers induce a reflex apnea (Coleridge and Coleridge, 1986). In dogs, this reflex apnea involves the inhibition of inspiratory neurons, expiratory neurons, and α- and γ-motoneurons in the intercostal nerves (Koepchen et al., 1977; Schmidt and Wellhoner, 1970). Such a reflex response in humans would explain the reflex inhibition of maximal inspiration consequent to acute O_3 exposure.

Data consistent with an O_3-induced stimulation of bronchial C fibers in human subjects recently has been published by Hazbun et al. (1993). These investigators observed a significant increase in substance P, the neurotransmitter released from the afferent endings of bronchial C fiber during excitation, in segmental airway washings of seven (2 female/5 male) healthy, nonsmoking subjects after a 1 h CE ($\dot{V}_E$ = 30 L/min/m^2 BSA) exposure to 0.25 ppm O_3. Substance P was not elevated in bronchoalveolar lavage (BAL) fluid after air exposure. In addition, the segmental airway substance P levels were significantly correlated ($r^2 = 0.89$; $p < 0.05$) with an elevated airway concentration of 8-epi-prostaglandin $F_{2\alpha}$, a marker of oxidative free radical reactions. These results are consistent with (1) an increased release of substance P secondary to an increased discharge of bronchial C fibers induced by O_3 inhalation, and (2) an O_3-induced inhibition of neutral endopeptidase, the enzyme that degrades substance P within the airways.

Lung C fibers have been shown to be stimulated by prostaglandin E_2 and other lung autacoids (Coleridge et al., 1978, 1976). Interestingly, Schelegle et al. (1987), Eschenbacher et al. (1989), and Ying et al. (1990) have shown that pretreatment with the cyclooxygenase inhibitor indomethacin reduces and, in some cases, totally abolishes O_3-induced pulmonary function decrements in human subjects. Schelegle et al. (1987) examined whether O_3-induced pulmonary function decrements could be inhibited by the prostaglandin synthetase inhibitor indomethacin in healthy human subjects. Fourteen college-age males completed six 1-h exposure protocols consisting of no drug, placebo, and indomethacin pretreatments, with FA and O_3 (0.35 ppm) exposure within each pretreatment. Pretreatments were delivered weekly in random order in a double-blind fashion. Exposures consisted of 1 h exercise on a cycle ergometer with work loads set to elicit a mean $\dot{V}_E$ of 60 L/min. Statistical analysis revealed significant effects for FVC and FEV_1 across pretreatment, with no drug versus indomethacin and placebo versus indomethacin comparisons being significant. These findings suggest that cyclooxygenase products of arachidonic acid, which are reduced by indomethacin inhibition of cyclooxygenase, play a role in the development of pulmonary function decrements. These and similar findings by Eschenbacher et al. (1989) and Ying et al. (1990) suggest that the release of some cyclooxygenase product consequent to O_3 inhalation plays a role in O_3-induced pulmonary function decrements. This idea is supported by the findings of Koren et al. (1991),

who obtained a positive correlation between O_3-induced pulmonary function decrements and the level of prostaglandin E_2 (PGE_2) in BAL fluid collected within 1 h after the end of exposure in human subjects who varied greatly in O_3 responsiveness.

The release of cyclooxygenase products of arachidonic acid from injured airway epithelium can thus be viewed as a link in a cascade of events, which begins with the initial reaction of O_3 with the tissues and ends with the observed pulmonary function responses. The apparent components of this chain of events include factors that influence (1) O_3 delivery to the tissue (e.g., the inhaled concentration, breathing pattern, and airway geometry); (2) O_3 reactions with components in airway surface liquid and epithelial cell membranes; (3) local tissue responses, including injury and inflammation; and (4) stimulation of neural afferents (bronchial C fibers) and the resulting reflex responses. It still is not understood how each event in this cascade contributes to the pulmonary responses induced by acute O_3 inhalation.

The influence that individual responsiveness has on this cascade of events has not been determined; however, recent data suggest that individual O_3 responsiveness may feed back and influence the distribution of O_3 dose within the lung. Gerrity et al. (1994) tested the hypothesis that O_3-induced rapid, shallow breathing helps to limit the dose of O_3 reaching the lower respiratory tract. They found that the degree of O_3-induced rapid, shallow breathing (25% decrease in V_T) was significantly correlated with a decrease in O_3 uptake efficiency of the lower respiratory tract. This observation may explain the recent data of Schelegle et al. (1991) and Aris et al. (1993a) that suggest individual responsiveness to O_3 as measured by FEV_1 decrements may be negatively correlated with the number of neutrophils (PMNs) present in BAL samples. However, the interrelationship among the responsiveness to O_3, the distribution of dose within the airway, and resulting airway inflammation is still poorly understood.

7.2.1.2 Subjects with Preexisting Disease

Introduction

Ten studies (König et al., 1980; Linn et al., 1982a; Koenig et al., 1985; Linn et al., 1978, 1983a; Solic et al., 1982; Kehrl et al., 1985; Superko et al., 1984; Silverman, 1979; Kulle et al., 1984) examining the pulmonary responses to acute O_3 exposures of less than 3 h in patients with preexisting disease were discussed in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). This section examines the effects of O_3 exposure on pulmonary function in subjects with preexisting disease by reviewing O_3 exposure studies that utilized subjects with (1) chronic obstructive pulmonary disease (COPD), (2) asthma, (3) allergic rhinitis, and (4) ischemic heart disease. Because of their important health implications, all of the available studies are reviewed and summarized in Table 7-2. Unless otherwise stated, the term "significant" is used to denote statistical significance at $p < 0.05$.

Subjects with Chronic Obstructive Pulmonary Disease

In five of the studies cited above, the O_3-induced pulmonary function responses of patients with mild to moderate COPD were examined (König et al., 1980; Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985). No significant changes in pulmonary function or symptoms were reported in any of the studies of the effects of O_3 in patients with COPD. Four of these studies (Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985) examined the

effects of O_3 concentrations between 0.1 and 0.3 ppm O_3 in 66 mild to moderate COPD patients using mild IE exposure protocols of 1 to 2 h duration. The total

Table 7-2. Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Condition	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Subjects with Chronic Obstructive Pulmonary Disease							
0.12	236	1 h IE (2 × 15 min light bicycle ergometry)	Tdb = 25 °C RH = 50%	18 M, 7 F	8 smokers, 14 ex-smokers, 3 nonsmokers; FEV_1/FVC = 32 to 66%	No significant changes in pulmonary function measurements; small significant decrease in arterial O_2 saturation.	Linn et al. (1982a)
0.18 0.25	353 490	1 h IE (2 × 15 min light bicycle ergometry)	Tdb = 25 °C RH = 50%	15 M, 13 F	15 smokers, 11 ex-smokers, 2 nonsmokers; FEV_1/FVC = 36 to 75%	No significant changes in pulmonary function measurements; no significant change in arterial O_2 saturation.	Linn et al. (1983a)
0.20	392	2 h IE (4 × 7.5 min light treadmill running)	Tdb = 22 °C RH = 40%	13 M	8 smokers, 4 ex-smokers, 1 nonsmoker; productive cough; FEV_1/FVC = 46 to 70%	No significant changes in pulmonary function measurements; small significant decrease in arterial O_2 saturation.	Solic et al. (1982)
0.30	588	2 h IE (4 × 7.5 min light treadmill running)	Tdb = 22 °C RH = 40%	13 M	9 smokers, 4 nonsmokers; FEV_1/FVC = 37 to 65%	No significant changes in pulmonary function measurements or arterial O_2 saturation.	Kehrl et al. (1985)
0.41	804	3 h daily (1 × 15 min light bicycle ergometry during each exposure) for 5 days	Tdb = 22 °C RH = 50%	17 M, 3 F	All smokers; productive cough; FEV_1/FVC = 56 to 82% and/or FEV_3/FVC = 75 to 93%	Decrease in FVC and FEV_3 with 0.41 ppm O_3 compared with FA exposure.	Kulle et al. (1984)
Subjects with Heart Disease							
0.20 0.30	392 588	40 min CE treadmill walking	NA	6 M	Coronary heart disease with angina pectoris threshold	No significant changes in pulmonary function measurements, exercise ventilatory pattern, oxygen uptake, or cardiovascular parameters.	Superko et al. (1984)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	[3] Observed Effect(s)	Reference
Subjects with Allergic Rhinitis							
0.18	353	2 h IE (4 × 15 min)	NA	26 M	History of allergic rhinitis	Increased respiratory symptoms, SR_{aw}, and reactivity to histamine with O_3 exposure and decreased FVC, FEV_1, and $FEF_{25-75\%}$ with O_3 exposure compared to FA.	McDonnell et al. (1987)
0.50	980	4 h rest	Tdb = 20 to 24 C RH = 40 to 48%	6 M, 6 F	History of seasonal allergic rhinitis; acute response to nasal challenge with antigen	Increase in upper and lower respiratory symptom scores, cell influx, epithelial cells with O_3 exposure compared to FA; no effect on acute allergic response to nasal antigen challenge between O_3 and FA exposure.	Bascom et al. (1990)
Adult Subjects with Asthma							
0.10 0.25 0.40	196 490 784	1 h light IE (2 × 15 min on treadmill, $\dot{V}_E$ = 27 L/min)	Tdb = 21 C RH = 40%	12 M, 9 F, 19 to 40 years old	Stable mild asthmatics with FEV_1 > 70% and methacholine responsiveness	No significant differences in FEV_1 or FVC were observed for 0.10 and 0.25 ppm O_3-FA exposures or postexposure exercise challenge; 12 subjects exposed to 0.40 ppm O_3 showed significant reduction in FEV_1.	Weymer et al. (1994)
0.12	236	1 h rest	NA	7 M, 8 F	Never smoked, mild stable asthmatics with exercise-induced asthma	Exposure to 0.12 ppm O_3 did not affect pulmonary function. Preexposure to 0.12 ppm O_3 at rest did not affect the magnitude or time course of exercise-induced bronchoconstriction.	Fernandes et al. (1994)
0.12	236	0.75 h IE $\dot{V}_E$ = 30 L/min (15 min rest, 15 min exercise, 15 min rest) followed by 15 min exercise inhaling 0.10 ppm SO_2	Tdb = 22 C RH = 75%	8 M, 5 F, 12 to 18 years old	Asthmatics classified on basis of positive clinical history and methacholine challenge. Asymptomatic at time of study.	Filtered air followed by SO_2 and O_3 alone did not cause significant changes in pulmonary function. Ozone followed by SO_2 resulted in significant decrease in FEV_1 (8%) and $\dot{V}_{max50\%}$ (15%) and a significant increase in R_T (19%).	Koenig et al. (1990)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Adult Subjects with Asthma (cont'd)							
0.12 0.24	236 472	1.5 h IE, $\dot{V}_E$ = 25 L/min	Tdb = 22°C RH = 65%	4 M, 4 F (nonasthmatics); 18 to 35 years old; 5 M, 5 F (asthmatics); 18 to 41 years old	Physician-diagnosed asthma confirmed with methacholine challenge test. All nonsmokers and asymptomatic at time of study. Nine were atopic.	No significant changes in pulmonary and nasal function measurements in either asthmatics or nonasthmatics. Significant increase in nasal lavage white cell count and epithelial cell following O_3 exposure in asthmatics only.	McBride et al. (1994)
0.12	236	6.5 h/day IE (6 × 50 min) (2 days of exposure), $\dot{V}_E$ = 28 L/min (asthmatic), $\dot{V}_E$ = 31 L/min (healthy)	NA	8 M, 7 F (nonasthmatics); 22 to 41 years old; 13 M, 17 F (asthmatics); 18 to 50 years old	Asthmatics classified on basis of positive clinical history, previous physician diagnosis, and low PD_{20}. Mild to severe asthmatics.	Significant increase in bronchial reactivity to methacholine in both asthmatics and nonasthmatics. FEV_1 decreased 8.6% in asthmatics and 1.7% in nonasthmatics, with difference not being significant.	Linn et al. (1994)
0.12	236	1 h rest	NA	4 M, 3 F, 21 to 64 years old	Mild, stable asthma	Increase in bronchial responsiveness to allergen; no change in baseline airway function.	Molfino et al. (1991)
0.20	392	2 h IE (4 × 15 min at 2× rest $\dot{V}_E$ cycle ergometry)	Tdb = 31°C RH = 35%	20 M, 2 F, 19 to 59 years old	Physician diagnosed asthma; 6 smokers, 9 ex-smokers, 7 nonsmokers	No significant changes in pulmonary function measurements; significant blood biochemical changes.	Linn et al. (1978)
0.25	490	2 h rest	NA	5 M, 12 F, 20 to 71 years old	Nonsmoking asthmatics selected from a clinical practice	No significant changes in pulmonary function measurements.	Silverman (1979)

Table 7-2 (cont'd). Ozone Exposure in Subjects with Preexisting Disease[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	[3] Observed Effect(s)	Reference
Adult Subjects with Asthma (cont'd)							
0.40	784	2 h IE (4 × 15 min cycle ergometry)	Tdb = 22 C RH = 50%	4 M, 5 F (normals), 19 to 31 years old; 4 M, 5 F (asthmatics), 18 to 34 years old	Asthmatics as diagnosed by a physician; history of chest tightness and wheezing	Decrease in FVC and IC with O_3 in asthmatics; increase in airway responsiveness to methacholine in asthmatics with O_3 and FA; asthmatic subjects had significantly greater decreases in FEV_1 and $FEF_{25-75\%}$ with O_3 exposure than did normal subjects.	Kreit et al. (1989) Eschenbacher et al. (1989)
Adolescent Subjects with Asthma							
0.12	235	1 h rest	Tdb = 22 C RH 75%	4 M, 6 F (normals), 13 to 18 years old; 4 M, 6 F (asthmatics), 11 to 18 years old	Asthmatics had a history of atopic extrinsic asthma and exercise-induced bronchospasm	Decrease in FRC with O_3 exposure in asthmatics; no consistent significant changes in pulmonary functional parameters in either group or between groups.	Koenig et al. (1985)
0.12	235	1 h IE (2 × 15 min treadmill walking at mean $\dot{V}_E$ = 32.5 L/min)	Tdb = 22 C RH 75%	5 M, 8 F (normals), 12 to 17 years old; 9 M, 3 F (asthmatics), 12 to 17 years old	Asthmatics selected from a clinical practice and had exercise- induced bronchospasm	Decrease in maximal flow at 50% of FVC in asthmatics with O_3 exposure compared to FA; no significant changes with combined O_3-NO_2 exposure.	Koenig et al. (1988)
0.12 0.18	235 353	40 min IE (1 × 10 min treadmill walking at mean $\dot{V}_E$ = 32.5 L/min)	NA	4 M, 9 F (normals), 14 to 19 years old; 8 M, 8 F (asthmatics), 12 to 19 years old	Asthmatics had allergic asthma, positive responses to methacholine, and exercise- induced bronchospasm	Decrease in FEV_1 and increase in R_T in normals and asthmatics with 0.12 and 0.18 ppm O_3 exposure compared to FA; no consistent differences between normals and asthmatics.	Koenig et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by rest and exercise; within groups listed from lowest to highest O_3 concentration.

exercise time in all four of these studies was 30 min, with intensity being variable (exercise $\dot{V}_E$ approximately 14 to 28 L/min). Linn et al. (1982a) observed a small but significant reduction in arterial oxygen saturation in 25 mild to moderate COPD patients at the end of the 0.12 ppm O_3 exposure for 1 h (absolute mean difference = 1.3%, $p < 0.05$). Similarly, Solic et al. (1982) observed a small reduction in arterial oxygen saturation in 13 mild to moderate COPD patients at the end of a 0.2-ppm O_3 exposure for 2 h (absolute mean difference = 0.48%, $p < 0.008$). In contrast, Kehrl et al. (1985) did not find a significant effect on arterial oxygen saturation in 13 mild to moderate COPD patients after exposure to 0.3 ppm O_3 using the same IE exposure protocol used by Solic et al. (1982). Similarly, Linn et al. (1983a) found no significant effect on arterial oxygen saturation in 28 mild to moderate COPD patients exposed to 0.18 and 0.25 ppm O_3 for 1 h. The combined observations of these studies indicate that persons with COPD are not responsive to O_3 concentrations of 0.3 ppm and lower in combination with mild exercise. However, this conclusion should be viewed within the context of the low total inhaled dose of O_3 involved in the above studies, in that studies in healthy subjects using similar total inhaled doses also have not shown significant pulmonary function effects. Interpretation of these studies also is complicated by the wide range of the pulmonary function impairment of the patients studied (FEV_1/FVC from 0.3 to 0.7), their variable smoking history, and the fact that these patients are older (60 years of age). The inconsistency of the observed small decreases in arterial oxygen saturation makes the interpretation of the clinical significance of this data difficult and uncertain.

Despite similar limitations, Kulle et al. (1984) observed small (<4%), statistically significant decreases in FVC and FEV_3 in 20 smokers (age range 31 to 51 years) diagnosed with mild chronic bronchitis exposed to 0.4 ppm O_3 for 3 h using an IE protocol (one 15-min exercise period beginning 1 h prior to end of exposure, $\dot{V}_E$ approximately 29 to 38 L/min). In addition, Kulle et al. (1984) observed that repeated daily exposure over a 5-day period led to an attenuation of these forced expiratory endpoints, and that this attenuation did not last longer than 4 days. The pulmonary responses induced by O_3 exposure in this study were associated only with mild symptoms.

Subjects with Asthma

Three studies examining the pulmonary responses to acute O_3 exposures in adult (Linn et al., 1978; Silverman, 1979) and adolescent (Koenig et al., 1985) asthmatics were discussed in the earlier criteria document (U.S. Environmental Protection Agency, 1986). Significant decrements in group mean pulmonary function were not observed for adult asthmatics exposed for 2 h at rest (Silverman, 1979) or with light IE (Linn et al., 1978) to O_3 concentrations of 0.25 ppm or less. However, it should be noted that, although group mean pulmonary function responses were not significantly affected in these studies, there were responsive asthmatic subjects who had obvious decrements in pulmonary function.

Koenig and co-workers (Koenig et al., 1985, 1987, 1988) conducted a series of studies examining the pulmonary responses of adolescent asthmatics and nonasthmatics (11 to 19 years of age) exposed to low levels of O_3. Koenig et al. (1985) found no significant changes in pulmonary function or symptoms in 10 adolescent normal and asthmatic subjects (four male, six female) who inhaled 0.12 ppm O_3 for 1 h at rest. The asthmatic subjects in this study were characterized as having histories of atopic (Type I, immunoglobulin E [IgE]-mediated) asthma and exercise-induced bronchospasm. Subsequently, in two separate studies of similar groups of adolescent asthmatics and nonasthmatics, Koenig et al. (1987, 1988)

observed no significant changes in pulmonary function or symptoms following exposure to 0.12 and 0.18 ppm O_3 with moderate IE up to 1 h, although a small significant decrease in flow at 50% of FVC was observed in the adolescent asthmatics exposed to 0.12 ppm O_3.

Kreit et al. (1989) and Eschenbacher et al. (1989) have demonstrated that exposure to 0.4 ppm O_3 with heavy IE (exercise $\dot{V}_E$ = 30 L/min/m^2 BSA) for 2 h elicits a significant decrease in FVC, FEV_1, FEV_1/FVC, and $FEF_{25\ 75\%}$ in both normal and asthmatic subjects. In these studies, O_3 exposure caused significantly greater decrements in FEV_1, FEV_1/FVC, and $FEF_{25\ 75\%}$ in asthmatic subjects. In contrast, Kreit et al. (1989) and Eschenbacher et al. (1989) found no significant difference between asthmatic and normal subjects in FVC and subjective symptoms. In addition, the effect of O_3 exposure on bronchial responsiveness as measured by the concentration of methacholine needed to increase SR_{aw} 100% ($PC_{1\ SRaw}$) was also studied. The asthmatic subjects had a significant decrease in $PC_{1\ SRaw}$ following FA and O_3 exposure. In comparison, the normal subjects had a significant decrease in $PC_{1\ SRaw}$ following O_3 exposure, with the percent decrease in mean $PC_{1\ SRaw}$ after O_3 exposure being similar in normal and asthmatic subjects, although the asthmatic patients' baseline $PC_{1\ SRaw}$ was significantly lower than that of the normal subjects. The findings of this study indicate that if the total inhaled dose is increased sufficiently by either increasing $\dot{V}_E$ during exposure or O_3 concentration, mild to moderate asthmatics will respond with a greater obstructive response than will normal subjects.

Linn et al. (1994) have reported responses of healthy (n = 15) and asthmatic (n = 30) subjects to 0.12 ppm O_3 and 100 μg/m^3 of respirable sulfuric acid (H_2SO_4) aerosol (MMAD = 0.5 μm; geometric standard deviation [σ_g] = 2), alone and in combination using the EPA prolonged-exposure protocol (see Section 7.2.2). These investigators observed a significant O_3-induced reduction in FEV_1 that was statistically significant and an increase in airway responsiveness to methacholine for all subjects combined. The asthmatic subjects demonstrated a statistically significant decrease in FEV_1 as a function of exposure duration regardless of pollutant exposure. In addition, there was a greater reduction in FEV_1 following O_3 alone in the asthmatics as compared to the nonasthmatics (Δ8.6% versus Δ1.7%), although this difference was not statistically significant. Despite the lack of a significant difference between asthmatics' and nonasthmatics' group mean FEV_1 responses with O_3 exposure, the responses observed in the asthmatics may be considered more important because their average FEV_1 was already significantly depressed by the underlying illness.

The findings of the above studies comparing the pulmonary function responses following O_3 exposure in asthmatic and nonasthmatic subjects suggest that asthmatics are at least as sensitive, if not more sensitive, to the acute effects of O_3 inhalation. The underlying mechanism that would explain a possible increased responsiveness of asthmatic subjects to O_3 is undefined. One possible mechanism could be that asthmatic subjects have an exaggerated airway inflammatory response to acute O_3 exposure. A study conducted by McBride et al. (1994) would support this hypothesis. McBride et al. (1994) exposed 10 asymptomatic asthmatic subjects with histories of allergic rhinitis and 8 nonallergic healthy subjects to FA and 0.12 and 0.24 ppm O_3 for 90 min using a light IE protocol ($\dot{V}_E$ = approximately 25 L/min). Pulmonary function tests, posterior rhinomanometry, and nasal lavage (NL) were performed before exposure and 10 min and 6 and 24 h after exposure. No significant changes in pulmonary or nasal function were found in either the allergic asthmatic or nonallergic nonasthmatic subjects. The allergic asthmatic subjects had a significant increase in the number of white blood cells in NL fluid 10 min and 24 h following the 0.24-ppm O_3 exposure. In

addition, a significant increase in epithelial cells was present 10 min after exposure to 0.24 ppm O_3 in the asthmatic subjects. No significant cellular changes were observed in the nonasthmatic subjects. These data indicate that the upper airways of asthmatic individuals are more sensitive to the acute inflammatory effects of O_3 than those of nonallergic nonasthmatic subjects.

The above studies compared the effects of O_3 inhalation on pulmonary function in asthmatic and normal subjects, but do not address the effect of preexposure to ambient concentrations of O_3 on the responsiveness of asthmatic subjects to other respiratory challenges, including other irritant gases, allergens, and exercise. Koenig et al. (1990) reported an increase in the bronchial response to an SO_2 challenge in a group of 13 asymptomatic adolescent asthmatic subjects following inhalation of 0.12 ppm O_3 for 45 min using a light to moderate IE protocol ($\dot{V}_E$ = approximately 30 L/min).

Molfino et al. (1991) investigated whether resting exposure to 0.12 ppm O_3 for 1 h potentiates the airway response to inhaled allergen in seven patients with mild asthma with seasonal symptoms of asthma and positive skin tests for ragweed or grass. This study was conducted over four week-long periods during the winter when ambient allergen levels were low. In each week, there were 3 consecutive study days. On Days 1 and 3, subjects underwent methacholine challenges, whereas, on Day 2, the subjects received one of four combined challenges in a single-blind design: (1) air breathing followed by inhalation of allergen diluent, (2) O_3 exposure followed by inhalation of allergen diluent, (3) air breathing followed by inhalation of allergen, and (4) O_3 exposure followed by inhalation of allergen. Molfino et al. (1991) observed no significant differences in baseline FEV_1 after O_3 exposure, but did observe a significant reduction in the provocative concentration of allergen required to reduce FEV_1 15%. This study was limited by its small number of subjects, and the results were confounded by possible ordering effects with the "O_3 exposure followed by allergen protocol" being the last protocol for all but one subject. Despite these limitations, the findings suggest that O_3 concentrations as low as 0.12 ppm may increase the bronchial responsiveness to allergen in atopic subjects.

In order to examine whether preexposure to O_3 results in exacerbation of exercise-induced asthma, two studies were conducted recently (Fernandes et al., 1994; Weymer et al., 1994). Fernandes et al. (1994) preexposed 15 stable mild asthmatics with exercise-induced asthma to 0.12 ppm O_3 for 1 h at rest followed by a 6-min exercise challenge test and found no significant effect on either the magnitude or time course of exercise-induced bronchoconstriction. Similarly, Weymer et al. (1994) observed that preexposure to either 0.10 or 0.25 ppm O_3 for 60 min while performing light IE did not enhance or produce exercise-induced asthma in 21 otherwise healthy adult subjects with stable mild asthma. Although the results of these studies would suggest that preexposure to O_3 neither enhances nor produces exercise-induced asthma in asthmatic subjects, the relatively low total inhaled doses used in the above studies limit the ability to draw any definitive conclusions.

Subjects with Allergic Rhinitis

McDonnell et al. (1987) exposed 26 adults (18 to 30 years of age) with allergic rhinitis to clean air and 0.18 ppm O_3 for 2 h using an IE protocol ($\dot{V}_E$ = 64 L/min at 15-min intervals). The study subjects with allergic rhinitis did not have a history of asthma-like symptoms. Following O_3 exposure, the subjects with allergic rhinitis exhibited significant increases in respiratory symptoms, airway reactivity to histamine, and SR_{aw} and significant

decreases in FVC, FEV_1, and $FEF_{25-75\%}$ when compared to clean air exposure. When compared to normal subjects without allergic rhinitis similarly exposed to 0.18 ppm O_3, the subjects with allergic rhinitis were no more responsive to O_3, based on symptoms, forced expiratory parameters, or airway reactivity to histamine aerosols, although subjects with allergic rhinitis did have a small but significantly greater increase in SR_{aw}. The data on subjects with allergic rhinitis and asthmatic subjects suggest that both of these groups have a greater rise in R_{aw} to O_3 with a relative order of airway responsiveness to O_3 being normal < allergic < asthmatic.

Bascom et al. (1990) conducted a study to characterize the upper respiratory response to acute O_3 inhalation, nasal challenge with antigen, and the combination of the two. Bascom et al. (1990) exposed 12 resting asymptomatic subjects with histories of allergic rhinitis in a randomized, crossover design on each of 2 days, separated by 2 weeks, to clean air or 0.5 ppm O_3 for 4 h. Following exposure, subjects underwent nasal challenge with four doses of antigen (1, 10, 100, and 1,000 protein nitrogen units of ragweed or grass). Upper and lower airway symptoms were rated and NL was performed before and after clean air and 0.5 ppm O_3 exposure, and following each antigen challenge. Exposure to O_3 caused significant increases in upper and lower airway symptoms, a mixed inflammatory cell influx with a sevenfold increase in NL PMNs, a 20-fold increase in eosinophils and a 10-fold increase in mononuclear cells as well as an apparent sloughing of epithelial cells. There was a significant increase in NL albumin concentration following O_3 exposure. When expressed as a change from the postexposure values, there was no significant difference between O_3 and clean air exposure in antigen-induced upper and lower airway symptoms, cells, albumin and mediators (histamine and TAME-esterase activity). These results suggest that acute exposure to O_3 does not alter the acute response to nasal challenge with antigen.

Subjects with Ischemic Heart Disease

One study has been conducted examining the cardiopulmonary effects of acute O_3 inhalation in patients with ischemic heart disease. Superko et al. (1984) exposed six middle-aged males with angina-symptom-limited exercise tolerance for 40 min to FA and to 0.2 and 0.3 O_3 while they were exercising continuously according to a protocol simulating their angina-symptom-limited exercise training prescription (mean $\dot{V}_E$ = 35 L/min). No significant pulmonary function impairment or evidence of cardiovascular strain induced by O_3 inhalation was observed. The low workloads were dictated by the patients' angina-symptom-limited exercise tolerance, and these low workloads acted to "protect" them from O_3-induced effects by limiting the total inhaled dose.

7.2.1.3 Influence of Gender, Age, Ethnic, and Environmental Factors

Gender Differences

As was noted in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), the pulmonary function responses to O_3 of only a small number of female subjects have been evaluated under controlled laboratory conditions. Although the database on females has expanded (see Table 7-3), there are still fewer data than for males. Most studies involving mixed groups of male and female subjects include too few female subjects to allow for meaningful comparisons between the responses of the sexes, or fail to consider the question at all. There are, however, a few studies that utilize only female subjects. Several studies

cited in the 1986 O_3 criteria document suggested that females might be more responsive to O_3 than males (Horvath et al., 1979; Gliner et al., 1983; Gibbons and Adams,

Table 7-3. Gender Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12 0.18 0.24 0.30 0.40	235 353 470 588 784	2.33 h $\dot{V}_E$ = 25 L/min/m^2 BSA (one exposure/subject)	Mean T = 22 °C Mean RH = 4% treadmill	30 to 33 F and 30 to 33 M in each concentration group; total of 372 individuals participated	Healthy NS, 18 to 35 years old, blacks and whites	Decrements in FEV_1, increases in SR_{aw} and cough, correlated with O_3 concentration. There were no significant differences between the responses of males and females.	Seal et al. (1993)
0.18	353	1 h (mouthpiece) CE $\dot{V}_E$ ≈ 47 L/min	T = 21 to 25 °C RH = 45 to 60% cycle	14 F	Mean FVC = 5.11 ± 0.53 L, NS, 20 to 24 years old	Significant concentration-response effect on FVC and FEV_1; lung size had no effect on percentage decrements in FVC or FEV_1.	Messineo and Adams (1990)
0.30	588			14 F	Mean FVC = 3.74 ± 0.30 L, NS, 19 to 23 years old		
0.20 0.30	392 588	1 h (mouthpiece) IE (20 min exercise) $\dot{V}_E$ ≈ 28 L/min for men $\dot{V}_E$ ≈ 23 L/min for women	T ≈ 22 °C RH ≈ 75% treadmill	9 M 10 F	NS, 55 to 74 years old NS, 56 to 74 years old	No changes in spirometry in men or women. Women had significant 13% increase in R_T following exposure, which was sustained at 20 min postexposure.	Reisenauer et al. (1988)
0.30	588	1 h (mouthpiece) CE $\dot{V}_E$ ≈ 70 L/min for men $\dot{V}_E$ ≈ 50 L/min for women	T = 21 to 25 °C RH = 45 to 60% cycle	20 M 20 F	NS, 18 to 30 years old NS, 19 to 25 years old	Significant decrements in FVC, FEV_1, and $FEF_{25-75\%}$ following O_3 exposure. No significant differences between men and women for spirometry or SR_{aw}.	Adams et al. (1987)

Table 7-3 (cont'd). Gender Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.45	882	2 h IE $\dot{V}_E$ 27.9 L/min for men $\dot{V}_E$ 25.4 L/min for women	T = 24 C RH = 58% cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	Range of responses in FEV_1: 0 to 12% (mean = 5.6%). No significant difference in responses of men and women. Tendency for women to have greater effects.	Drechsler-Parks et al. (1987a,b)
0.45	882	2 h IE Mean $\dot{V}_E$ = 28.5 L/min for men Mean $\dot{V}_E$ = 26.1 L/min for women	Mean T = 23.1 C Mean RH = 46.1% cycle/treadmill	10 M 6 F	Healthy NS, 60 to 89 years old Healthy NS, 64 to 71 years old	Mean decrement in FEV_1 = 5.7%. Decrements in FVC and FEV_1 were the only pulmonary functions significantly altered by O_3 exposure. No significant differences between responses of men and women.	Bedi et al. (1989)
0.48	941	2 h IE $\dot{V}_E$ 25 L/min	T = 21 C WBGT cycle	10 F	Healthy NS, 19 to 36 years old	Mean decrement in FEV_1 = 22.4%. Significant decrements in all spirometry measurements. Results not significantly different from a similar study on males (Drechsler-Parks et al., 1984).	Horvath et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]WBGT = 0.7 $T_{wet\ bulb}$ + 0.3 $T_{dry\ bulb\ or\ globe}$.

1984; Lauritzen and Adams, 1985). DeLucia et al. (1983), on the other hand, did not find significant differences in the responses of young men and young women to O_3 exposure.

Messineo and Adams (1990) hypothesized that differences previously observed between the responses of males and females exposed to O_3 were related to differences in lung size between the sexes. They addressed this issue by selecting two groups of 14 women each. One group had a mean FVC of 5.11 L, and the other group had a mean FVC of 3.74 L. All subjects were 19 to 24 years of age and were healthy nonsmokers who had not lived in a high-air-pollution area for at least 6 mo. The subjects completed three 1-h CE ($\dot{V}_E$ = 47 L/min) exposures: (1) FA, (2) 0.18 ppm O_3, and (3) 0.30 ppm O_3. The mouthpiece exposures were presented in random order, at least 4 days apart, and all were performed when the subject was in the follicular phase of her menstrual cycle. Two subjects in the small-lung group and one in the large-lung group were unable to complete the 0.30 ppm O_3 exposure. Both groups had similar O_3-induced percentage decrements (9 to 10% following exposure to 0.18 ppm O_3 and 23 and 26% following exposure to 0.30 ppm O_3 for the small- and large-lung groups, respectively) in all measures of lung function, regardless of lung size, leading to the conclusion that lung size, per se, is not systematically related to percentage decrements in FEV_1 consequent to O_3 exposure.

Horvath et al. (1986) exposed 10 healthy, young, nonsmoking females, 19 to 36 years of age (mean age 23.6 years) to 0.48 ppm O_3 or FA for 2 h while they exercised intermittently at a target ventilation of 25 L/min. The subjects engaged in three 20-min cycle ergometer exercise periods alternated with four 15-min rest periods. The exposures were a minimum of 1 week apart. The responses of these subjects were compared with those of a group of 10 young males who earlier had completed the same protocol (Drechsler-Parks et al., 1984). There were no statistically significant differences in the responses based on gender. The female subjects had decrements of 18.8, 22.4, and 30.8% in FVC, FEV_1, and $FEF_{25\ 75\%}$, respectively, compared to 19.8, 25.0, and 31.9% for the male subjects. On an individual basis, 4 of the 10 males and 3 of the 10 females had decrements of 30% or more in FEV_1 following the exposure to 0.48 ppm O_3. One male subject did not respond to the O_3 exposure. It was noted, however, that the female subjects inhaled an absolute dose of O_3 about 22% less than the male subjects due to a slightly lower exercise $\dot{V}_E$ and the inherently lower resting $\dot{V}_E$ of females compared to males. However, when O_3 dose was related to BSA or to FVC, the females inhaled slightly higher relative doses of O_3 than the males.

Adams et al. (1987) compared the responses of 20 young men (18 to 30 years of age) and 20 young women (19 to 35 years of age) exposed to 0.3 ppm O_3 via mouthpiece. All subjects were healthy nonsmokers with clinically normal pulmonary function. None had a history of significant allergies, and none had resided in a high-air-pollution area for at least 3 mo. The subjects completed 1-h CE exposures (mean $\dot{V}_E$ 70 L/min for males and 50 L/min for females) to FA and 0.3 ppm O_3. The exposures were given in random order and were separated by a minimum of 5 days. Ozone exposure induced significant decrements in FVC, FEV_1, and $FEF_{25\ 75\%}$ compared to FA exposure. Three females and four males were unable to complete the O_3 exposure. Females experienced mean decrements of 14.2, 20.3, and 24.5% in FVC, FEV_1, and $FEF_{25\ 75\%}$, respectively, compared to mean decrements of 15.8% in FVC, 23.8% in FEV_1, and 35.7% in $FEF_{25\ 75\%}$ for males. There were no statistically significant differences between the spirometry or SR_{aw} responses related to gender. Because the female subjects inhaled a substantially smaller absolute dose of O_3 due to the considerably lower exercise $\dot{V}_E$, yet had similar decrements in pulmonary function compared to men, the

authors concluded that females are more responsive to O_3 than males. In this study, the female subjects inhaled a lower relative dose of O_3 compared to males, when expressed on the basis of BSA, but a similar relative dose when expressed on the basis of FVC.

Seal et al. (1993) reported on 372 healthy black and white men and women between 18 and 35 years of age who each were assigned to complete one 2.33-h exposure to FA, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3. Subjects exercised intermittently on a motor-driven treadmill at a work load inducing a $\dot{V}_E$ of about 23 L/min/m^2 BSA for women and about 24.5 L/min/m^2 BSA for men. Although female subjects inhaled about 22% less total dose of O_3 than males in each exposure-concentration group, there were no significant differences in the changes in FEV_1 (see Figure 7-2), SR_{aw}, or cough ratings between males and females among either blacks or whites. Women also inhaled a lower absolute dose of O_3 than men.

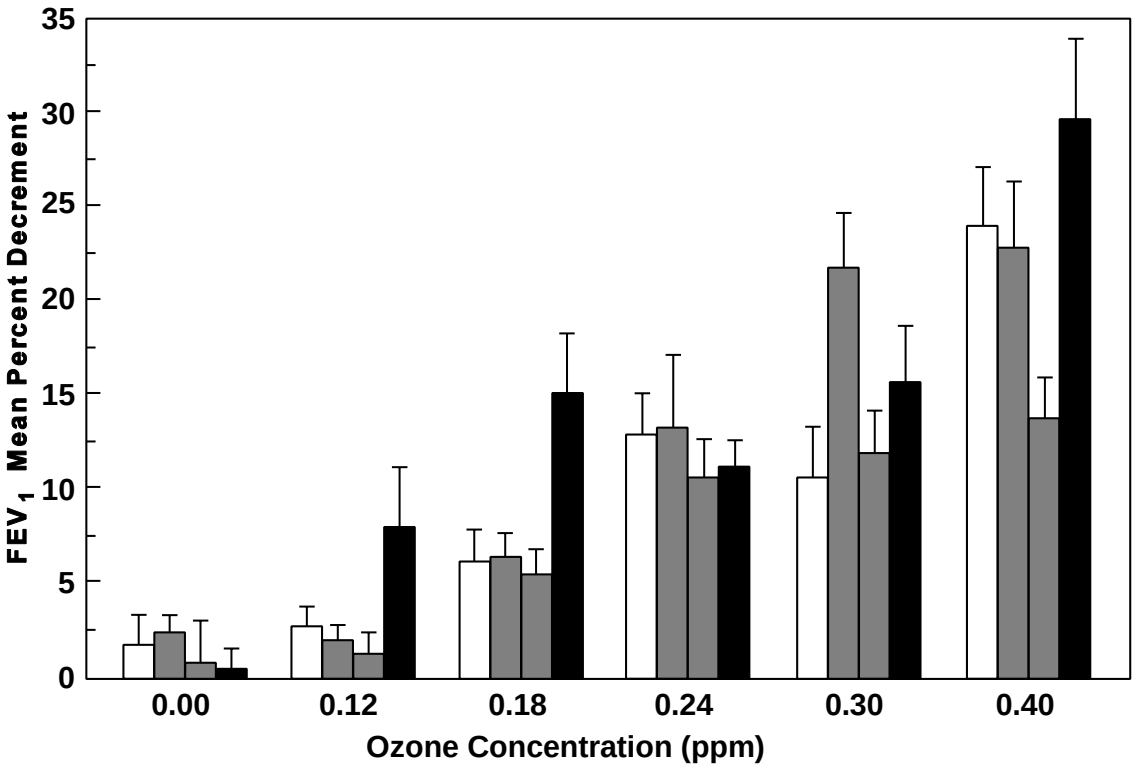

Figure 7-2. Mean percent change (± standard error of the mean) in post-minus prevalues of forced expiratory volume in 1 s (FEV_1) for each gender-race group. Open bars = white women; cross-hatched bars = black women; hatched bars = white men; solid bars = black men.

Source: Seal et al. (1993).

Drechsler-Parks et al. (1987a,b) compared the responses of eight men and eight women between 51 and 76 years of age to FA and 0.45 ppm O_3. The subjects were all healthy nonsmokers who were long-term residents of a relatively low pollution area. The subjects participated in 2-h IE (20 min rest/20 min exercise at $\dot{V}_E$ = 25 L/min) exposures that were presented in random order and were separated by at least 1 week. Except for FEV_3, there were no statistically significant differences between the responses of the men and women subjects, although women had slightly larger mean decrements in FVC and FEV_1 than men. Individual decrements in FVC and FEV_1 ranged from 0 to about 12% for both male and female subjects. Based on FEV_1, two females and three males had no response to the O_3 exposure. Male subjects inhaled a somewhat larger absolute effective dose of O_3 due to higher exercise and resting $\dot{V}_E$. When $\dot{V}_E$ was normalized to BSA, females inhaled a larger dose of O_3 than males. When $\dot{V}_E$ was normalized to FVC, the relative inhaled doses of O_3 were similar.

Reisenauer et al. (1988) reported on the pulmonary function responses of 9 men and 10 women between 55 and 74 years of age who were exposed to 0.0, 0.2, and 0.3 ppm O_3. The three exposures were presented in random order and at the same time of day for each subject. The subjects were exposed via mouthpiece for 1 h, during which seven men exercised for 10 min and rested for 50 min, and the other two men and all of the women alternated two 20-min rest periods and two 10-min exercise periods. Ventilation rates were about 28 L/min for men and 23 L/min for women, although, when $\dot{V}_E$ was normalized to BSA, the relative $\dot{V}_E$ for males and females was similar. All data were pooled, regardless of the total exercise time. There were no significant changes in any parameter of pulmonary function in the males. Females had no significant changes in any spirometric parameter, but, following the 0.3-ppm O_3 exposure, did have a small (13%) increase in total respiratory resistance (R_T), which remained at this level 20 min postexposure.

Bedi et al. (1989) reported on the responses of 10 men and 6 women (60 to 89 years of age) exposed for 2 h to FA or 0.45 ppm O_3 for 3 consecutive days. Only the first O_3 day results will be discussed in this section; the issue of repeated exposures is addressed in Section 7.2.1.4. Exposures were conducted at the same time of day, on consecutive days, with the FA exposure always conducted first. The subjects alternated 20-min exercise periods (mean $\dot{V}_E$ = 28.5 L/min for men and 26.1 L/min for women) and 20-min rest periods throughout the 2-h chamber exposures. When $\dot{V}_E$ was normalized to BSA, women inhaled slightly higher relative doses of O_3; but when normalized to FVC, women inhaled a slightly lower relative dose of O_3 than men. There were no statistically significant group mean differences between the responses of men and women subjects. The mean decrements in FVC and FEV_1 following the O_3 exposure for the 16 subjects were 2.8 and 5.7%, respectively. In an exploratory analysis, the subjects were divided into two groups based on whether their decrement in FEV_1 following the first O_3 exposure compared to the FA exposure was 5% or <5%. There were eight subjects in each group, with the sensitive group consisting of two females and six males. The mean post-O_3 exposure decrement in FEV_1 was 320 mL for the sensitive group, versus 21 mL for the nonresponsive group. Similar patterns of response were evident in FVC and FEV_3. There were no significant changes in any flow parameter, maximum voluntary ventilation (MVV), expiratory reserve volume, or functional residual capacity.

The question as to whether there is a difference in sensitivity to O_3 between men and women remains unresolved. Different conclusions depend on whether $\dot{V}_E$ is normalized to body or lung size in calculating the inhaled doses of O_3. The subgroups studied by Bedi et al. (1989) included six males and two females, suggesting that older males may be more sensitive

to O_3 than older females. However, Reisenauer et al. (1988) found a significant increase in R_T only in women. Horvath et al. (1986), Adams et al. (1987), and Drechsler-Parks et al. (1987a,b) suggested that because their female subjects had similar pulmonary function responses to their male subjects, even though the females inhaled less O_3, females were more sensitive than males. Messineo and Adams (1990) suggested that some factor other than absolute lung size accounted for observed differences between males and females; their two groups of females with widely different lung sizes experienced similar decrements in pulmonary function following equivalent exposures. Although the currently available literature suggests that females may be somewhat more sensitive to O_3 than males, the question is not settled. Further, comparative studies have included only small subject groups, except for Seal et al. (1993), and often only group mean data are presented, with little information about individual responses.

Hormonal Influences

Seal et al. (1995) compared the pulmonary function responses of 48 white and 55 black women (18 to 35 years of age) whose menstrual phase was known at the time of a single 2.3-h exposure to 0.18, 0.24, 0.30, or 0.40 ppm O_3. Subjects performed intermittent treadmill exercise ($\dot{V}_E$ = 20 L/min/m^2 BSA) during the first 2 h of exposure. There were no significant effects for SR_{aw} or cough that could be related to menstrual cycle. There was a race $\times$ menstrual phase interaction for FEV_1. However, when the groups of black and white women were analyzed separately, there was no significant primary effect for menstrual cycle phase. The significance of the observed interaction between race and menstrual cycle phase is unknown.

Weinmann et al. (1995) compared the pulmonary function responses of six healthy, nonsmoking women to a 130-min exposure to 0.35 ppm O_3, 4 to 8 days after the onset of menses and 4 to 8 days after ovulation. Subjects performed intermittent exercise at a workload that induced a $\dot{V}_E$ of 10 $\times$ FVC. Ovulation was confirmed by a blood progesterone test. Spirometry was performed pre- and 25-min post-O_3 exposure. Although resting $\dot{V}_E$ was the same during both exposures, exercise load had to be reduced 30% during the luteal phase in order to match the ventilatory response to exercise during the follicular phase. There were no significant effects related to phase of the menstrual cycle. The authors concluded that menstrual phase does not need to be considered in experimental design. One problem with the study is that the postexposure measurements were made 25 min after the conclusion of the exposure. Typically, pulmonary function decrements begin to reverse once exposure ends; thus, any pulmonary function changes that did occur could be expected to be reduced at 25-min post-O_3 exposure, compared to immediately after exposure.

Acute O_3 exposure has been shown to cause short-term airway inflammation (see Section 7.2.4) induced by PGs, among other inflammatory substances. It also has been demonstrated that progesterone inhibits PG production in the uterine endometrium, which fluctuates as the progesterone concentration varies throughout the menstrual cycle. Fox et al. (1993) investigated the hypothesis that O_3 exposure during the follicular phase, when progesterone concentration is lowest, might result in greater pulmonary function responses due to reduced anti-inflammatory influences of progesterone. Nine nonsmoking women completed 1-h mouthpiece exposures to FA and 0.3 ppm O_3 while exercising continuously ($\dot{V}_E$ about 50 L/min) during both the follicular and mid-luteal phases of two to four ovulatory menstrual cycles. There were no differences in any pulmonary function responses to FA related to

menstrual phase, nor was there a difference in the mean FVC decrements following the follicular or mid-luteal phase O_3 exposures. The O_3-induced decrements in FEV_1 and $FEF_{25\ 75\%}$ were significantly larger during the follicular phase (17.3 and 23.1%, respectively) than during the mid-luteal phase (13.4 and 15.3%, respectively). The authors speculated that the difference between the FEV_1 and $FEF_{25\ 75\%}$ responses to the two O_3 exposures could be due to differences in circulating progesterone and the effect of progesterone on prostaglandin activity.

Available data (see Table 7-4) do not permit a conclusion regarding the influence of the menstrual cycle on responses to O_3 exposure. Two of the three studies available, Fox et al. (1993) and Weinmann et al. (1995), were performed with small groups of subjects and resulted in opposite conclusions. Seal et al. (1995) compared race (black versus white) and menstrual phase, obtaining a significant interaction between race and phase, but post-hoc analysis failed to establish a basis for the interaction, leaving the implications of the study unclear.

Age Differences

It has been hypothesized that age may be a factor in responsiveness to O_3. Although children make up a large proportion of the population, few controlled laboratory studies of the pulmonary function effects of any air pollutant have been reported on subjects under age 18. Field and epidemiological studies (see Section 7.4) attempting to relate ambient air pollutant exposure to pulmonary function in children have suggested that children may be more responsive to ambient air pollution than young adults.

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) included only one laboratory exposure study in which children were the subjects. McDonnell et al. (1985a) evaluated the pulmonary function responses of 23 boys between 8 and 11 years of age to 0.00 and 0.12 ppm O_3 in random order. The boys alternated 15-min rest and exercise periods ($\dot{V}_E$ = 35 L/min/m^2 BSA) for the first 120 min of the 150-min exposure. Forced expiratory spirometry and respiratory symptoms were measured before exposure and at 125 min of exposure, whereas R_{aw} was measured before exposure began and after 145 min of exposure. The group mean decrement in FEV_1 following the O_3 exposure was 3.4%, compared to 4.3% for a group of young adult males who earlier had completed the same protocol (McDonnell et al., 1983). It should be noted that the absolute $\dot{V}_E$ for the children (39.4 L/min) and adults (65.0 L/min) was similar when normalized for BSA (about 35 L/min/m^2 BSA). Assuming that adjusting ventilation for differences in BSA is an appropriate normalizing technique, these children appeared to experience O_3-induced pulmonary effects similar to adults. The children reported no symptoms, but the adults reported a small, but statistically significant, increase in cough following O_3 exposure.

Although controlled laboratory studies of the effects of exposure to air pollutants are rarely performed with children as subjects, a few, more recent studies are discussed below (see Table 7-5). Avol et al. (1987) have reported on the pulmonary function responses of 33 healthy boys and 33 healthy girls having a mean age of 9.4 years. The children completed exposures to purified air and outdoor ambient air that was drawn into an environmental chamber. Ambient temperature averaged about 33 °C. Exposures were 1 h in duration, were separated by a minimum of 2 weeks, and were conducted from June through September, beginning in the early afternoon when ambient air pollutant concentrations generally peak. The subjects performed continuous exercise throughout the

Table 7-4. Hormonal Influences on Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	References
0.12 0.24 0.30 0.40	235 470 588 784	2.3 h IE $\dot{V}_E$ = 20 L/min/m^2 BSA	NA	48 WF, 55 BF	Healthy NS, 18 to 35 years old	Significant menstrual cycle phase × race interaction for FEV_1. No significant menstrual cycle phase effect when blacks and whites were analyzed separately. No significant menstrual phase effects for SR_{aw} or cough score.	Seal et al. (1995)
0.30	588	1 h CE $\dot{V}_E$ ≈ 50 L/min	NA	9 F	Healthy NS, regular menstrual cycles, 20 to 34 years old	FEV_1 decreased 13.1% during the mid-luteal phase and 18.1% during the follicular phase. Decrement in $FEF_{25-75\%}$ was significantly larger during the follicular phase than the mid-luteal phase. Changes in FVC were similar in both phases.	Fox et al. (1993)
0.35	686	130 min	NA	9 F	Healthy NS, regular menstrual cycles, 18 to 35 years old	Changes in FVC, FEV_1, $FEF_{25-75\%}$, $\dot{V}_{max50\%}$, and $V_{max25\%}$ were similar during both the follicular and luteal phases.	Weinmann et al. (1995)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

Table 7-5. Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.113[c] + other ambient pollutants	221	1 h CE $\dot{V}_E$ ≈ 22 L/min	T = 32.7 °C RH ≈ 43% cycle	33 M, 33 F	NS for both groups, mean age = 9.4 years old	No differences in responses of boys and girls. Similar decrements (<5% on average) following both purified air and ambient air (O_3 at 0.11 ppm) exposures.	Avol et al. (1987)
0.12	235	1 h (mouthpiece) IE $\dot{V}_E$ = 4 to 5 × resting	T = 22 °C RH = 75% treadmill	5 M, 7 F	Healthy NS, 12 to 17 years old	No significant changes in any pulmonary function in healthy subjects.	Koenig et al. (1988)
0.12 0.18	235 353	40 min (mouthpiece) IE 10 min exercise at $\dot{V}_E$ = 32.6 L/min; 40 min (mouthpiece) IE 10 min exercise at $\dot{V}_E$ = 41.3 L/min	NA treadmill	3 M, 7 F 4 M, 6 F	Healthy NS, 14 to 19 years old	No significant change in FEV_1; increased R_T with exposure to 0.18 ppm O_3. Some subjects responded to 5 to 10 mg/mL methacholine after 0.18-ppm O_3 exposure, whereas none responded to 25 mg/mL methacholine at baseline bronchochallenge.	Koenig et al. (1987)
0.18 0.24 0.30 0.40	353 470 588 784	2.3 h IE $\dot{V}_E$ = 20 L/min/m^2 BSA	NA	48 WF, 55 BF	Healthy NS, 18 to 35 years old, black and white	Older women had smaller changes in FEV_1 than younger women. No age- related differences in SR_{aw} or cough score.	Seal et al. (1993)
0.20 0.30	392 588	1 h (mouthpiece) IE (20 min) $\dot{V}_E$ ≈ 28 L/min for men $\dot{V}_E$ ≈ 23 L/min for women	T ≈ 22 °C RH ≈ 75% treadmill	9 M, 10 F	Healthy NS, 55 to 74 years old	No change in any spirometry measure. Women had 13% increase in R_T after 0.30-ppm exposure.	Reisenauer et al. (1988)

Table 7-5 (cont'd). Age Differences in Pulmonary Function Responses to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.45	882	2 h IE $\dot{V}_E$ ≥ 26 L/min	T ≥ 23 °C RH = 53% cycle	8 M 8 F	Healthy NS, 51 to 76 years old	Mean decrement in FEV_1 = 5.6 ± 13%; range of decrements = 0 to 12%.	Drechsler-Parks et al. (1987a,b)
0.45	882	2 h IE Mean $\dot{V}_E$ = 28.5 L/min for men Mean $\dot{V}_E$ = 26.1 L/min for women	T = 23 °C RH = 46% cycle/treadmill	10 M 6 F	Healthy NS, 60 to 89 years old	Mean decrement in FEV_1 = 5.7%; eight subjects had a 5% or greater difference between their response to O_3 and FA, and the other eight had less than a 5% difference between their responses to FA and 0.45 ppm O_3.	Bedi et al. (1989)
0.45	882	2 h IE $\dot{V}_E$ ≥ 26 L/min	T ≥ 24 °C RH = 63% cycle	8 M 8 F	Healthy NS, 51 to 69 years old Healthy NS, 56 to 76 years old	13 subjects had decrements in FEV_1 on three separate exposures to 0.45 ppm within 5% of their mean response to the three exposures. The other three subjects were not reproducible. Symptom reports did not correlate well with pulmonary function changes.	Bedi et al. (1988)
0.45	882	1 h CE $\dot{V}_E$ ≥ 26 L/min 2 h IE $\dot{V}_E$ ≥ 26 L/min	T ≥ 23 °C RH = 58% cycle/treadmill	7 M 5 F	Healthy NS, 60 to 79 years old (all in 60s except one 79 years old)	Comparison of 1-h CE protocol and 2-h IE protocol indicated no difference between the changes in pulmonary function following the two protocols.	Drechsler-Parks et al. (1990)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Ozone concentration is the mean of a range of ambient concentrations.

hour of exposure. Boys and girls exercised at similar $\dot{V}_E$, 22 to 23 L/min. It should be noted that the ambient exposure included the full range of air pollutants present in the outdoor air mix on the days of the exposures, except for small fractions of O_3 and particles lost in the inlet duct. Concentrations of O_3, nitrogen dioxide (NO_2), total suspended particulate (TSP), particulate nitrate, particulate sulfate, particulate sodium, and particulate ammonium were measured throughout the exposures. The O_3 concentration during the ambient air exposures averaged 0.113 ppm, whereas it averaged 0.003 ppm during the purified air exposures. The children consistently had similar declines in pulmonary function with time, following both FA and ambient air exposures. Typical mean decrements in FVC and FEV_1 were 50 mL or less. The investigators also have published similar studies on adolescents and adults (Avol et al., 1984, 1985a). The responses of the adolescents and adults to both exposures were not substantially different from those of the children whose results are reported here. The authors further noted that the children seemed to have difficulty performing consistent, reproducible pulmonary function tests, a factor that could have impacted on these results.

Several studies comparing the pulmonary function responses of healthy and asthmatic adolescents to O_3 exposure have appeared in the literature. The responses of the asthmatics are presented in Section 7.2.1.2; only data on normal adolescent subjects will be discussed in this section.

Koenig et al. (1987) reported on 20 adolescents, 14 to 19 years of age, who were exposed for 40 min to air or 0.12 or 0.18 ppm O_3 via a mouthpiece system. Ten subjects were exposed to each O_3 concentration, but not all subjects were exposed to both concentrations. None of the healthy subjects had a history of asthma or allergies, and all had pulmonary function within the predicted range, based on age, sex, and height. There was a 5- to 7-min break in exposure for pulmonary function test performance following 30 min of resting exposure, followed by a 10-min exercise period ($\dot{V}_E = 32.6 \pm 6.4$ L/min for the 0.12-ppm O_3 exposure and 41.3 ± 9.3 L/min for the 0.18-ppm O_3 exposure). Changes in FEV_1 were not significant following any exposure. After exposure to 0.18 ppm O_3, R_T was increased 15%.

Koenig et al. (1988) also have reported on the pulmonary function responses of another group of 12 healthy adolescents (12 to 17 years of age) to 1-h exposures to air and 0.12 ppm O_3. The subjects were exposed by mouthpiece to air and 0.12 ppm O_3 while alternating 15-min periods of exercise ($\dot{V}_E = 32.8 \pm 6.0$ L/min) with 15-min periods of rest ($\dot{V}_E = 8.8 \pm 1.2$ L/min). Tests of pulmonary function included forced expiratory spirometry and R_T. Healthy subjects had no significant alterations in any parameter of pulmonary function consequent to exposure to air or 0.12 ppm O_3.

Although few data are available on the responses of healthy adolescents exposed to O_3, the limited existing data do not identify adolescents as being either more or less responsive than young adults.

At the time the 1986 O_3 criteria document was released, no studies specifically evaluating the pulmonary function responses of older adults had been reported. Several studies (Folinsbee et al., 1985; Adams et al., 1981) that included a few middle-aged individuals among the subjects were suggestive that there might be a decrease in O_3 responsiveness with advancing age. Several reports have since appeared, collectively suggesting that, collectively, healthy older adults (i.e., over 50 years of age) generally are minimally responsive to O_3, although some individuals remain responsive to O_3.

Drechsler-Parks et al. (1987a) reported on eight men and eight women between 51 and 76 years of age who were exposed for 2 h to FA or 0.45 ppm O_3. The subjects were

healthy nonsmokers with normal baseline pulmonary function. The chamber exposures involved alternating 20-min rest and exercise periods ($\dot{V}_E$ averaged 27.9 $\pm$ 0.29 L/min for men and 25.4 $\pm$ 0.80 L/min for women). Exposures were presented in random order at least 1 week apart. The only significant difference related to sex was in FEV_3, in which women had larger decrements than men. There were no significant decrements in any other parameter of pulmonary function related to O_3 exposure, except when the data of all 16 subjects were pooled, significant mean decrements of 5.3 $\pm$ 1.3% in FVC, and 5.6 $\pm$ 1.3% in FEV_1 were observed. The range of individual decrements in FEV_1 was from 0 to 12%. Two women and three men had no response following the O_3 exposure. The subjects reported more symptoms following the O_3 exposure than the FA exposure. Seven subjects reported cough, nine reported sore throat, and six reported chest tightness. The authors compared their results on older adults to the results from a group of young adults who had completed the same protocol. All of the older subjects inhaled slightly higher doses of O_3 than the younger subjects (10.23×10^4 L for older men versus 10.12×10^4 L for younger men and 8.48×10^4 L for older women versus 7.94×10^4 L for younger women). However, older men had a mean decrement in FEV_1 of 4.2% versus 23.7% for younger men, whereas older women had a mean decrement in FEV_1 of 7.0% versus 14.7% for younger women. The decrements of the older subjects also were compared to published values for the young adults, with the older subjects studied consistently showing smaller changes in pulmonary functions than the young adults. These comparisons indicated that these older adults were less responsive to O_3 exposure than typical young adults, in terms of pulmonary function changes and symptom reports.

Reisenauer et al. (1988) reported on the pulmonary function responses of 19 healthy adults between 55 and 74 years of age. All were nonsmokers with baseline pulmonary function within the predicted normal range. None had a history of asthma, atopy, or cardiovascular disease, and none responded to a baseline methacholine bronchochallenge. Subjects were exposed by mouthpiece to 0.0, 0.20, or 0.30 ppm O_3 for 1 h. Seven men rested for 50 min and exercised for 10 min, whereas the other two men and all women alternated two 20-min rest periods with two 10-min exercise periods; $\dot{V}_E$ was approximately 28 L/min for men and 23 L/min for women. The three exposures were presented in random order at the same time of day for each individual, but the separation between exposures is not stated. The only significant change in pulmonary function was a 13% increase in R_T with exposure to 0.30 ppm O_3 in women only, which was sustained for at least 20 min postexposure. The authors concluded, based on the increase in R_T in the women, that older adults were at increased risk for pulmonary function changes with near-ambient O_3 exposure. However, R_T is a highly variable parameter, and no other changes were significant. Given the large number of variables tested, this isolated result possibly is related to the large number of statistical tests performed. In contrast, the results of Koenig et al. (1987) from 10 healthy adolescents exposed to 0.18 ppm O_3 using a similar protocol, reported a mean decrement in FEV_1 of 2% and an increase of 10.5% in R_T at 2 to 3 min postexposure. At 7 to 8 min postexposure, the increase in R_T was 15.3%. Comparison of these results to those of Reisenauer et al. (1988) at 0.20 ppm O_3 supports the contention that younger individuals are more responsive to O_3 than older individuals, in that no changes in spirometry were noted in the older adults exposed to 0.30 ppm O_3, although older women showed increased R_T with 0.30 ppm O_3 exposure, whereas the adolescents had a mean decrement of 1% in FEV_1 and a mean increase of 16% in R_T with exposure to 0.18 ppm O_3.

Bedi et al. (1988) reported that older men and women 51 to 76 years of age who completed three exposures to 0.45 ppm O_3 did not respond equivalently to each of three exposures. The subjects were healthy nonsmokers with baseline pulmonary function within predicted normal limits. The subjects alternated 20-min exercise ($\dot{V}_E$ was approximately 26 L/min) and 20-min rest periods throughout the 2-h chamber exposures. There was a minimum of 1 week between exposures, but separations ranged from 1 to 4 weeks between exposures 1 and 2, and between 1 and 7 weeks between exposures 2 and 3. Analysis of variance indicated no difference between the group mean responses to the three exposures. The data were then subjected to a correlation analysis, which led to the conclusion that the responses within an individual subject were not reproducible. McDonnell et al. (1985b), on the other hand, found good reproducibility of pulmonary function responses after exposure to various concentrations of O_3 between 0.12 and 0.40 ppm in young adult males between 18 and 30 years of age.

Seal et al. (1993) compared the pulmonary function responses of 48 white and 55 black women (18 to 35 years) who each completed a 2.3-h exposure to 0.18, 0.24, 0.30, or 0.40 ppm O_3. The subjects participated in only one exposure each while exercising intermittently ($\dot{V}_E$ = 20 L/min/m^2 BSA) during the first 2 h of the exposure. Older subjects within the age range tested had smaller decrements in FEV_1 than younger subjects.

One simple method to estimate the O_3 exposure dose is to calculate the product of O_3 concentration (parts per million), $\dot{V}_E$ (liters per minute), and exposure duration (minutes). Research on young adults (Folinsbee et al., 1978; Adams et al., 1981) has demonstrated that the order of relative importance of the three factors is O_3 concentration, $\dot{V}_E$, and exposure duration. Drechsler-Parks et al. (1990) investigated the relative role of the three components of effective dose in 12 healthy, nonsmoking adults between 61 and 79 years of age. The subjects were exposed to both FA and 0.45 ppm O_3, once while they performed a 1-h continuous exercise protocol and once while they performed a 2-h IE protocol in which they alternated 20-min exercise periods and 20-min rest periods. Mean $\dot{V}_E$ ranged from 25.2 to 27.3 L/min among the four exposures. Exposures were separated by at least 1 week. Regardless of protocol, O_3 exposure induced significant decrements in FEV_5, FEV_1 (7.7 and 10.6% for the 1- and 2-h exposures, respectively), FEV_3, and peak expiratory flow rate (PEFR) compared to FA exposure. There were significant decrements in $FEF_{25-75\%}$ (0.37, 0.46, 0.49, and 0.47 L/s, respectively), $FEF_{5\%}$, $FEF_{25\%}$, and MVV following all four exposures. The only significant difference between the responses to the 1- and 2-h O_3 protocols was in FEV_5. The total number of symptoms reported was 10 for the 1-h FA exposure, 6 for the 1-h O_3 exposure, and 12 for both the 2-h FA and 2-h O_3 exposures. It appears that resting ventilation during the 2-h protocol had a smaller effect compared to exercise ventilation. This supports earlier reports that the O_3 concentration is the most significant factor among the three factors that contribute to effective dose (Adams et al., 1981; Folinsbee et al., 1978; Hackney et al., 1975).

Available data, although on a limited number of subjects, consistently indicate that responsiveness to O_3 is decreased in persons over 50 years of age compared to younger adults. Although there are few data available on adults in their thirties and forties, the statistical modeling study of McDonnell et al. (1993) on subjects from 18 to 32 years of age suggests that responsiveness to O_3 is already diminishing by age 30, and that the most responsive individuals are likely to be less than 25 years of age. The results of Bedi et al. (1988) suggest that older adults may be less reproducible in their responses to O_3 than younger adult males (McDonnell

et al., 1985b); however, this finding is based on only 16 subjects and should be confirmed before being considered conclusive.

Ethnic and Racial Factors

Young white males have been the most frequently studied population in published reports on pulmonary function responses to O_3. There is concern, however, that responses to O_3 may be influenced by ethnic differences based on the observation that blacks have smaller lungs than whites for a given standing or sitting height (Rossiter and Weill, 1974; McDonnell and Seal, 1991). Thus, an equivalent inhaled volume of O_3 could result in a larger O_3 dose per unit of lung tissue in blacks compared to whites, potentially inducing greater effects in blacks than whites exposed to O_3 under the same conditions. Seal et al. (1993) evaluated the pulmonary function responses of 372 individuals, black, white, male, and female ($n > 90$ per group), between 18 and 35 years of age who were exposed to 0.00, 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3. Each subject was assigned randomly to an exposure group and participated in only one experimental session. The protocol involved a 2.33-h exposure to the assigned condition. During the first 2 h of exposure, the subjects alternated 15-min rest periods and 15-min exercise periods ($\dot{V}_E = 25$ L/min/m^2 BSA). Spirometric and plethysmographic measurements were made at 5 and 20 min following the final exercise period. The initial nonparametric analysis of the percentage changes in FEV_1 indicated that FEV_1 responses increased with increasing O_3 concentration, and a group effect occurred that was independent of O_3 concentration. There was an O_3 effect, but no group effect or group $\times$ O_3 interaction for SR_{aw}, indicating an increase in SR_{aw} with increasing O_3 concentration. Both group and O_3 effects were significant for cough, but the interaction was not significant. A post hoc analysis, using a different statistical method on the absolute changes in FEV_1, indicated that the black males experienced significant decrements in FEV_1 following exposure to 0.12 ppm O_3, whereas black women and white men and women did not have significant decrements in FEV_1 at O_3 concentrations below 0.18 ppm. These results are not easily explained because there was no gender difference among whites and no racial difference among women. Furthermore, the black men had significantly greater decrements in FEV_1 at only some of the O_3 concentrations studied (see Figure 7-2). Although the results can be considered suggestive of an ethnic difference, more subjects must be studied before the issue of ethnic difference in O_3 responsiveness can be more definitive. It should be noted that, although this study included a large number of subjects, each subject participated in only one experiment. Thus, the range of individual responsiveness could have been different between groups.

Environmental Factors

A number of environmental factors, such as ambient temperature and humidity, season of the year, route of inhalation, and smoking history have been hypothesized to potentially impact on responses to O_3 exposure in additive or synergistic ways. None of these potentially interacting agents has been addressed adequately in the extant O_3 literature. Although O_3 concentrations in Los Angeles, for example, generally are highest on hot, dry days, most research on responses to O_3 exposure has been conducted under temperature and humidity conditions not substantially different from those typical of indoor environments. The few studies that included temperature and humidity as experimental factors have produced equivocal results (see Chapter 10, Section 10.2.9 in the 1986 O_3 criteria document). No new

reports of temperature or humidity effects have appeared since the 1986 O_3 criteria document (U.S. Environmental Protection Agency, 1986).

Earlier studies discussed in the 1986 O_3 criteria document have suggested that cigarette smokers are less responsive to O_3 than nonsmokers. Since then, the question as to whether reactivity to O_3 returns with cessation of cigarette smoking has been addressed by Emmons and Foster (1991). Thirty-four individuals with no history of asthma or obvious respiratory disease who enrolled in a smoking cessation program were assigned randomly to an O_3 group (n = 18) or an FA group (n = 16). The subjects ranged from 24 to 58 years of age and had a group mean smoking history of 33.9 $\pm$ 13 pack-years. Most of the subjects had baseline pulmonary functions somewhat below predicted values, based on age, height, and sex. Subjects completed 2-h exposures to 0.42 ppm O_3 or FA, as assigned, prior to beginning the smoking cessation program. The subjects rested during the exposures, except for 5 min of exercise at 150 kg □ m/min (no $\dot{V}_E$ given) at the beginning of the last 30 min of exposure. Nine subjects in the O_3 group and six in the FA group completed the 6-mo smoking cessation program and repeated their assigned exposures at the end of the program. Prior to beginning the smoking cessation program, both the FA and O_3 groups had pre- to postexposure changes in FVC, FEV_1, and $FEF_{25\ 75\%}$ within the variability of repeated tests. The O_3 group had a significant mean change in $FEF_{25\ 75\%}$ of □22.5%, comparing post- to preexposure, whereas the FA group had a nonsignificant □12% change. Changes in FVC and FEV_1 were not significant in either group. It should be noted that smoking cessation led to a group mean improvement in baseline $FEF_{25\ 75\%}$ of 22.9%. The post-O_3 exposure values for $FEF_{25\ 75\%}$ were similar following the initial and the post-smoking cessation exposures. Thus, the difference in the $FEF_{25\ 75\%}$ decrement with O_3 exposure post-smoking cessation was largely due to the improvement that ensued from 6 mo of abstinence from smoking. The results of Emmons and Foster (1991) suggest that active smoking blunts responsiveness to O_3 and that cessation of smoking for 6 mo leads to improved baseline pulmonary function and possibly the reemergence of O_3 responsiveness.

7.2.1.4 Repeated Exposures to Ozone

Repeated daily exposure to O_3 in the laboratory setting leads to attenuated changes in spirometry and symptom responses that were initially termed "adaptation" (Hackney et al., 1977a). A series of repeated exposure studies, performed in various laboratories, was reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). The spirometric responses to repeated O_3 exposure typically showed that the response was increased on the second exposure day to concentrations in the range of 0.4 to 0.5 ppm O_3 in exposures accompanied by moderate exercise (see Table 7-6). Thus, the response was enhanced on the second consecutive day. Mechanisms for enhanced responses had not been established, although it was hypothesized that persistence of O_3-induced damage for greater than 24 h may have contributed to the larger Day 2 response. An enhanced Day 2 response was less obvious or absent in exposures that were repeated at lower concentrations or that caused relatively small group mean O_3-induced decrements in spirometry. Two reports (Bedi et al., 1985; Folinsbee et al., 1986) indicated that enhanced spirometric responsiveness was present within 12 h, lasting for at least 24 h and possibly 48 h, but was clearly absent after 72 h. After 3 to 5 days of consecutive daily exposures to O_3, responses were markedly diminished or absent. One study (Horvath et al., 1981) suggested that the rapidity of this

decline in response was related to the magnitude of the subjects' initial responses to O_3 or their "sensitivity". Finally, the persistence of the attenuation of spirometric and symptom

Table 7-6. Changes in Forced Expiratory Lung Volume After Repeated Daily Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m³	Exposure Duration and Activity[c]	Number and Gender of Subjects	Percent Change in FEV_1 on Consecutive Exposure Days: First	Second	Third	Fourth	Fifth	References
0.12	235	6.6 h, IE (40)	17 M	−12.79	−8.73	−2.54	−0.6	0.2	Folinsbee et al. (1994)
0.20	392	2 h, IE (30)	10 M	+1.4	+2.7	−1.6	—	—	Folinsbee et al. (1980)
0.20	392	2 h, IE (18 and 30)	8 M, 13 F	−3.0	−4.5	−1.1	—	—	Gliner et al. (1983)
0.20	392	2 h, IE (18 and 30)	9	−8.7	−10.1	−3.2	—	—	Gliner et al. (1983)[e]
0.20	392	1 h, CE (60)	15 M	−5.02	−7.8	—	—	—	Brookes et al. (1989)
0.25	490	1 h, CE (63)	4 M, 2 F	−20.2	−34.8	—	—	—	Folinsbee et al. (1986)
			5 M, 2 F	−18.8	—	−22.3	—	—	
0.35	686	2 h, IE (30)	10 M	−5.3	−5.0	−2.2	—	—	Folinsbee et al. (1980)
0.35	686	1 h, CE (60)	8 M	−31.0	−41.0	−33.0	−25.0	—	Foxcroft and Adams (1986)
0.35	686	1 h, CE (60)	10 M	−16.1	−30.4	—	—	—	Schonfeld et al. (1989)
			10 M	−14.4	—	−20.6	—	—	
0.35	686	1 h, CE (60)	15 M	−15.9	−24.6	—	—	—	Brookes et al. (1989)
0.40	784	3 h, IE (4-5 × resting)	13 M	−9.2	−10.8	−5.3	−0.7	−1.0	Kulle et al. (1982)[f]
0.40	784	3 h, IE (4-5 × resting)	11 F	−8.8	−12.9	−4.1	−3.0	−1.6	Kulle et al. (1982)[f]
0.40	784	2 h, IE (65)	8 M	−18.0	−29.9	−21.1	−7.0	−4.4	Folinsbee et al. (1995)
0.42	823	2 h, IE (30)	24 M	−21.1	−26.4	−18.0	−6.3	−2.3	Horvath et al. (1981)
0.45	882	2 h, IE (27)	1 M, 5 F	−13.3	—	−22.8	—	—	Bedi et al. (1985)
0.45	882	2 h, IE (27)	10 M, 6 F	−5.8	−5.6	−1.9	—	—	Bedi et al. (1989)
0.47	921	2 h, IE (3 × resting)	8 M, 3 F	−11.4	−22.9	−11.9	−4.3	—	Linn et al. (1982b)[g]
0.50	980	2 h, IE (30)	8 M	−8.7	−16.5	−3.5	—	—	Folinsbee et al. (1980)
0.50	980	2.5 h, IE (2 × resting)	6	−2.7	−4.9	−2.4	−0.7	—	Hackney et al. (1977a)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.
[c]Exposure duration and intensity of IE or CE were variable; $\dot{V}_E$ (number in parentheses) given in liters per minute or as a multiple of resting ventilation.
[d]For a more complete discussion of these studies, see Table 7-7 and U.S. Environmental Protection Agency (1986).
[e]Subjects were especially sensitive on prior exposure to 0.42 ppm O_3 as evidenced by a decrease in FEV_1 of more than 20%. These nine subjects are a subset of the total group of 21 individuals used in this study.
[f]Bronchial reactivity to a methacholine challenge also was studied.
[g]Seven subjects completed entire experiment.

responses has been studied (Horvath et al., 1981; Linn et al., 1982b; Kulle et al., 1982). These studies indicate that the attenuation of response is relatively short-lived, being partially reversed within 3 to 7 days and typically abolished within 1 to 2 weeks. Repeated exposures separated by 1 week (for up to 6 weeks) apparently do not cause any lessening of the spirometric response (Linn et al., 1982b).

Folinsbee et al. (1995) (also see Devlin et al., 1995) exposed a group of 15 healthy males to 0.4 ppm O_3 for 2 h/day on 5 consecutive days. Subjects performed heavy IE ($\dot{V}_E$ = 60 to 70 L/min, 15 min rest/15 min exercise). Decrements in FEV_1 averaged 18.0, 29.9, 21.1, 7.0, and 4.4% on the 5 exposure days. Baseline preexposure FEV_1 decreased from the first day's preexposure measurement and was depressed by an average of about 5% on the third day. This study illustrates that, with high-concentration and heavy-exercise exposures, spirometry and symptom responses are not completely recovered within 24 h.

Besides the absence of pulmonary function responses after several days of O_3 exposure, symptoms of cough and chest discomfort usually associated with O_3 exposure generally are absent (Folinsbee et al., 1980, 1994; Linn et al., 1982b; Foxcroft and Adams, 1986). In addition, airway responsiveness to methacholine is increased with an initial O_3 exposure (Holtzman et al., 1979; Folinsbee et al., 1988), may be further increased with subsequent exposures (Folinsbee et al., 1994), and shows a tendency for the increased response to diminish with repeated exposure (Kulle et al., 1982; Dimeo et al., 1981). A number of possible explanations for the initially enhanced and then lessened response may be related to changes that are occurring in pulmonary epithelia as a consequence of O_3 exposure. Inflammatory responses (Koren et al., 1989a), epithelial damage, and changes in permeability (Kehrl et al., 1987) could be invoked to explain at least a portion of these responses. By blocking spirometric and symptom responses with indomethacin pretreatment, Schonfeld et al. (1989) demonstrated that in the absence of an initial spirometric response such effects were not enhanced by repeated exposure. However, the mechanisms of these responses with regard to repeated exposures in humans remains to be elucidated.

Recent studies of repeated O_3 exposures have addressed some other features of the responses (see Table 7-7). A series of reports from the Rancho Los Amigos group in California have examined changes in response to O_3 as a result of the season of the year in the South Coast Air Basin of Los Angeles, CA. The purpose of this research (Linn et al., 1988; also Hackney et al., 1989; Avol et al., 1988) was to determine whether responsive subjects (n = 12), identified during an initial screening following a period of low ambient O_3 exposure, would remain responsive after regular ambient exposure during the "smog season". Responses of so-called "nonresponsive" subjects (n = 13) also were examined across the year. The subjects were exposed to 0.18 ppm O_3 on four occasions, spring, fall, winter, and the following spring. Only 17 subjects (8 responders) participated in the final spring exposures. The marked difference in FEV_1 response between responsive and nonresponsive subjects seen initially (−12.4% versus +1%) no longer was present after the summer smog season (fall test) or 3 to 5 mo later (winter test). However, when the reduced subset of subjects was exposed during the following spring, the responsive subjects again had significantly larger changes in FEV_1. Seasonal changes in FEV_1 response to O_3 in the responsive and nonresponsive subjects are shown below.

Table 7-7. Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12	235	6.6 h 50 min exercise/10 min rest, 30 min lunch $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH five consecutive daily exposures	17 M	Healthy NS	FEV_1 responses were maximal on first day of exposure (−13%), less on second day (−9%), absent thereafter. Symptom responses only the first 2 days. Methacholine airway responsiveness was at least doubled on all exposure days, but was highest on the second day of ozone. Airway responsiveness was still higher than air control after 5 days of ozone exposure. Trend to lessened response, but it was not achieved after 5 days.	Folinsbee et al. (1994) (also see Table 7-9)
0.18	353	2 h IE (heavy) $\dot{V}_E$ ≈ 60 to 70 L/min (35 L/min/m² BSA)	31 °C 35% RH (screen exposures in spring 1986; second exposures in summer/fall 1986 and winter 1987 and spring 1987 for responders and nonresponders only)	59 adult Los Angeles residents 12 responsive 13 nonresponsive	Responders: Age = 19 to 40 years; 6 atopic, 2 asthmatic, 4 normal Nonresponders: Age = 18 to 39 years, 13 normal	Responders had ΔFEV_1 = 12.4% after initial screening; nonresponders had no change. Responders had nonsignificant response in late summer or early winter, but were responsive again in early spring (spring 1986, −385 mL; Autumn 1986, −17 mL; winter 1987, +16 mL; spring 1987, −347 mL). Nonresponders did not change with season. Suggests that responders responses may vary with ambient exposure, but nonresponders generally remain nonresponsive.	Linn et al. (1988) (also see Hackney et al., 1989)
0.20/0.20 0.35/0.20 0.35/0.35	392/392 686/392 686/686	1 h CE at 60 L/min	21 to 25 °C 40 to 60% RH (three 2-day sets of exposures)	15 M	Healthy aerobically trained NS, FVC = 4.24 to 6.98 L	Consecutive days of exposure to 0.20 ppm produced similar responses on each day (−5.02, −7.80); 0.35/0.20 ppm pair caused increased response to 0.20 ppm on second day (−8.74); 0.35/0.35 ppm caused much increased response on Day 2 (−15.9, −24.6). Symptom responses were worse on the second exposure to 0.35 ppm, but not with second exposure to 0.20 ppm.	Brookes et al. (1989)

Table 7-7 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.35	686	1 h CE (see paper for details)	22 to 25 °C 35 to 50% RH (1 day FA; 1 day O_3; 4 days consecutive exposure to O_3)	8 M	Aerobically trained healthy NS (some were known O_3 sensitive), age = 22.4 ± 2.2 years	Largest FEV_1 decrease on second of 4 days O_3 exposure (40% mean decrease). Trend for adaptation not complete in 4 days. $\dot{V}O_{2max}$ decreased with single acute O_3 exposure (6%) but was not significant after 4 days of O_3 exposure (4%). Performance time was less after acute O_3 (211 s) exposure than after FA (253 s).	Foxcroft and Adams (1986)
0.35	686	60 min CE $\dot{V}_E$ = 60 L/min	21 to 25 °C 40 to 60% RH (two exposures for each subject separated by 24, 48, 72, or 120 h)	40 M (4 groups of 10)	NS; nonallergic, non-Los Angeles residents for >6 mo; age 25 years	No differences between responses to exposures separated by 72 or 120 h. Enhanced FEV_1 response at 24 h (16.1% vs. 30.4%). Possible enhanced response at 48 h (14.4% vs. 20.6%). Similar trends observed for respiratory pattern and SR_{aw}.	Schonfeld et al. (1989)
0.45	882	2 h IE (3 × 20 min exercise) $\dot{V}_E$ = 26 L/min	23.3 °C 62.5% RH (three exposures with a minimum 1-week interval)	8 M, 8 F	Healthy NS, 61 years old for M and 65 years old for F (FVC = 4.97 L for M and 3.11 L for F)	Spirometric changes were not reproducible from time to time after ozone exposure ($r < 0.50$). Repeat exposures to air yielded consistent responses.	Bedi et al. (1988)
0.45	882	2 h IE (3 × 20 min exercise) $\dot{V}_E$ = 27 L/min	23.3 °C 63% RH Exposed for 3 consecutive days, not exposed for 2 days, then exposed to 0.45 ppm again for 1 day	10 M, 6 F	Healthy NS, 60 to 89 years old (median age = 65 years; mean FVC = 3.99 L; mean FEV_1 = 3.01 L; FEV_1/FVC range = 61 to 85%)	Overall increase in symptoms, but no single symptom increased significantly. FVC decreased 111 mL and 104 mL on Days 1 and 2, respectively. FEV_1 fell by 171 and 164 mL, and FEV_3 fell by 185 and 172 mL. No significant changes on Days 3 and 4 or with FA. FEV_1 changes were 5.8, 5.6, 1.9, and 1.7% on the four O_3 days.	Bedi et al. (1989)

Table 7-7 (cont'd). Pulmonary Function Effects with Repeated Exposures to Ozone[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	g/m						
0.45 (+ 0.30 PAN)	882	2 h IE (20 min rest, 20 min exercise) $\dot{V}_E$ = 27 L/min	22 C 60% RH 5 days consecutive exposure to PAN + O_3	3 M, 5 F	Healthy NS, Mean age = 24 years	FEV_1 decreased 19% with O_3 alone, 15% on Day 1 of O_3 + PAN, 5% on Day 5 of O_3 + PAN, 7% 3 days after 5 days of O_3 + PAN, 15% after 5 days of O_3 + PAN. Similar to O_3 adaptation studies, O_3 responses peaked after 2 days, were depressed 3 days later, and responses returned 7 days later. PAN probably had no effect on adaptation to O_3.	Drechsler-Parks et al. (1987b) (also see Table 7-13)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

	ΔFEV_1 Spring (mL)	ΔFEV_1 Fall (mL)	ΔFEV_1 Winter (mL)	ΔFEV_1 Spring (mL)
Responders	−385	−17	+16	−347
Nonresponders	+28	+90	+34	+81

These results suggest a seasonal variability in response that may be attributed to increased ambient O_3 exposure during the summer months. It must be noted that the responders included subjects who had a history of complaints from ambient air pollution. Furthermore, this group included a significant proportion of allergic individuals whose seasonal allergies could have contributed to their varying responses. Historically, however, studies with the subjects drawn from the population of Los Angeles have reported reduced responses to O_3 exposure in the laboratory compared to nonresidents (Hackney et al., 1976, 1977b).

Brookes et al. (1989) reexamined a hypothesis previously tested by Gliner et al. (1983), that repeated exposure to one concentration can alter response to subsequent exposure to a different O_3 concentration. Gliner et al. (1983) previously had shown that the response to 0.40 ppm O_3 was not influenced by previously being exposed to 0.20 ppm O_3 for 2 h on 3 consecutive days. Brookes et al. (1989) tested whether exposure to 0.20 or 0.35 ppm O_3 would change subsequent response to 0.20 or 0.35 ppm O_3. They found increased responses to 0.20 ppm for both preexposures (ΔFEV1 = −5.02, −7.80, and −8.74% for 0.20 ppm acutely, 0.20 ppm after 0.20 ppm, and 0.20 ppm after 0.35 ppm, respectively), but this trend was significant only for the higher concentration. Although not statistically significant, the response increase seen on the second exposure day at 0.20 ppm is similar to that seem by Gliner et al. (1983). These observations suggest that, although preexposure to low concentrations of O_3 may not influence response to higher concentrations, preexposure to a high concentration of O_3 may significantly increase response to a lower concentration on the following day.

Schonfeld et al. (1989) confirmed previous observations of Bedi et al. (1985) and Folinsbee et al. (1986) that the period of enhanced responsiveness to O_3 following an initial exposure persists for about 24 to 48 h but is absent by 72 h after the initial exposure. In a series of paired exposures to 0.35 ppm with continuous heavy exercise separated by intervals of 1, 2, 3, or 4 days, they found that the responses to the second exposure were clearly increased at 24 h (ΔFEV1 = −16.1 and −30.4% for the first and second exposures, respectively) and possibly also at 48 h (ΔFEV1 = −14.4 and −20.6%). Similar trends were observed for other physiological variables such as SRaw and respiratory pattern during exercise. With a 3- or 4-day interval between exposures, the responses to the two exposures were similar.

Foxcroft and Adams (1986) demonstrated that decrements in exercise performance seen after a 1-h exposure to 0.35-ppm (continuous heavy exercise) were less after 4 consecutive days of O_3 exposure than they were after a single acute exposure. Maximal aerobic power and performance time on a progressive bicycle exercise test were reduced 6% and 42 s, respectively, from FA control, after a single 0.35-ppm exposure. After 4 consecutive days of 1-h exposures, the maximal aerobic power was reduced only 4% and the performance time by only 14 s; these differences from FA control were not statistically

significant. Despite the change in exercise performance, Foxcroft and Adams (1986) did not show the attenuation of FEV_1 response seen in many previous studies (Folinsbee et al., 1980; Linn et al., 1982b). However, these investigators selected known O_3-sensitive subjects whose FEV_1 decrements exceeded 30% on the first 3 days of exposure. The large magnitude of these responses, the trend for the responses to decrease on the third and fourth day, the decreased symptom responses, and the observations of Horvath et al. (1981) that O_3-sensitive subjects adapt slowly, suggest that attenuation of response would have occurred if the exposure series had been continued for another 1 or 2 days. These observations support the contention advanced by Horvath et al. (1981) that the progression of attenuation of response is a function of "O_3 sensitivity". Furthermore, these results suggest that exercise responses after O_3 exposure may be limited, either voluntarily or involuntarily, more by subjective symptoms than by alterations in gas exchange consequent to changes in ventilatory function.

Bedi et al. (1989) examined the responses of elderly subjects (median age, 65 years) to four exposures to 0.45 ppm O_3 for 2 h with mild IE. The first three exposures were on consecutive days, with the fourth exposure following the third by 3 days. Changes in FEV_1 on the first two exposure days averaged −5.8 and −5.6%, about half the response expected in a group of healthy young males (−12.7%; Folinsbee et al., 1978). There were no significant changes in FEV_1 on the third (−1.9%) and fourth (−1.7%) exposure days. Symptom responses were negligible, although there was an overall increase in symptoms on the first day of O_3 exposure compared to air exposure. Despite the high concentration of the exposure, there was no enhancement of the spirometry response on the second day of exposure. Although similar observations have been made in previous studies producing small changes in spirometry (Folinsbee et al., 1980, 1994) with repeated exposures, the responses of older subjects are not sufficiently understood to explain these responses. Bedi et al. (1988) had previously reported that responses to O_3 in the older subjects tended to be less reproducible, although this factor alone could not explain these responses.

Drechsler-Parks et al. (1987b) examined the response to repeated exposures to 0.45 ppm O_3 plus 0.30 ppm peroxyacetyl nitrate (PAN). Exposures to O_3 and O_3 plus PAN yielded similar changes in spirometry (ΔFEV_1 = −19 and −15%, respectively). Thus, PAN did not increase responses to O_3. Repeated exposure to the PAN plus O_3 mixture resulted in similar changes to those seen with O_3 exposure alone. Responses in FEV_1 exceeded −30% on the second exposure and fell to less than −5% after the fifth day. The attenuation of response persisted 3 days after the repeated exposures, but was absent after 7 days. These observations suggest that PAN does not influence the attenuation of response to repeated O_3 exposure. If the PAN responses are considered negligible, this study confirms the observation that the attenuation of O_3 responses with chamber exposures lasts no longer than 1 week.

Repeated multihour exposure to low concentrations of O_3 has been examined (Horvath et al., 1991; Folinsbee et al., 1994; Linn et al., 1994). Horvath et al. (1991) exposed subjects for 2 consecutive days to 0.08 ppm using the 6.6-h prolonged-exposure protocol (see Section 7.2.2). They observed small pre- to postexposure changes in FEV_1 (−2.5%) on the first exposure, but no change on the second day. Linn et al. (1994) observed a 1.7% decrease in FEV_1 in healthy subjects after a 6.5-h exposure to 0.12 ppm. A second consecutive exposure yielded even smaller (<1%) responses. With exposure to a mixture of O_3 plus 100 $\mu g/m^3$ of H_2SO_4 aerosol, there was a 4.2% decrease in FEV_1 on the first exposure day. In a group of asthmatics exposed under similar conditions, the FEV_1 response on the first day was −8.6% (O_3) and −11.6% (O_3 plus acid). After adjustment for the exercise effect

(□4.6%), the responses (□4 and □7%) were still greater than those of nonasthmatics. Responses were slightly reduced on the second day of exposure.

Folinsbee et al. (1994) exposed 17 subjects to 0.12 ppm O_3 for 6.6 h on 5 consecutive days. Spirometry responses were typified by changes in FEV_1 that reached □13% on the first day and □9% on the second day of exposure. No significant differences in spirometry responses between FA and subsequent O_3 exposures were observed. Symptom responses were also greatest on the first exposure day and were largely absent from the third day on. Methacholine responsiveness was tested using a single dose of methacholine and then by comparing changes in R_{aw} as the ratio of SR_{aw} after methacholine aerosol to that after saline aerosol. The responses to FEV_1 and methacholine testing are shown below.

	Day 1	Day 2	Day 3	Day 4	Day 5	Clean Air
□%FEV_1	□12.79	□8.73	□2.54	□0.6	+0.2	+1.1
SR_{aw} Ratio	3.67	4.55	3.99	3.24	3.74	2.22

Methacholine responsiveness was increased (over the clean air response) throughout the 5 days of O_3 exposure, although it reached a peak on the second day, and, in some subjects, there was a trend for responsiveness to decrease after 5 days. These results suggest that repeated exposure to low levels of O_3, despite the attenuation of symptoms and pulmonary function changes, is not without hazard. It is likely that some epithelial damage persists that contributes to the enhanced response to methacholine throughout the exposure series. However, it must be noted that, in this study, subjects initially were selected based on their FEV_1 response to 0.16 ppm O_3 for 4 h. This may in part explain the greater FEV_1 responses seen in this study, but there was no correlation between individual FEV_1 decrements and changes in methacholine responsiveness. Furthermore, the Horvath et al. (1991) subjects were exposed only to 0.08 ppm, and they were somewhat older than the Folinsbee et al. (1994) subjects; the Linn et al. (1994) subjects, on the other hand, had lower ventilation during exercise and were residents of Los Angeles accustomed to exposure to these levels of O_3 (see Chapter 4 for typical O_3 concentrations).

Based on studies cited here and in the previous criteria document (U.S. Environmental Protection Agency, 1986), several conclusions can be drawn about repeated 1- to 2-h O_3 exposures. Repeated exposures to O_3 can cause an enhanced (i.e., greater) response on the second day of exposure. This enhancement appears to be dependent on the interval between the exposures (24 h causes the greatest increase) and is absent with intervals □3 days. An enhanced response also appears to depend to some extent on the magnitude of the initial response. Small responses to the first O_3 exposure are less likely to result in an enhanced response on the second day of O_3 exposure. Repeated daily exposure also results in attenuation of spirometric responses, typically after 3 to 5 days of exposure. This attenuated response persists for less than 1 or as long as 2 weeks. In temporal conjunction with the spirometry changes, symptoms induced by O_3, such as cough and chest discomfort, also are attenuated with repeated exposure. Ozone-induced changes in airway responsiveness attenuate more slowly than spirometric and symptom responses. Attenuation of the changes in airway responsiveness also persist longer than changes in spirometry, although this has been studied only on a limited basis. In longer-duration, lower-concentration studies that do not cause an

enhanced second-day response, the attenuation of response to O_3 appears to proceed more rapidly.

7.2.1.5 Effects on Exercise Performance

Introduction

An early epidemiological study examining race performances in high school cross-country runners (Wayne et al., 1967) suggested that exercise performance is depressed by inhalation of ambient oxidant air pollutants. Wayne et al. (1967) suggested that the detrimental effects of oxidant air pollutants on race performance may have been related to increased R_{aw} or to the associated discomfort in breathing, thus limiting runners' motivation to perform at high levels. The effects of acute O_3 inhalation on exercise performance have been evaluated in numerous controlled human studies. These studies can be divided into two categories: (1) those that examine the effects of acute O_3 inhalation on maximal oxygen uptake and (2) those that examine the effects of acute O_3 inhalation on the ability to complete strenuous continuous exercise protocols up to 1 h in duration. Five studies (Folinsbee et al., 1977; Horvath et al., 1979; Folinsbee et al., 1984; Adams and Schelegle, 1983; Savin and Adams, 1979) examining the effects of acute O_3 exposures on exercise performance were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986). This section summarizes the studies reviewed in that document and reviews more recent studies that examine the effect of acute O_3 inhalation on maximal oxygen uptake and endurance performance. Studies are also summarized in Table 7-8.

Effect on Maximal Oxygen Uptake

Three studies (Folinsbee et al., 1977; Horvath et al., 1979; Savin and Adams, 1979) examining the effects of acute O_3 exposures on $\dot{V}O_{2max}$ were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986). Of these studies, only Folinsbee et al. (1977) observed that $\dot{V}O_{2max}$ was significantly decreased (10.5%) following a 2-h exposure to 0.75 ppm O_3 with light IE. Reductions in $\dot{V}O_{2max}$ were accompanied by a 9.5% decrease in maximum attained workload, a 16% decrease in maximum ventilation, and a 6% decrease in maximum heart rate. The 16% decrease in maximum ventilation was associated with a 21% decrease in V_T. In addition, the O_3 exposure resulted in a 22.3% decrease in FEV_1 and subjective symptoms of cough and chest discomfort. In contrast, Horvath et al. (1979) did not observe a change in $\dot{V}O_{2max}$ or other maximum cardiopulmonary endpoints in male and female subjects exposed at rest to 0.75 ppm O_3 for 2 h, although FVC was significantly decreased (10%). Similarly, Savin and Adams (1979) observed no effect on maximum attained workload or $\dot{V}O_{2max}$ in nine subjects exposed to 0.3 ppm O_3 while performing a progressively incremented exercise test to volitional fatigue lasting 30 min. In addition, Savin and Adams (1979) observed no significant effect on pulmonary function, performance time, maximum heart rate, or anaerobic threshold, although maximum ventilation was significantly reduced 7%.

More recent findings of Foxcroft and Adams (1986) and Gong et al. (1986) support the earlier observations of Folinsbee et al. (1977). Foxcroft and Adams (1986) observed significant ($p < 0.05$) reductions in performance time (16.7%), $\dot{V}O_{2max}$ (6.0%), maximum ventilation (15.0%), and maximum heart rate (5.6%) in eight aerobically trained males during a rapidly incremented $\dot{V}O_{2max}$ test following 50-min exposure to 0.35 ppm O_3 with CE (exercise $\dot{V}_E$ = 60 L/min). Similarly, Gong et al. (1986) found significant

Table 7-8. Ozone Effects on Exercise Performance[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] µg/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.06-0.07 0.12-0.13	120-140 245-260	CE ($\dot{V}_E$ = 30 to 120 L/min) 16 to 28 min progressive maximum exercise protocol	Tdb = 23 to 24.5 °C RH = 50 to 53%	12 M, 12 F	Athletic	Reduced maximum performance time and increased respiratory symptoms during O_3 exposure.	Linder et al. (1988)
0.12 0.18 0.24	235 353 470	1 h competitive simulation exposures at mean $\dot{V}_E$ = 87 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Highly trained competitive cyclists	Decrease in exercise time of 7.7 min and 10.1 min for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm O_3, respectively; decrease in FVC and FEV_1 for 0.18- and 0.24-ppm O_3 exposure compared with FA exposure.	Schelegle and Adams (1986)
0.12 0.20	235 392	1 h CE $\dot{V}_E$ = 89 L/min	Tdb = 31 °C	15 M, 2 F	Highly trained competitive cyclists	Decrease in $\dot{V}_{Emax}$, $\dot{V}O_{2max}$, V_{Tmax}, workload, ride time, FVC, and FEV_1 with 0.20 ppm O_3 exposure, but not significant with 0.12-ppm O_3 exposure, as compared to FA exposure.	Gong et al. (1986)
0.20 0.35	392 686	1 h CE or competitive simulation at mean $\dot{V}_E$ = 77.5 L/min	Tdb = 23 to 26 °C RH = 45 to 60%	10 M	Well-trained distance runners	V_T decreased and f increased with continuous 50-min O_3 exposures; decrease in FVC, FEV_1, and $FEF_{25-75\%}$ from FA to 0.20 ppm and FA to 0.35-ppm O_3 exposure in all conditions; three subjects unable to complete continuous and competitive protocol at 0.35 ppm O_3.	Adams and Schelegle (1983)
0.21	412	1 h CE at 75% $\dot{V}O_2$max	Tdb = 19 to 21 °C RH = 60 to 70%	6 M, 1 F	Well-trained cyclists	Decrease in FVC, FEV_1, $FEF_{25-75\%}$, and MVV with 0.21 ppm O_3 compared with FA exposure.	Folinsbee et al. (1984)
0.25	490	1 h CE $\dot{V}_E$ = 63 L/min	Tdb = 20 °C RH = 70%	19 M, 7 F	Active nonathletes	FVC, FEV_1, and MVV all decreased with 0.25-ppm O_3 exposure compared with FA.	Folinsbee et al. (1986)
0.25 0.50 0.75	490 980 1,470	2 h rest	NA	8 M, 5 F		FVC decreased with 0.50- and 0.75-ppm O exposure compared with FA; 4% nonsignificant decrease in mean $\dot{V}O_{2max}$ following 0.75 ppm O_3 compared with FA exposure.	Horvath et al. (1979)
0.35	686	50 min CE $\dot{V}_E$ = 60 L/min	NA	8 M	Trained nonathletes	V_T decreased, f increased with 50-min O_3 exposures; decrease in FVC, FEV_1, $FEF_{25-75\%}$, performance time, $\dot{V}O_{2max}$, $\dot{V}_{Emax}$, and HR_{max} from FA to 0.35-ppm O_3 exposure.	Foxcroft and Adams (1986)

Table 7-8 (cont'd). Ozone Effects on Exercise Performance[a]

Ozone Concentration[b]		Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
ppm	μg/m³						
0.75	1,470	2 h IE (4 × 15 min light [50 W] bicycle ergometry)	NA	13 M	4 light S, 9 NS	Decrease in FVC, FEV_1, ERV, IC, and $FEF_{50\%}$ after 1-h 0.75-ppm O_3 exposure; decrease in $\dot{V}O_{2max}$, $\dot{V}_{Tmax}$, $\dot{V}_{Emax}$, maximal workload, and heart rate following 0.75-ppm O_3 exposure compared with FA.	Folinsbee et al. (1977)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

reductions in performance time (29.7%), $\dot{V}O_{2max}$ (16.4%), maximum ventilation (18.5%), and maximum workload (7.8%) in 17 top-caliber endurance cyclists during a rapidly incremented $\dot{V}O_{2max}$ test following 1-h exposure to 0.2 ppm O_3 with very heavy CE ($\dot{V}_E$ = 90 L/min) and the addition of ambient heat stress (31 °C). In both studies (Foxcroft and Adams, 1986; Gong et al., 1986), the reductions in maximal exercise endpoints were accompanied by significant decrements in pulmonary function and marked subjective symptoms of respiratory discomfort. More recently, Linder et al. (1988) observed small decrements in performance time during a progressive maximal exercise test at O_3 concentrations as low as 0.06 ppm. These small effects were associated with increased respiratory symptoms and small, inconsistent changes in FEV_1. Hence, it appears that maximal oxygen uptake is reduced if it is preceded by an O_3 exposure entailing a sufficient total inhaled dose of O_3 to result in significant pulmonary function decrements or subjective symptoms of respiratory discomfort.

Effect on Endurance Exercise Performance

Two studies (Adams and Schelegle, 1983; Folinsbee et al., 1984) that addressed the effects of acute O_3 exposures on the ability of highly trained subjects to complete strenuous continuous exercise protocols were discussed in the 1986 EPA criteria document (U.S. Environmental Protection Agency, 1986).

Adams and Schelegle (1983) exposed 10 well-trained distance runners to FA and 0.20 and 0.35 ppm O_3 while the runners exercised on a bicycle ergometer at workloads simulating either a 1-h steady-state "training" bout or a 30-min warm-up followed immediately by a 30-min "competitive bout". The exercise levels in the steady-state training bout were of sufficient magnitude (68% of their $\dot{V}O_{2max}$) to increase mean $\dot{V}_E$ to 80 L/min. The $\dot{V}_E$ averaged over the entire competitive simulation was also 80 L/min, whereas the mean $\dot{V}_E$ during the 30-min competitive bout was 105 L/min. Subjective symptoms increased as a function of O_3 concentration for both training and competitive protocols. In the competitive protocol, four runners exposed to 0.20 ppm O_3 and nine exposed to 0.35 ppm O_3 indicated that they could not have performed maximally. Three subjects were unable to complete both the training and competitive protocols at 0.35 ppm O_3, and a fourth failed to complete only the competitive ride.

Folinsbee et al. (1984) exposed six well-trained men and one well-trained woman to 0.21 ppm O_3 while they exercised continuously on a bicycle ergometer for 1 h at 75% of their $\dot{V}O_{2max}$ ($\dot{V}_E$ = 81 L/min). Following O_3 exposure, FVC and FEV_1 were reduced significantly and the subjects reported symptoms of laryngeal and tracheal irritation and chest soreness and tightness when taking deep breaths. Anecdotal reports obtained from the cyclists suggested that their performance would have been limited if they experienced similar symptoms during competition.

Avol et al. (1984) exposed 50 well-conditioned cyclists to 0.00, 0.08, 0.16, 0.24, and 0.32 ppm O_3 for 1 h in ambient heat (32 °C) while they exercised continuously ($\dot{V}_E$ = 57 L/min). Reductions in FEV_1 and symptoms, initially detected at 0.16 ppm O_3, increased in a concentration- dependent manner. Three and 16 cyclists could not complete the 1-h exposure to 0.16 and 0.24 ppm O_3, respectively, without a reduction in workload. Similarly, in their study of the effects of O_3 exposure on $\dot{V}O_{2max}$, Gong et al. (1986) reported that 6 of 17 highly trained endurance cyclists were not able to complete 1-h exposure to 0.2 ppm O_3 with very heavy CE ($\dot{V}_E$ = 90 L/min) and the addition of ambient heat stress (31 °C).

In a study designed to determine the effects of the inhalation of low ambient O_3 concentrations on simulated competitive endurance performance, Schelegle and Adams (1986) exposed 10 highly trained endurance athletes to 0.12, 0.18, and 0.24 ppm O_3 while they were performing a 1-h "competitive" protocol. The competitive protocol used in this study was similar to that used by Adams and Schelegle (1983) except that the workload during the final 30-min competitive bout was more intense; it was selected based on the maximum workload (approximately 86% of their $\dot{V}O_{2max}$, mean $\dot{V}_E$ = 120 L/min) each subject could maintain for 30 min while breathing FA. All subjects completed the FA exposure, whereas one, five, and seven subjects could not complete the 0.12-, 0.18-, and 0.24-ppm O_3 exposures, respectively. Following 0.18- and 0.24-ppm O_3 exposures, FVC and FEV_1 were reduced significantly ($p < 0.05$), and subjective symptoms were elevated significantly ($p < 0.05$). No significant effect of O_3 was found for metabolic or ventilatory pattern responses. Similarly, Folinsbee et al. (1986) found that highly trained runners experienced a reduced run time on a treadmill (speed and grade set at approximately 80% of their subjects $\dot{V}O_{2max}$) when exposed to 0.18 ppm O_3 compared with FA. These subjects did have significantly elevated symptoms of respiratory discomfort and significantly decreased FVC and FEV_1, whereas arterial oxygen saturation at the end of the run was not affected by O_3 exposure.

Determining the mechanisms leading to the observed decrements in maximal oxygen uptake and the inability to complete strenuous exercise protocols is problematic. As stated by Astrand and Rodahl (1977) "the capacity for prolonged rhythmic muscular exercise is limited by an interrelated composite of cardiorespiratory, metabolic, environmental, and psychological factors." Many investigators cited above have concluded that the observed reductions in exercise performance appeared to be due to symptoms limiting the ability of their subjects to perform. However, in every case, this is a conclusion achieved by exclusion and not by the demonstration of a causal relationship. Other factors could also contribute to O_3-induced decrements in exercise performance. One possibility is that stimulation of neural receptors in the airways may result in an inhibition of alpha-motor nerve activity to respiratory muscles during inspiration (Koepchen et al., 1977; Schmidt and Wellhoner, 1970), resulting in the observed decrease in V_T and, at the same time, increasing the subject's sensation of respiratory effort. This mechanism would not be directly related to symptoms of discomfort but, because of the common role of airway neural afferents, may be difficult to discern from the effects of symptoms of respiratory discomfort. Indeed, a reflex inhibition of the ability to inspire would be consistent with the reduced V_T following O_3 exposure in subjects performing maximal exercise and would be consistent with the development of a physiologically induced ventilatory limitation to maximal oxygen uptake.

7.2.2 Pulmonary Function Effects of Prolonged (Multihour) Ozone Exposures

Since 1988, a series of studies has described the responses of subjects exposed to relatively low (0.08 to 0.16 ppm) O_3 concentrations for durations of 4 to 8 h (see Table 7-9). These studies have demonstrated statistically significant changes in spirometry, R_{aw}, symptoms, and airway responsiveness during and after exposures. As in studies conducted at higher concentrations of O_3 for shorter periods of time, there is broad variability in response.

The only related study cited in the previous criteria document (U.S. Environmental Protection Agency, 1986) was that of Kerr et al. (1975), who exposed

Table 7-9. Pulmonary Function Effects After Prolonged Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	157 196 235	6.6 h IE (6 × 50 min) $\dot{V}_E$ ≈ 39 L/min	18 °C 40% RH	22 M	Healthy NS, 18 to 33 years old	FVC and FEV_1 decreased throughout the exposure; FEV_1 decrease at end exposure was 7.0, 7.0, and 12.3%, respectively. FEV_1 change > 15% occurred in 3, 5, and 9 subjects at 0.08, 0.10, and 0.12 ppm, respectively. Methacholine responsiveness increased by 56, 89, and 121%, respectively.	Horstman et al. (1990)
		See Horstman et al. (1990) and Folinsbee et al. (1988)				A lognormal model was fitted to FEV_1 data. Model parameters indicate O_3 concentration had greater effect than $\dot{V}_E$ or duration (estimated exponent for $[O_3]$ ≈ 4/3).	Larsen et al. (1991)
0.08 0.10	157 196	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 40 L/min	18 °C 40% RH	38 M	Healthy NS, mean age 25 years old	FEV_1, decreased 8.4% at 0.08 ppm and 11.4% at 0.10 ppm. Symptoms of cough, PDI, and SB increased with O_3 exposure.	McDonnell et al. (1991)
0.08	157	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 35 to 38 L/min (1 day of air, 2 days of O_3)	25 °C 48% RH	5 F, 6 M	Healthy NS, 30 to 45 years old	FVC decreased 2.1%, FEV_1 decreased 2.2% on first day of O_3 exposure; no change on second O_3 day.	Horvath et al. (1991)
0.12	235	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 42.6 L/min	18 °C 40% RH (1 exposure to clean air; 1 exposure to O_3)	10 M	Healthy NS, 18 to 33 years old	FEV_1 decreased by 13% after 6.6 h. FVC dropped 8.3%. Cough and PDI increased with O_3 exposure. Airway responsiveness to methacholine doubled after O_3 exposure.	Folinsbee et al. (1988)
(a) 0.12 (b) Varied from 0.0 to 0.24 (increased by 0.06 ppm/h then decreased by 0.06 ppm/h)	235	8 h IE (8 × 30 min) $\dot{V}_E$ = 40 L/min	22 °C 40% RH < 3 μg/m³ TSP	23 M	Healthy NS, 20 to 35 years old	(a) FEV_1 decreased 5% by 6 h and remained at this level through 8 h. (b) FEV_1 change mirrored O_3 concentration change with a lag time of ≈ 2 h. Max decrease of 10.2% after 6 h. FEV_1 change was reduced in last 2 h of exposure.	Hazucha et al. (1992)

Table 7-9 (cont'd). Pulmonary Function Effects After Prolonged Exposures to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.12	235	6.5 h/day IE (6 × 50 min) (2 days of exposure) $\dot{V}_E$ = 28 L/min (asthmatic) $\dot{V}_E$ = 31 L/min (healthy)	21 °C 50% RH	15 (8 M, 7 F) 30 (13 M, 17 F)	Healthy NS, 22 to 41 years old Asthmatic NS, 18 to 50 years old	Bronchial reactivity to methacholine increased with O_3 exposure in healthy subjects. FEV_1 decreased 2% (pre- to postexposure) in healthy subjects and 7.8% in asthmatics. Responses were generally less on the second day. Two healthy subjects and four asthmatics had FEV_1 decreases > 10%.	Linn et al. (1994)
0.12	235	6.6 h IE (6 × 50 min) $\dot{V}_E$ = 38.8 L/min	18 °C 40% RH (5 consecutive days of exposure to O_3, 1 day exposure to CA)	17 M	Healthy NS, mean age 25 ± 4 years old	FEV_1 decreased by 12.8, 8.7, 2.5, and 0.6 and increased by 0.2 on Days 1 to 5 of O_3 exposure, respectively. Methacholine airway responsiveness increased by > 100% on all exposure days. Symptoms increased on the first O_3 day, but were absent on the last 3 exposure days.	Folinsbee et al. (1994)
0.16	314	4 h IE (4 × 50 min) $\dot{V}_E$ = 38.9 L/min	18 °C 40% RH (one exposure to O_3, no control exposure)	15 M	Healthy NS, mean age 25 ± 4 years old	FVC decreased 9.5% and FEV_1 decreased 16.6%. FEV_1/FVC ratio decreased from 0.79 to 0.73.	

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

subjects for 6 h to 0.5 ppm O_3, with only two brief 15-min periods of moderate exercise ($\dot{V}_E$ = 44 L/min) during the exposure. Small changes in spirometry were observed. Because of the minimal extent of exercise and the high O_3 concentration, these results cannot be compared to the more recent studies.

The first prolonged O_3 exposure study involving low concentrations and a substantial amount of "moderate exercise"[1] was reported by Folinsbee et al. (1988). The basic protocol used by these investigators has been used in a number of subsequent investigations and therefore merits describing in some detail. The exposures lasted 6 h and 35 min (6.6 h). Except for a 35-min lunch break (during which O_3 exposure continued at rest) after 3 h, the subjects exercised at a moderate level (with a ventilation of about 40 L/min) for 50 min of each hour. Pulmonary function tests were conducted during the 10-min rest period and at the beginning and end of exposure. The exposure was intended to simulate a day of heavy outdoor work or play. For convenience, this protocol is referred to as the EPA prolonged-exposure protocol.

In this study (Folinsbee et al., 1988), a group of 10 subjects was exposed to clean air and 0.12 ppm O_3 for 6.6 h. Forced vital capacity and FEV_1 decreased in a roughly linear fashion throughout the exposure and had fallen by 8.3 and 13%, respectively, by the end of the exposure. Symptoms of cough and chest discomfort were increased, and airway responsiveness to methacholine was approximately doubled after O_3 exposure. There was a wide range of response, three subjects had FEV_1 decrements of 25% or greater, and the three least sensitive subjects had less than 5% change in FEV_1.

In order to extend these initial observations, Horstman et al. (1990) used the same protocol to expose a group (n = 22) of subjects to clean air and three different O_3 concentrations (0.08, 0.10, and 0.12 ppm). At 0.12 ppm O_3, responses were similar to those observed in the previous study, with the exception that the symptom responses were smaller in the new group of subjects. A similar (but of smaller magnitude) pattern of response in spirometry, R_{aw}, and airway responsiveness was seen at the two lower concentrations. The mean FEV_1 responses during the four exposures are shown in Figure 7-3. The responses were dependent on concentration and exposure duration (ventilation was not varied) and averaged 7, 8, and 13% at the three O_3 concentrations. Larsen et al. (1991) used these data (Horstman et al., 1990) to develop a "dose-response" relationship for percent change in FEV_1 as a function of O_3 concentration and exposure duration. The lognormal multiple linear regression model suggested that FEV_1 responses were approximately linear with duration of exposure but that O_3 concentration plays a slightly more important role. The exponent of approximately 4/3 suggests that doubling O_3 concentration would be similar to increasing exposure duration by about 2˝ times.

A series of additional exposures were conducted at 0.08 and 0.10 ppm O_3 to study changes in cells and inflammatory mediators from BAL (see Section 7.2.4), but pulmonary function was measured as well. McDonnell et al. (1991) reported an 8.4% decrease in FEV_1 at 0.08 ppm and an 11.4% decrease at 0.10 ppm. These responses were slightly larger than those seen in the previous Horstman et al. (1990) study. The duration-FEV_1 response data were fit to a three-parameter logistic model, which significantly improved the amount of

[1]The "moderate" exercise descriptor is based on previously published EPA guidelines for representative types of exercise (see Table 10-3, U.S. Environmental Protection Agency, 1986). Note, however, that exercise continued at this level (40 L/min) for 6 to 8 h should be considered as "heavy" or "strenuous work or play".

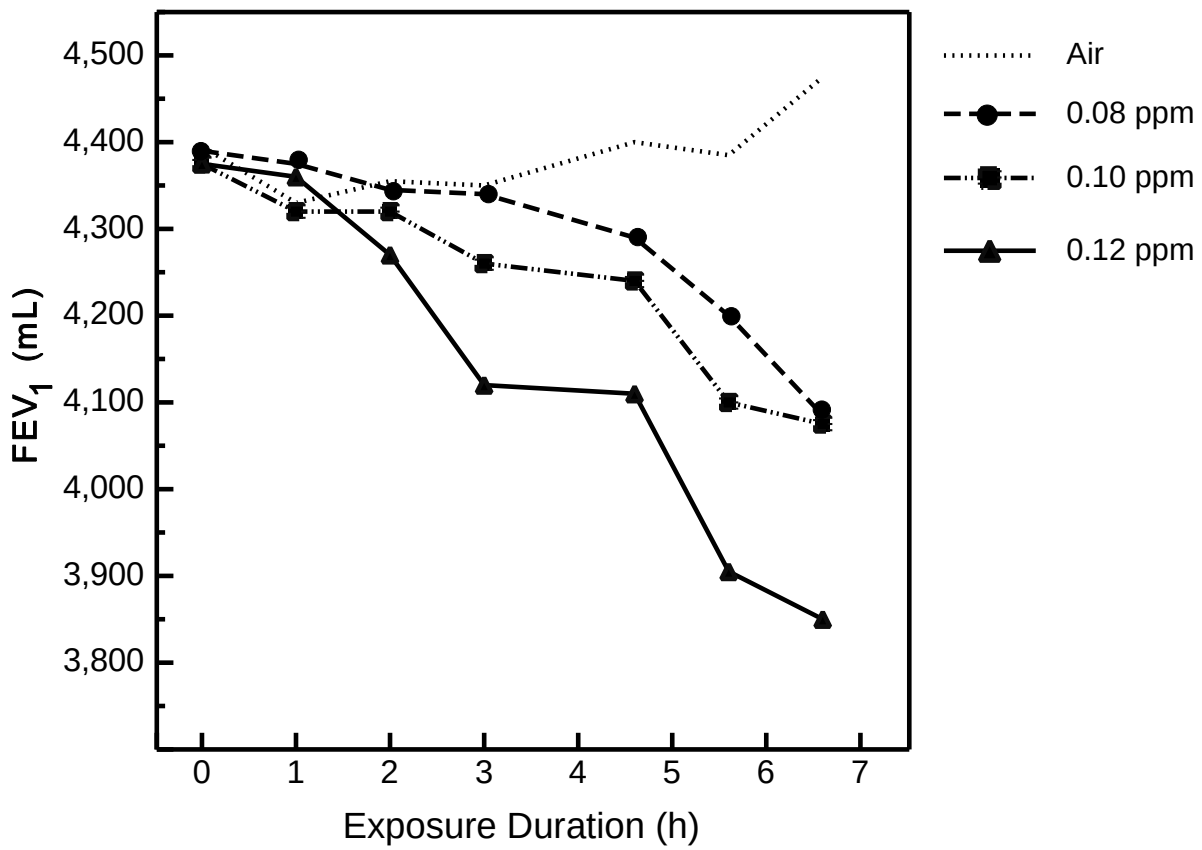

Figure 7-3. The forced expiratory volume in 1 s (FEV_1) is shown in relation to exposure duration at different ozone concentrations. A 35-min resting exposure period was interposed between the end of the third hour and the beginning of the fourth hour. There were six 50-min exercise periods (minute ventilation ≈39 L/min) during the exposure; these measurements were made 5 min after the end of each exercise. The total exposure duration was 6.6 h. The standard error of the mean (not shown) for these FEV_1 averages ranged from 120 to 160 mL.

Source: Horstman et al. (1990).

variance explained by the model compared to a linear model; this is consistent with exploratory analyses in the Folinsbee et al. (1988) report. The reasonably good fit to the logistic model suggests that the O_3-pulmonary function response relationship may have a sigmoid shape. The primary importance of this observation is that it suggests that there is a response plateau. That is, for a given O_3 concentration and exercise ventilation level (i.e., dose rate), and after a certain length of exposure, the FEV_1 response tends not to increase further (i.e., plateau) with increasing duration of exposure.

In the fourth study in this series (Folinsbee et al., 1994), 17 subjects were exposed to 0.12 ppm O_3 for 6.6 h on 5 consecutive days. Subjects who were not responsive to O_3 were not selected to participate in this study. Responses in FEV_1 on the first of these exposures averaged 12.8%. Again, symptom responses were modest with a significant increase in lower respiratory symptoms on the first exposure day. A significant increase in airway responsiveness to methacholine also was shown. The response to the repeated exposures is discussed in Section 7.2.1.4. In addition, 15 subjects were exposed to 0.16 ppm for 4 h using the same hourly exposure protocol as described above. In these subjects, FVC decreased 9.5%, and FEV_1 declined 16.6%.

Folinsbee et al. (1991) took the FEV_1 response data from all four studies conducted at the EPA Health Effects Research Laboratory, using the same prolonged-exposure protocol, and examined the distribution of responses among the subjects at the three concentrations. This response distribution is illustrated graphically in Figure 7-4, which illustrates that FEV_1 decrements as large as 30 to 50% have been observed with prolonged exposure to O_3 concentrations 0.12 ppm. This response distribution allows one to determine the number or percentage of subjects with responses in excess of a certain level. The proportion of subjects with an FEV_1 decrease in excess of 10% is shown in Figure 7-4. With air exposure, no one exceeded this response level; however, 46% of the subjects exposed to 0.12 ppm O_3 had a >10% drop in FEV_1 after 6.6 h.

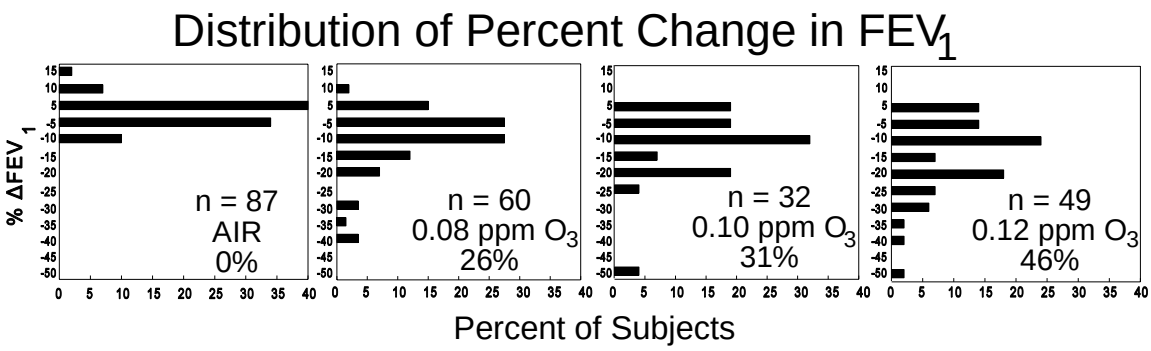

Figure 7-4. The distribution of response for 87 subjects exposed to clean air and at least one of 0.08, 0.10, or 0.12 ppm ozone (O_3) is shown here. The O_3 exposures lasted 6.6 h, during which time the subjects exercised for 50 min of each hour with a 35-min rest period at the end of the third hour. Decreases in forced expiratory volume in 1 s (FEV_1) are expressed as percent change from baseline. For example, the bar labeled "10" indicates the percent of subjects with a decrease in FEV_1 of >5% but 10%, and the bar labeled "5" indicates improvement in FEV_1 of >0% but 5%. Each panel of the figure indicates the percentage of subjects at each O_3 concentration with a decrease of FEV_1 in excess of 10%.

This response distribution also illustrates the wide range of response to O_3 under these exposure conditions and reinforces the observation by others (McDonnell et al., 1983; Horvath et al., 1981) of a substantial range of individual response to O_3.

Horvath et al. (1991) examined the responses of healthy men and women (ages 30 to 45) to 0.08 ppm O_3 for 6.6 h using the EPA prolonged-exposure protocol. When compared with the clean air exposure, FEV_1 decreased about 5% with O_3 exposure. However, the variability of this response among this small heterogeneous group of subjects was enough to preclude statistical significance of this observation. No significant changes were observed with a second exposure on the next day. On the first day of O_3 exposure, 7 of the 11 subjects reported chest tightness. The authors point out that the range of variability in response in their study was similar to that reported by Folinsbee et al. (1988) and Horstman et al. (1990), although fewer subjects experienced large negative changes in FEV_1. One possible explanation for the differences between the findings of Horvath et al. (1991) and Horstman et al. (1990) may be that the subjects in Horvath's study were significantly older, which may result in reduced responses to O_3, as Drechsler-Parks et al. (1987a) have shown (see Section 7.2.1.3). The ventilation during exercise (37 to 39 L/min) was similar to that reported by Horstman et al. (1990). An additional FEV_1 measurement was made in this study at the end of the lunch period (i.e., after 40 min of rest). At this time, the small decrements in FEV_1 seen after the third exercise were reversed, and the FEV_1 was similar to the response in FA at the same time point. Although spirometry was not measured at this time in the other prolonged-exposure studies (Folinsbee et al., 1988; Horstman et al., 1990), it was noted that the decline in FEV_1 was attenuated between the third and fourth postexercise measurement. These observations suggest that the subjects' lung function may indeed have improved during the lunch rest period.

Linn et al. (1994) have reported responses of 45 healthy and asthmatic subjects to 0.12 ppm O_3 using the EPA prolonged-exposure protocol. In healthy subjects, they observed a small (1.7%) decrease in FEV_1, which was statistically significant, and an increase in airway responsiveness to methacholine. The functional responses in asthmatics (e.g., a 7.8% decrease in FEV_1) were greater than those of the healthy subjects. They observed smaller responses on a second consecutive day of exposure, as did Horvath et al. (1991) and Folinsbee et al. (1994). The ventilation averaged 31 and 28 L/min in the healthy and asthmatic subjects, respectively. The FEV_1 responses observed in this study, although statistically significant, are much lower than those observed by EPA investigators (Folinsbee et al., 1988; Horstman et al., 1990; Folinsbee et al., 1994). The smaller responses may be due to previous ambient exposures, lower ventilations, or a larger proportion of O_3-insensitive subjects in Los Angeles. Only 1 of 15 healthy subjects experienced an FEV_1 decrement in excess of 10%, whereas 9 of 30 asthmatics had FEV_1 decrements in excess of 10%. Asthmatic responses ranged from 12% to 35%.

To further explore the factors that determine responsiveness to O_3, Hazucha et al. (1992) designed a protocol to examine the effect of varying, rather than constant, O_3 concentrations. In this study, subjects were exposed to a constant level of 0.12 ppm O_3 for 8 h and to an O_3 level that increased linearly from 0 to 0.24 ppm for the first 4 h and then decreased linearly from 0.24 to 0 over the second 4 h of the 8 h exposure (triangular concentration profile). Subjects performed moderate exercise for the first 30 min of each hour. The overall exposure dose for these two exposures, calculated as the $C \times T \times \dot{V}_E$, was almost identical (difference $< 1\%$). With exposure to the constant 0.12 ppm O_3, the FEV_1 declined

approximately 5% by the fifth hour of exposure and remained at that level for the remainder of the exposure. These responses are illustrated in Figure 7-5. This observation clearly indicates a response plateau, suggested in other studies (Horstman et al., 1990), with an exposure regimen that produces relatively small changes in lung function.

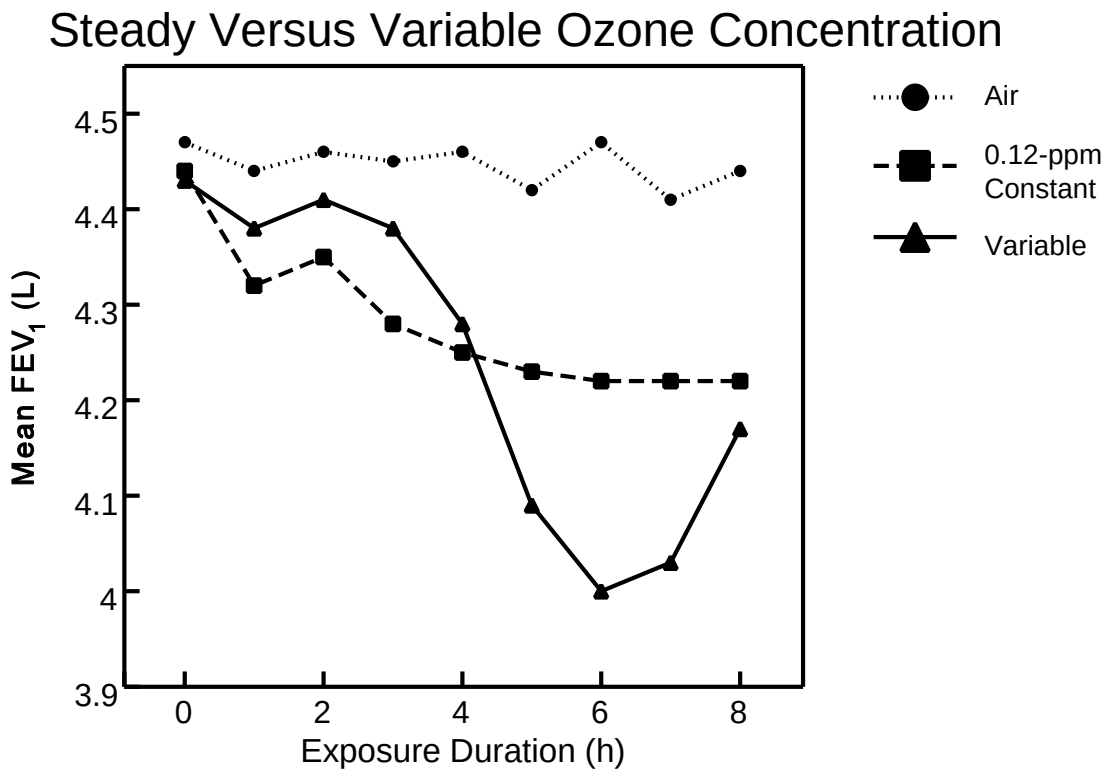

Figure 7-5. The forced expiratory volume in 1 s (FEV_1) is shown in relation to exposure duration (hours) under three exposure conditions. Subjects exercised (minute ventilation ≈40 L/min) for 30 min during each hour; FEV_1 was measured at the end of the intervening rest period. Standard error of the mean for these FEV_1 averages (not shown) ranged from 120 to 150 mL.

Source: Hazucha et al. (1992).

With the triangular O_3 concentration profile, the FEV_1 decreased almost twice as much after 6 h of exposure. The initial response over the first 3 h was minimal, and then there was a substantial decrease in FEV_1, corresponding to the higher average O_3 concentration, that reached a nadir after 6 h. Despite continued exposure to a lower O_3 concentration (<0.12 ppm), the FEV_1 began to improve and was reduced by only 5.9% at the end of the 8-h exposure. (However, note that the average O_3 concentration in the eighth hour was 0.03 ppm). This study illustrates two important points. First, a response plateau occurs. It is intuitively obvious that there must be a limit to the acute decrease that can occur in FEV_1. However, from this study, it is also clear that the response plateau must be dependent on the O_3 concentration because much larger decreases in FEV_1 occur with exposure to O_3 concentrations higher than 0.12 ppm. Second, the response to O_3 exposure is dependent on the dose rate (some function of C and $\dot{V}_E$) and the cumulative dose (some function of dose rate and T), at least when the O_3 concentration is varied. This study also affirms the observation (Folinsbee et al., 1978; Adams et al., 1981; Hazucha, 1987; Larsen et al., 1991) that

O_3 concentration is a more important factor in determining O_3 responses than is either exposure duration or the volume of air breathed during the exposure.

7.2.3 Increased Airway Responsiveness

Increased airway responsiveness indicates that the airways are predisposed to bronchoconstriction induced by a variety of stimuli (e.g., specific allergens, SO_2, cold air, etc.). Airway responsiveness is usually measured by having the individual forcefully exhale into a spirometer designed to measure expiratory flow rates (e.g., FEV_1) or, less commonly, by measuring R_{aw} in a body plethysmograph. In order to determine the level of airway responsiveness, airway function is measured before and immediately after the inhalation of small amounts of an aerosolized bronchoconstrictor drug (e.g., methacholine or histamine). The dose of the bronchoconstrictor drug is increased in a step-wise fashion until a predetermined degree of airway response (e.g., a 20% drop in FEV_1 or a 100% increase in R_{aw}) has occurred. The dose of the bronchoconstrictor drug that produced the aforementioned response is often referred to as the "PD_2 " (i.e., the provocative dose that produced a 20% drop in FEV_1) or the "PD_1 " (i.e., the provocative dose that produced a 100% increase in R_{aw}).

A high level of bronchial responsiveness is a hallmark of asthma. However, varying degrees of increased airway responsiveness may occur in other lung disease (e.g., chronic bronchitis or viral respiratory infections) or in healthy asymptomatic individuals. The range of nonspecific bronchial responsiveness, as expressed by the PD_2 for example, is at least 1,000-fold from the most sensitive asthmatics to the least sensitive healthy subjects (see Figure 7-6). The average PD_2 for healthy subjects is 10 to 100 times that of mild to moderate asthmatics (Chatham et al., 1982; Cockcroft et al., 1977). Atopic or allergic individuals without asthma (intermediate in responsiveness between healthy subjects and mild asthmatics) typically have a lower PD_2 than healthy individuals (Townley et al., 1975; Cockcroft et al., 1977). Increasing severity of asthma, as indicated by increasing symptoms or medication usage, is associated with decreasing PD_2 . Mild asthmatics may have a PD_2 that is 10 times higher than that of moderate or severe asthmatics (Cockcroft et al., 1977). A low PD_2 in nonasthmatics also is associated with increased symptoms and a reduced baseline FEV_1 (Kennedy et al., 1990). The average changes in airway responsiveness induced by O_3 range from 150 to 500%. This means that, in a healthy subject exposed to O_3, a PD_2 of 20 units would decrease to a PD_2 between 13 and 4 units. Therefore, with a pronounced O_3-induced change in airway responsiveness, a healthy subject could move from the normal range into the upper half of the mild asthmatic range of airway responsiveness.

Increases in airway responsiveness are an important consequence of exposure to O_3. Results of studies reporting changes in airway responsiveness following O_3 exposure are summarized in Table 7-10. These studies vary with regard to exposure regimens, type and dose of bronchoconstrictive agent, and subject population. Increased airway responsiveness associated with O_3 exposure was first reported by Golden et al. (1978), who studied histamine-responsiveness in eight healthy men after exposure to 0.6 ppm O_3 for 2 h at rest and found that the histamine-induced ΔR_{aw} for the group was 300% greater 5 min after O_3 exposure than at baseline. Two of their subjects, however, had an increased response to histamine 1 week or greater after exposure, raising the possibility that high O_3 levels can result in more persistent increases in airway responsiveness. Later, Holtzman et al. (1979) found in 16 nonasthmatic subjects that a 10-breath methacholine or histamine challenge

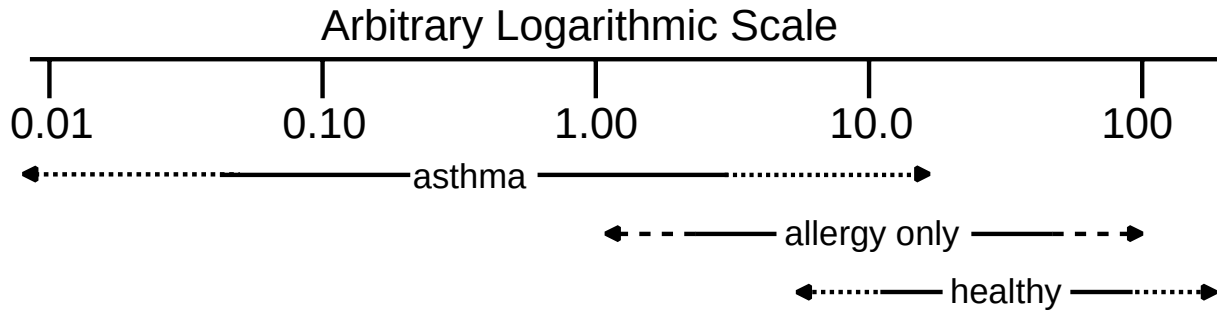

Figure 7-6. Airway function can be measured before and immediately after the inhalation of an aerosolized bronchoconstrictor drug like methacholine. The provocative dose that produces a 20% drop in forced expiratory volume in 1 s has been used to express the range of nonspecific bronchial responsiveness.

increased SRaw almost twice as much after O_3 as after air exposure, but this effect resolved after 24 h. Atopic subjects showed similar increases in responsiveness to histamine after O_3 exposure. The authors concluded that the increased nonspecific bronchial responsiveness after O_3 exposure was not related to atopy. König et al. (1980) found increased responsiveness to inhaled acetylcholine after a 1-h exposure to 627 and 1,960 $\mu g/m^3$ (0.32 and 1.00 ppm, respectively). Folinsbee and Hazucha (1989) found increased airway responsiveness in 18 females 1 and 18 h after a 70-min exposure to 0.35 ppm O_3 when compared to air. Taken together, these studies suggest that O_3-induced increases in airway responsiveness usually resolve 18 to 24 h after exposure, but may persist in some individuals for longer periods.

Dimeo et al. (1981) were the first to investigate "adaptation" to the increases in airway responsiveness following O_3 exposure. Over 3 days of a 2 h/day exposure to 0.40 ppm O_3, they found progressive attenuation of the increases in airway responsiveness such that, after the third day of O_3 exposure, histamine airway responsiveness was no longer different from the sham exposure levels. Kulle et al. (1982) extended these findings by exposing two groups of healthy volunteers (n = 48) to 0.40 ppm O_3 for 3 h/day for 5 days in a row and found that there was a significantly enhanced response to methacholine after the first 3 days of exposure, but this response slowly normalized by the end of the fifth day. Thus, the attenuation of O_3-induced increases in airway responsiveness followed the same time course as attenuation of other pulmonary function changes.

Gong et al. (1986) demonstrated increased airway responsiveness to histamine at 0.2 ppm O_3 in 17 vigorously exercising elite cyclists who were exposed for 1 h. Folinsbee et al. (1988) found an approximate doubling of the mean methacholine responsiveness in a group of healthy volunteers exposed for 6.6 h to 0.12 ppm O_3. However, on an individual basis, no relationship was found between O_3-induced changes in airway responsiveness and those in FVC and FEV_1, suggesting that changes in airway responsiveness and lung volume occurred by different mechanisms. Horstman et al. (1990) extended Folinsbee's observations by demonstrating significant decreases in the PD_1 in 22 healthy subjects immediately after a

Table 7-10. Increased Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] □g/m	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	157 196 235	6.6 h IE at □39 L/min	18 □C 40% RH	22 M	Healthy NS, 18 to 32 years old	33, 47, and 55% decreases in cumulative dose of methacholine required to produce a 100% increase in SR_{aw} after exposure to O_3 at 0.08, 0.10, and 0.12 ppm, respectively.	Horstman et al. (1990)
0.10 0.32 1.00	196 627 1,960	2 h	NA	14	Health NS, 24 ± 2 years old	Increased airway responsiveness to methacholine immediately after exposure at the two highest concentrations of O_3.	König et al. (1980)
0.12 0.20	235 392	1 h at $\dot{V}_E$ = 89 L/min followed by 3 to 4 min at □150 L/min	31 □C 35% RH	15 M, 2 F	Elite cyclists, 19 to 30 years old	Greater than 20% increase in histamine responsiveness in one subject at 0.12 ppm O_3 and in nine subjects at 0.20 ppm O_3.	Gong et al. (1986)
0.12	235	6.6 h with IE at □25 L/min/m^2 BSA	NA	10 M	Healthy NS, 18 to 33 years old	Approximate doubling of mean methacholine responsiveness after exposure. On an individual basis, no relationship between O_3-induced changes in airway responsiveness and FEV_1 or FVC.	Folinsbee et al. (1988)
0.12 ppm O_3-100 ppb SO_2 0.12 ppm O_3-0.12 ppm O_3 Air-100 ppb SO_2		45 min in first atmosphere and 15 min in second IE	75% RH 22 □C	8 M, 5 F	Asthmatic, 12 to 18 years old	Greater declines in FEV_1 and $\dot{V}_{max50\%}$ and greater increase in respiratory resistance after O_3-SO_2 than after O_3-O_3 or air-SO_2.	Koenig et al. (1990)
Air-antigen 0.12 ppm O_3-antigen		1 h at rest	NA	4 M, 3 F	Asthmatic, 21 to 64 years old	Increased bronchoconstrictor response to inhaled ragweed or grass after O_3 exposure compared to air.	Molfino et al. (1991)
0.35	686	70 min with IE at 40 L/min	NA	18 F	Healthy NS, 19 to 28 years old	PD_{100} decreased from 59 CIU after air exposure to 41 CIU and 45 CIU, 1 and 18 h after O_3 exposure, respectively.	Folinsbee and Hazucha (1989)

Table 7-10 (cont'd). Increased Airway Responsiveness Following Ozone Exposures[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m³	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics[c]	Observed Effect(s)	Reference
0.20 0.40 0.40	392 784 784	2 h with IE at 2 × resting 2 h with IE at 2 × resting 2 h/day for 3 days	22 °C 55% RH	12 M, 7 F	Healthy NS, 21 to 32 years old	110% increase in ΔSR_{aw} to a 10-breath histamine (1.6%) aerosol challenge after exposure to O_3 at 0.40 ppm, but no change at 0.20 ppm. Progressive adaptation of this effect over 3-day exposure.	Dimeo et al. (1981)
0.40	784	3 h/day for 5 days in a row		13 M, 11 F	Healthy NS, 19 to 46 years old	Enhanced response to methacholine after first 3 days, but this response normalized by Day 5.	Kulle et al. (1982)
0.40	784	2 h with IE at $\dot{V}_E$ = 53 to 55 L/min	22 °C 50% RH	8 M, 10 F	9 asthmatics (5 F, 4 M), 9 healthy (5 F, 4 M), 18 to 34 years old	Decreased $PC_{100SR_{aw}}$ from 33 mg/mL to 8.5 mg/mL in healthy subjects after O_3. $PC_{100SR_{aw}}$ fell from 0.52 mg/mL to 0.19 mg/mL in asthmatic subjects after exposure to O_3 and from 0.48 mg/mL to 0.27 mg/mL after exposure to air.	Kreit et al. (1989)
0.60	1,176	2 h at rest	NA	5 M, 3 F	Healthy NS, 22 to 30 years old	300% increase in histamine-induced ΔR_{aw} 5 min after O_3 exposure; 84 and 50% increases 24 h and 1 week after exposure ($p > 0.05$), respectively. Two subjects had an increased response to histamine 1 week after exposure.	Golden et al. (1978)
0.60	1,176	2 h with IE at 2 × resting	22 °C 55% RH	11 M, 5 F	9 atopic, 7 nonatopic, NS, 21 to 35 years old	Ten-breath methacholine or histamine challenge increased SR_{aw} ≥150% in 16 nonasthmatics after O_3. On average, the atopic subjects had greater responses than the nonatopic subjects. The increased responsiveness resolved after 24 h. Atropine premedication blocked the O_3-induced increase in airway responsiveness.	Holtzman et al. (1979)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

6.6-h exposure to concentrations of O_3 as low as 0.08 ppm. Because methacholine challenges were not conducted at later time points in any of these studies, the duration of the increased airway responsiveness after ambient-level O_3 exposure could not be determined.

No doubt exists that O_3, even at ambient concentrations, produces acute increases in airway responsiveness. Whether O_3 exposure causes protracted increases in airway responsiveness in healthy individuals, induces asthma, or predisposes individuals to asthma is a more difficult question to answer (see Section 7.4.2). However, the increases in airway responsiveness following O_3 exposure, even if short in duration, may have important clinical implications. Several studies have been conducted specifically to determine the significance of acute increases in airway responsiveness after O_3 exposure. These studies, designed to test the hypothesis that an O_3 exposure heightens the response to a subsequent bronchoconstrictor challenge, have exposed asthmatics to O_3 or air and, then, to a known bronchoconstrictor agent to compare the pulmonary function changes after O_3 to those after air. Kreit et al. (1989) were first to investigate the change in airway responsiveness that occurs after O_3 exposure in individuals with asthma. They exposed nine mild asthmatics (baseline $PC_{1\ SRaw} < 1.5$ mg/mL) for 2 h to 0.40 ppm O_3 with IE and found that the baseline $PC_{1\ SRaw}$ declined from 0.52 to 0.19 mg/mL after O_3 as compared to 0.48 to 0.27 mg/mL after air. Koenig et al. (1990) demonstrated that a 45-min exposure to 0.12 ppm O_3 followed by a 15-min exposure to 100 ppb SO_2 caused greater changes in FEV_1, respiratory resistance, and $\dot{V}_{max5\ \%}$ in 14 adolescent asthmatics than did an air-SO_2 exposure combination.

Molfino et al. (1991) examined the effects of a 1-h resting exposure to 0.12 ppm O_3 on the response to a ragweed or grass allergen inhalation challenge. Asthmatic subjects were exposed twice to air and twice to O_3, once per week over a period of 4 weeks. Two allergen challenges were performed, once after air and once after O_3 exposure. The other air and O_3 exposures were followed by a placebo challenge. A ragweed allergen extract was used for six of the seven subjects. The order of experiments was not randomized (in an effort to avoid unexpectedly severe reactions); six of the seven subjects were exposed to the ozone-allergen condition last and five of the seven were exposed to the air-placebo condition first. Allergen responsiveness was expressed as the allergen concentration needed to cause a 15% reduction in FEV_1 or PC_{15}. The PC_{15} was lower after the O_3 exposure than after the air exposure ($p = 0.04$). These observations suggest that allergen-specific airway responsiveness is increased after O_3 exposure. Although it is expected that specific bronchial reactivity will be increased by O_3 exposure based on the marked increases in nonspecific bronchial responsiveness induced by O_3 exposure, such a response would not have been anticipated under these mild exposure conditions where lung function or symptomatic responses have not been observed. The lack of randomization in this study makes it difficult to assess the validity of conclusions based on the statistical analysis. These results are provocative but should be considered preliminary until this experiment can be repeated.

Ozone may be a clinically important co-factor in the response to airborne bronchoconstrictor substances in individuals with asthma. It is plausible that this phenomenon could contribute to increased asthma exacerbations and, even, consequent increased hospital admissions (see Section 7.4.1). Whether the increased airway responsiveness following O_3 exposure produces an accentuated bronchoconstrictor response to inhaled allergens or SO_2 in healthy individuals or those with lung diseases other than asthma is unknown.

Several studies have been undertaken to determine the mechanism of O_3-induced increases in airway responsiveness (also see Chapter 6). Early experiments in dogs (Lee et al.,

1977) and humans (Golden et al., 1978) suggested an important role for vagal reflexes because vagal nerve cooling and atropine inhibited the increase in histamine-induced bronchoconstriction caused by O_3. Ozone exposure increased bronchomotor responses to cholinergic stimuli (e.g., acetylcholine and methacholine) in dogs (Holtzman et al., 1983) and humans (Seltzer et al., 1986). Subsequent studies, however, revealed that bilateral vagotomy did not inhibit O_3-induced hyperresponsiveness to subcutaneous histamine in guinea pigs (Gordon et al., 1984). These data provide strong evidence that O_3-induced increased airway responsiveness is mediated, at least in part, by cholinergic receptors on airway smooth muscle cells. Interestingly, Gordon et al. also noted that isometric tension in guinea pig tracheal smooth muscle and lung parenchymal strips in response to histamine and carbachol was not affected by exposure to O_3, suggesting that O_3 affected the in vivo milieu surrounding the smooth muscle rather than produced direct effects on the smooth muscle itself.

It can be hypothesized that the increased epithelial permeability caused by O_3 (see Chapter 6) may allow greater penetration of bronchoconstrictor substances, including methacholine and histamine, and that this would lead to increased airway responsiveness. However, Roum and Murlas (1984) suggested that the increased epithelial permeability after O_3 could not totally explain this phenomenon because parenteral cholinergic challenge after O_3 more reproducibly caused bronchospasm than did inhalation challenge with methacholine. The increased responsiveness to parenteral compared to inhaled cholinergic challenge may, however, have been due to increased bronchial blood flow after O_3 exposure. Therefore, the findings of Roum and Murlas do not exclude increased epithelial permeability as the cause of increased airway responsiveness after O_3 exposure.

Holtzman et al. (1983) first pointed out that O_3-induced acute inflammation may be important in the induction of the increased airway responsiveness. In mongrel dogs exposed to O_3, they found bronchial wall PMN infiltration in those animals that developed increased airway responsiveness to acetylcholine, but not in animals that failed to develop increased airway responsiveness. O'Byrne et al. (1984) later demonstrated that hydroxyurea simultaneously decreased peripheral blood leukocyte counts, decreased PMN influx into bronchial tissue, and prevented increased airway responsiveness in dogs exposed to O_3. Both O_3-induced increased airway responsiveness and bronchial tissue PMN influx returned 6 weeks after treatment was discontinued when peripheral leukocyte counts had normalized. Seltzer et al. (1986) found a larger percentage of PMNs (30.8% versus 8.0%) in BAL fluid after O_3 exposure in their subjects that had a greater than threefold decrease in the provocative concentration, which caused a □8 L × cm H_2O/L/s increase in SR_{aw} for methacholine, compared to those subjects that had less than a twofold decrease. These data suggest a possible association between inflammation and increased airway responsiveness after O_3 exposure.

An early study in dogs (O'Byrne et al., 1984) suggested that oxygenation products of arachidonic acid that are sensitive to inhibition by the anti-inflammatory drug indomethacin play a role in O_3-induced hyperresponsiveness without affecting the influx of PMNs. In the first of several human studies with a PG inhibitor, indomethacin did not attenuate the increase in airway responsiveness in subjects exposed to 0.4 ppm O_3 for 2 h (Ying et al., 1990), but did ameliorate the effect of O_3 on spirometric endpoints. Kleeberger and Hudak (1992) observed a marked reduction in PMN influx in O_3-exposed mice given indomethacin without any change in O_3-induced increases in permeability, as indicated by BAL protein. However, Hazucha et al. (1996) found no effect of ibuprofen on PMN levels or protein in the BAL fluid of O_3-exposed humans (also see Section 7.2.4.5). Seltzer et al. (1986) and Koren et al. (1989a,b)

found that O_3 increases a large number of BAL inflammatory mediators (including PGE_2, $PGF_{2\alpha}$, and thromboxane B_2 [TXB_2]), one or more of which may play a role in the increase in airway responsiveness after O_3 exposure.

The role of reactive oxygen metabolites or neuropeptide mediators in the increase in airway responsiveness after O_3 has not been investigated. Furthermore, there has been no direct assessment of alterations in nerve afferents, changes in neurotransmitter concentrations, changes in smooth muscle postsynaptic receptors, or modulation of nerve signal transmission by inflammatory mediators as these pertain to the increase in airway responsiveness after O_3. In conclusion, although the mechanism of O_3-induced increases in airway responsiveness is not completely understood, it appears to be associated with a number of cellular or biochemical changes in the airway (see Section 7.2.4 and Tables 7-11 and 7-12). Because these alterations are part of a complex process, it comes as no surprise that the mechanistic studies on O_3-induced increases in airway responsiveness have not pinpointed an isolated derangement.

7.2.4 Inflammation and Host Defense

7.2.4.1 Introduction

In general, inflammation can be considered as the host response to injury, and the induction of inflammation can be accepted as evidence that injury has occurred. Several outcomes are possible: (1) inflammation can resolve entirely; (2) continued acute inflammation can evolve into a chronic inflammatory state; (3) continued inflammation can alter the structure or function of other pulmonary tissue, leading to diseases such as fibrosis or emphysema; (4) inflammation can alter the body's host defense response to inhaled microorganisms, particularly in potentially vulnerable populations such as the very young and old; and (5) inflammation can alter the lung's response to other agents such as allergens or toxins. It is also possible that the profile of response can be altered in persons with preexisting pulmonary disease (e.g., asthma or COPD) or smokers. At present, it is known that short-term exposure of humans to O_3 can cause acute inflammation and that long-term exposure of laboratory animals results in a chronic inflammatory state (see Chapter 6). However, the relationship between repetitive bouts of acute inflammation in humans caused by O_3 and the development of chronic respiratory disease is unknown.

The previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) contained no studies in which inflammation was measured in humans exposed to O_3. Fiberoptic bronchoscopy since has been used to sample cells and fluids lining the respiratory tract of humans for many markers (Reynolds, 1987). Bronchoalveolar lavage primarily samples the alveolar region of the lung; however, the use of small volume lavages (Rennard et al., 1990) or balloon catheters also allows sampling of the airways. Nasal lavage allows sampling of cells and fluid removed from the nasal passages.

In the past 6 years, several studies have analyzed BAL and NL cells and fluid from humans exposed to O_3 for markers of inflammation and lung damage (see Tables 7-11 and 7-12). The presence of PMNs in the lung has long been accepted as a hallmark of inflammation and has been taken as the major indicator that O_3 causes inflammation in the

Table 7-11. Bronchoalveolar Lavage Studiesof Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] μg/m³	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
0.08 0.10	157 196	6.6 h	IE (40 L/min) six 50-min exercise periods + 10 min rest; 35 min lunch	18 M, 18 to 35 years old	BAL fluid 18 h after exposure to 0.1 ppm O_3 had significant increases in PMNs, protein, PGE_2, fibronectin, IL-6, lactate dehydrogenase, and α-1 antitrypsin compared with the same subjects exposed to FA. Similar but smaller increases in all mediators after exposure to 0.08 ppm O_3 except for protein and fibronectin. Decreased phagocytosis of yeast by alveolar macrophages was noted at both concentrations.	Devlin et al. (1990, 1991) Koren et al. (1991)
0.20	392	4 h	IE (50 min at 40 L/min, 10 min rest)	15 M, 13 F, 21 to 39 years old	Bronchial lavage, bronchial biopsies, and BAL done 18 h after exposure. BAL shows changes similar to other studies. Airway lavage shows increased cells, LDH, IL-8. Biopsies show increased number of PMNs.	Aris et al. (1993a)
0.30	588	1 h (mouth-piece)	CE (60 L/min)	5 M	Significantly elevated PMNs in the BAL fluid 1, 6, and 24 h after exposure, with peak increases at 6 h.	Schelegle et al. (1991)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	BAL fluid 18 h after exposure had significant increases in PMNs, protein, albumin, IgG, PGE_2, plasminogen activator, elastase, complement C3a, and fibronectin.	Koren et al. (1989a,b)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	Macrophages removed 18 h after exposure had changes in the rate of synthesis of 123 different proteins as assayed by computerized densitometry of two-dimensional gel protein profiles.	Devlin and Koren (1990)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	BAL fluid 18 h after exposure contained increased levels of the coagulation factors, tissue factor, and factor VII. Macrophages in the BAL fluid had elevated tissue factor mRNA.	McGee et al. (1990)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	10 M, 18 to 35 years old	BAL fluid 1 h after exposure to 0.4 ppm O_3 had significant increases in PMNs, protein, PGE_2, TXB_2, IL-6, LDH, α-1 antitrypsin, and tissue factor compared with the same subjects exposed to FA. Decreased phagocytosis of yeast by alveolar macrophages.	Koren et al. (1991)

Table 7-11 (cont'd). Bronchoalveolar Lavage Studies of Inflammatory Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b]		Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s) [3]	Reference
ppm	g/m					
0.40	784	2 h/day for 5 days, 2 h either 10 or 20 days later	IE (40 L/min) at 15-min intervals	16 M, 18 to 35 years old	BAL done immediately after fifth day of exposure and again after exposure 10 or 20 days later. Most markers of inflammation (PMNs, IL-6, IL-8, protein, 1-antitrypsin, PGE_2, fibronectin) showed complete attenuation; markers of damage (LDH, elastase) did not. Reversal of attenuation was not complete for some markers, even after 20 days.	Devlin et al. (1995)
0.40	784	2 h	IE (60 L/min) at 15-min intervals	10 M	Subjects given 800 mg ibuprofen or placebo 90 min before exposure. Subjects given ibuprofen had less of a decrease in FEV_1 after O_3 exposure. BAL fluid 1 h after exposure contained similar levels of PMNs, protein, fibronectin, LDH, -1 antitrypsin, LTB_4, and C3a in both ibuprofen and placebo groups. However, subjects given ibuprofen had decreased levels of IL-6, TXB_2, and PGE_2.	Hazucha et al. (1996)
0.40 0.60	784 1,176	2 h	IE (83 W for women, 100 W for men) at 15-min intervals	7 M, 3 F, 23 to 41 years old	BAL fluid 3 h after exposure had significant increases in PMNs, PGE_2, TXB_2, and PGF_{2} at both O_3 concentrations.	Seltzer et al. (1986)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

Table 7-12. Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)[3]	Reference
Nasal Lavage Studies						
0.12 0.24	235 470	90 min	IE (20 L/min) at 15-min intervals	5M, 5F, asthmatic; 4M, 4F, nonasthmatic; 18 to 41 years old	NL done immediately and 24 h after exposure. Increased number of PMNs at both times in asthmatic subjects exposed to 0.24 ppm O_3; no change in nonasthmatic subjects. No change in lung or nasal function.	McBride et al. (1994)
0.30	588	6 h/day for 5 consecutive days	IE (light treadmill)	24 M (12 O_3, 12 air)	Subjects inoculated with type 39 rhinovirus prior to exposure. NL was performed on the morning of Days 1 to 5, 8, 15, and 30. No difference in virus titers in NL fluid of air and O_3-exposed subjects at any time tested. No difference in PMNs or interferon gamma in NL fluid, or in blood lymphocyte proliferative response to viral antigen.	Henderson et al. (1988)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	11 M, 18 to 35 years old	NL done immediately before, immediately after, and 22 h after exposure. Increased numbers of PMNs at both times after exposure; increased levels of tryptase, a marker of mast cell degranulation, immediately after exposure; increased levels of albumin 22 h after exposure.	Graham and Koren (1990) Koren et al. (1990)
0.50	980	4 h on 2 consecutive days	Resting	41 M (21 O_3, 20 air-exposed), 18 to 35 years old	NL done immediately before and after each exposure and 22 h after the second exposure. Increased levels of PMNs at all times after the first exposure, with peak values occurring immediately prior to the second exposure.	Graham et al. (1988)
0.50	980	4 h	Resting	6 M, 6 F, allergic rhinitics, 31.4 ± 2.0 (SD) years old	NL done immediately after exposure. Increased upper and lower respiratory symptoms and increased levels of PMNs, eosinophils, and albumin in NL fluid.	Bascom et al. (1990)

Table 7-12 (cont'd). Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b] ppm	Ozone Concentration[b] g/m	Exposure Duration	Activity Level ($\dot{V}_E$)	Number and Gender of Subjects	Observed Effect(s)	Reference
Clearance Studies						
0.20 0.40	392 784	2 h	IE (light treadmill)	7 M, 27.2 ± 6.0 (SD) years old	Subjects inhaled radiolabeled iron oxide particles immediately before exposure. Concentration-dependent increase in rate of particle clearance 2 h after exposure, although clearance was confined primarily to the peripheral airways at the lower O_3 concentration.	Foster et al. (1987)
0.40	784	1 h	CE (40 L/min)	15 M or F, 18 to 35 years old	Subjects inhaled radiolabeled iron oxide particles 2 h after exposure. No O_3-induced difference in clearance of particles during the next 3 h or the following morning.	Gerrity et al. (1993)
0.40	784	2 h	IE (70 L/min) at 15-min intervals	8 M, 20 to 30 years old	Subjects inhaled ^{99m}Tc-DTPA 75 min after exposure. Significantly increased clearance of ^{99m}Tc-DTPA from the lung in O_3-exposed subjects. Subjects had expected changes in FVC and SR_{aw}.	Kehrl et al. (1987)
0.50	784	2.25 h	IE (70 L/min) at 15-min intervals	16 M, 20 to 30 years old	Similar design and results as earlier study (Kehrl et al., 1987). For the combined studies the average rate of clearance was 60% faster in O_3-exposed subjects.	Kehrl et al. (1989)
In Vitro Studies						
0.25 0.50	490 980	6 h	Human nasal epithelial cells		Increased in ICAM-1, IL-6, IL-1, and TNF expression at 0.5 ppm. No increase in IL-8 expression. No increases at 0.25 ppm.	Beck et al. (1994)
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line		Concentration-dependent increased secretion of PGE_2, TXB_2, $PGF_{2\alpha}$, LTB_4, and LTD_4. More secretion basolaterally than apically.	McKinnon et al. (1993)
0.25 0.50 1.00	490 980 1,960	1 h	Airway epithelial cell line and alveolar macrophages		Increased secretion of IL-6, IL-8, and fibronectin by epithelial cells, even at lowest O_3 concentration. No O_3-induced secretion of these compounds by macrophages.	Devlin et al. (1994)

Table 7-12 (cont'd). Additional Studies of Inflammatory and Host Defense Effects from Controlled Human Exposure to Ozone[a]

Ozone Concentration[b]		Exposure	Activity Level	Number and Gender of		
ppm	g/m[3]	Duration	($\dot{V}_E$)	Subjects	Observed Effect(s)	Reference
In Vitro Studies (cont'd)						
0.30 1.00	588 1,960	1 h	Alveolar macrophages		Concentration-dependent increases in PGE_2 production, and decreases in phagocytosis of sheep erythrocytes. No O_3-induced secretion of IL-1, TNF, or IL-6.	Becker et al. (1991)

[a]See Appendix A for abbreviations and acronyms.
[b]Listed from lowest to highest O_3 concentration.

lungs of humans. Soluble mediators of inflammation (or its resolution) such as cytokines and arachidonic acid metabolites also have been measured in the BAL fluid of humans exposed to O_3. Cytokines that have been reported most often are interleukin (IL)-6 and IL-8, although IL-1 and tumor necrosis factor (TNF) also have been studied. Soluble metabolites of arachidonic acid involved in inflammation and host defense (e.g., PGE_2 and $PGF_{2\alpha}$, thromboxane, and leukotrienes [LTs] such as LTB_4) also have been reported in the BAL fluid of humans exposed to O_3. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties and may be involved in increased airway hyperreactivity observed following O_3 exposure.

Under normal circumstances, the epithelia lining the large and small airways develop tight junctions and restrict the penetration of exogenous particles and macromolecules from the airway lumen into the interstitium and blood, as well as restrict the flow of plasma components into the airway lumen. However, several studies (see Table 7-12) show that O_3 disrupts the integrity of the epithelial cell barrier in human airways, as measured by increased passage of radiolabeled compounds out of the airways, as well as passage of markers of plasma influx such as albumin, immunoglobulin, and other proteins into the airways. In addition, markers of epithelial cell damage such as lactate dehydrogenase (LDH) activity also have been measured in the BAL fluid of humans exposed to O_3.

Inflammatory cells of the lung such as alveolar macrophages (AMs), monocytes, and PMNs also constitute an important component of the pulmonary host defense system. In their unstimulated state, they present no danger to surrounding pulmonary cells and tissues, but upon activation, they are capable of generating free radicals and enzymes with microbicidal capabilities, but they also have the potential to damage nearby cells. Animal studies have demonstrated that O_3 decreases host defense system function (see Chapter 6, Section 6.2.3).

Other soluble factors that have been studied include those involved with fibrin deposition and degradation (Tissue Factor, Factor VII, and plasminogen activator), potential markers of fibrogenesis (fibronectin, platelet derived growth factor), and components of the complement cascade (C3a).

7.2.4.2 Inflammation Assessed by Bronchoalveolar Lavage

Seltzer et al. (1986) were the first to demonstrate that exposure of humans to O_3 resulted in inflammation in the lung. In this study, 10 volunteers were exposed to 0.4 or 0.6 ppm O_3 for 2 h while undergoing exercise, and BAL was performed 3 h later. Bronchoalveolar lavage fluid from subjects exposed to O_3 contained 7.8-fold more PMNs compared with BAL fluid from the same subjects exposed to FA. Additionally, BAL fluid from O_3-exposed subjects contained increased levels of PGE_2, $PGF_{2\alpha}$, and TXB_2 compared to fluid from air-exposed subjects. Koren et al. (1989a,b) also described inflammatory changes in the lungs of 11 subjects exposed to 0.4 ppm O_3 for 2 h while undergoing IE at 70 L/min in a study designed to simulate adults working outdoors or children actively playing. Bronchoalveolar lavage was performed 18 h after O_3 exposure. Subjects exposed to O_3 had an eightfold increase in PMNs in the BAL fluid, confirming the observations of Seltzer et al. In addition, Koren et al. reported a twofold increase in BAL fluid protein, albumin, and immunoglobulin G (IgG) levels, suggestive of increased epithelial cell permeability as a result of O_3 exposure. There was also a 12-fold increase in IL-6 levels in the BAL fluid. Interleukin-1 and TNF were not present in detectable levels in the BAL fluid of any subject. There was, however, a twofold increase in the proinflammatory eicosanoid PGE_2, as well as a

twofold increase in the complement component C3a. This study also provided evidence for stimulation of fibrogenic processes in the lung by demonstrating significant increases in two components of the coagulation pathway, Tissue Factor and Factor VII (McGee et al., 1990), as well as urokinase plasminogen activator and fibronectin (Koren et al., 1989a). Taken together, these two studies demonstrate that exposure of humans to moderate levels of O_3 results in an inflammatory reaction in the lung, as evidenced by substantial increases in PMNs and proinflammatory compounds. Furthermore, these studies demonstrate that both cells and mediators capable of damaging pulmonary tissue are increased after O_3 exposure, as are compounds that play a role in fibrotic and fibrinolytic processes.

Although animal studies have shown that the terminal bronchioles are a major site of O_3-induced inflammation, few human studies have confirmed this finding because BAL primarily samples cells and fluid in the terminal bronchioles and alveoli. However, isolated lavage of the mainstream bronchus using balloon catheters or the more traditional BAL using small volumes of saline have the ability to preferentially measure O_3-induced changes in the large airways. In one study, isolated airway lavage was performed on 14 subjects 18 h after exposure to 0.2 ppm O_3 while undergoing moderate exercise (Aris et al., 1993a). Increases in total lavagable cells, LDH activity, and IL-8 were reported. In contrast, Schelegle et al. (1991), observed no increase in PMNs in the bronchial fluid; however, bronchial biopsies showed increased numbers of PMNs in airway tissue.

The data suggestive of O_3-induced changes in epithelial cell permeability described by Koren et al. (1989a, 1991) and Devlin et al. (1991) support earlier work in which epithelial cell permeability, as measured by increased clearance of radiolabled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) from the lungs of humans exposed to O_3, was demonstrated (Kehrl et al., 1987). In that study, eight healthy subjects who inhaled ^{99m}Tc-DTPA just prior to exposure to air or 0.4 ppm O_3 for 2 h while undergoing heavy exercise (65 L/min) had increased clearance of the compound. Kehrl et al. (1989) reported similar observations on an additional 16 subjects. For the combined group of 24 subjects exposed for 2 h to 0.4 ppm O_3, the average clearance rate was 60% faster than that observed after air exposure, strongly suggesting increased permeability from the airway lumen and alveolar space to the blood and interstitial spaces. The average O_3-induced decrement in FVC in these subjects was 10%. These changes in permeability most likely are associated with acute inflammation and potentially could allow better access of inhaled antigens and other substances to the submucosa.

Studies in which human AM and airway epithelial cells were exposed to O_3 in vitro suggest that most of the components found in increased levels in the BAL fluid of O_3-exposed humans are produced by epithelial cells. Macrophages exposed to 0.3 and 1.0 ppm (but not 0.1 ppm) O_3 for 1 h showed small increases in PGE_2, but no change in superoxide anion or cytokine production (Becker et al., 1991). In contrast, airway epithelial cells exposed in vitro to 0.1, 0.25, 0.5, and 1.0 ppm O_3 for 1 h showed large concentration-dependent increases in PGE_2, TXB_2, LTB_4, LTC_4, and LTD_4 (McKinnon et al., 1993). These cells also showed increases in IL-6, IL-8, and fibronectin at O_3 concentrations as low as 0.1 ppm (Devlin et al., 1994). Interestingly, macrophages removed 18 h later from subjects exposed to 0.4 ppm O_3 for 2 h while undergoing intermittent heavy exercise (Koren et al., 1989a) showed changes in the rate of synthesis of 123 different proteins as measured by quantitative computerized densitometry of two-dimensional gel protein profiles. However, AMs exposed to O_3 in vitro showed changes only in the rate of synthesis of six proteins, suggesting that most of the

changes seen in the in vivo-exposed AMs were due to actions resulting from mediators released by other cells following O_3 exposure, which then altered macrophage function.

Numerous studies have shown that humans exposed to O_3 for 5 consecutive days experience decrements in pulmonary function on the first and second days, but the decrements diminish with each succeeding day so that by the fifth day, no such effects are observed (see Section 7.2.1). However, these studies did not address the question of whether repeated exposure to O_3 also resulted in attenuation of inflammation or lung damage. Animal studies suggest that although some markers of inflammation may be diminished, underlying damage to lung epithelial cells continues (Tepper et al., 1989). In a recent study (Devlin et al., 1995), humans were exposed to 0.4 ppm O_3 for 5 consecutive days (2 h/day while undergoing IE) and then were exposed to O_3 a single time either 10 or 20 days later. The results show that numerous indicators of inflammation (e.g., PMN influx, IL-6, IL-8, PGE_2, BAL protein, fibronectin, macrophage phagocytosis) show attenuation (i.e., there is a complete disappearance of response, and values are no different from those observed in the same individual after 5 days of exposure to FA). Ten days later, some of these markers regained full susceptibility, but others did not regain susceptibility even after 20 days. In agreement with animal studies, some markers (LDH, elastase) never show attenuation, indicating that tissue damage may continue to occur during repeated exposure.

7.2.4.3 Inflammation Induced by Ambient Levels of Ozone

Devlin et al. (1991) reported an inflammatory response in humans exposed to levels of O_3 at or below 0.12 ppm. In this study, 10 volunteers were exposed to 0.08 and 0.10 ppm O_3 for 6.6 h while undergoing moderate exercise (40 L/min) and underwent BAL 18 h later. An additional eight subjects were exposed to 0.08 ppm O_3. Increased numbers of PMNs and levels of IL-6 were found at both O_3 concentrations. There also were increases in most of the other compounds reported by Koren et al. (1989a,b), including fibronectin and PGE_2. Alveolar macrophage phagocytic capability was also monitored in this study, and it was reported that macrophages removed from humans exposed to both O_3 concentrations had decreased ability to phagocytize *Candida albicans* opsonized with complement. Comparison of the magnitude of inflammatory changes observed in this study and by Koren et al. (1989a,b), when normalized for differences in concentration, duration of exposure, and ventilation, suggest that lung inflammation from O_3 may occur as a consequence of exposure to ambient levels while exercising. Although the mean changes in IL-6, PGE_2, and PMNs reported by Devlin et al. (1991) were small, there was a considerable range of response among the individuals participating in the study. Thus, although some of the study population showed little or no response to O_3, others had increases in IL-6 or PMNs that were as large as or larger than those reported by Koren et al. (1989a,b) when subjects were exposed for 2 h to 0.4 ppm O_3. Interestingly, those individuals who had the largest increases in inflammatory mediators in this study did not necessarily have the largest decrements in pulmonary function, suggesting separate mechanisms underlying these two responses to O_3. These data suggest that, although the population as a whole may have a small inflammatory response to low levels of O_3, there may be a significant subpopulation that is very sensitive to these low levels of O_3. Furthermore, even a small inflammatory response (if it recurs) in the population as a whole should not be discounted.

7.2.4.4 Time Course of Inflammatory Response

The time course of the inflammatory response to O_3 in humans has not been explored fully. Studies in which BAL was performed 1 h (Devlin et al., 1990; Koren et al., 1991) or 3 h (Seltzer et al., 1986) after exposure to 0.4 ppm O_3 demonstrate that the inflammatory response is quickly initiated, and other data (Koren et al., 1989a,b) indicate that, even 18 h after exposure, inflammatory mediators such as IL-6 and PMNs are still substantially elevated. However, a comparison of these studies shows there are differences in the magnitude of response of some indicators, depending on when BAL is performed after O_3 exposure. Ozone-induced increases in PMNs, IL-6, and PGE_2 are greater 1 h after O_3 exposure, whereas BAL levels of fibronectin and plasminogen activator are greater 18 h after exposure. Still other compounds (protein, Tissue Factor) are equally elevated both 1 and 18 h after O_3 exposure. Schelegle et al. (1991) exposed five subjects to FA or 0.3 ppm O_3 for 1 h with a ventilation of 60 L/min. Each subject was exposed to O_3 on three separate occasions, and BAL was performed 1, 6, or 24 h after exposure. In addition, BAL was separated into two fractions: the first 60 mL wash was designated the "proximal airways" fraction (PA), and the remaining three 60 mL washes were pooled and designated the "distal airways and alveolar surface" fraction (DAAS). The percent of PMNs in the PA sample was statistically elevated at 1, 6, and 24 h after O_3 exposure, with a peak response at 6 h. The percent of PMNs in the DAAS sample was elevated at only the 6 and 24 h time points, with equivalent elevations at each time.

7.2.4.5 Effect of Anti-Inflammatory Agents on Ozone-Induced Inflammation

Previous studies (Schelegle et al., 1987; Eschenbacher et al., 1989) have shown that indomethacin, an anti-inflammatory agent that inhibits the production of cyclooxygenase products of arachidonic acid metabolism, is capable of blunting the well-documented decrements in pulmonary function observed in humans exposed to O_3. In a recent study, 10 healthy male volunteers were given 800 mg ibuprofen, another anti-inflammatory agent that blocks cyclooxygenase metabolism, or a placebo 90 min prior to a 2-h exposure to 0.4 ppm O_3. An additional 200 mg was administered following the first hour of exposure. Bronchoalveolar lavage was performed 1 h after the exposure. As expected, subjects given ibuprofen had blunted decrements in lung function following O_3 exposure compared to the same subjects given a placebo (Hazucha et al., 1996). Bronchoalveolar lavage fluid from subjects given ibuprofen also had reduced levels of the cyclooxygenase product PGE_2 as well as IL-6, but no decreases were observed in PMNs, fibronectin, permeability, LDH activity, or macrophage phagocytic function (Hazucha et al., 1995). These data suggest that although anti-inflammatory agents may blunt O_3-induced decrements in FEV_1 and increases in PGE_2, most inflammatory mediators are elevated in the BAL of these subjects.

7.2.4.6 Use of Nasal Lavage To Assess Ozone-Induced Inflammation in the Upper Respiratory Tract

Bronchoalveolar lavage has proven to be a powerful research tool to analyze changes in the lung following exposure of humans to xenobiotics. However, because BAL is expensive, somewhat invasive, and requires specialized personnel and facilities, it usually is done only with small numbers of subjects and in selected medical centers. Therefore, there is increasing interest in the use of NL as a tool in assessing O_3-induced inflammation in the upper respiratory tract, which is the primary portal for inspired air, and therefore the first region of the respiratory tract to come in contact with airborne xenobiotics. Nasal lavage is simple and

rapid to perform, is noninvasive, and allows collection of multiple sequential samples from the same person. Graham et al. (1988) reported increased levels of PMNs in the NL fluid of 21 humans exposed to 0.5 ppm O_3 at rest for 4 h on 2 consecutive days, with NL performed immediately before and immediately after each exposure as well as 22 h after the second exposure. Nasal lavage fluid contained elevated numbers of PMNs at all postexposure times tested, with peak values occurring immediately prior to the second day of exposure. There were no changes in PMN numbers at any time in 20 subjects exposed to clean air for 2 consecutive days. Bascom et al. (1990) exposed 12 subjects with allergic rhinitis to 0.5 ppm O_3 at rest for 4 h, followed immediately by NL. They reported a sevenfold increase in PMNs, a 20-fold increase in eosinophils, and a 10-fold increase in mononuclear cells following O_3 exposure, as well as a 2.5-fold increase in albumin. Graham and Koren (1990) compared inflammatory mediators present in both the NL and BAL fluids of humans exposed to O_3. The same 11 subjects who were exposed to 0.4 ppm O_3 for 2 h with BAL performed 18 h later, as described earlier (Koren et al., 1989a,b), also underwent NL immediately before, immediately after, and 18 h after each exposure (Graham and Koren, 1990). There were significant increases in PMNs in the NL fluid taken both immediately after exposure and on the next day. Increases in NL and BAL PMNs were similar (6.6- and eightfold, respectively), demonstrating a qualitative correlation between changes in the lower airways as assessed by BAL and the upper respiratory tract as assessed by NL. Furthermore, all individuals who had increased PMNs in BAL fluid also had increased PMNs in NL fluid, although the NL PMN increase could not quantitatively predict the BAL PMN increase. Albumin, a marker of epithelial cell permeability, was increased 18 h later, but not immediately after exposure. There were no changes in PGE_2, plasminogen activator, LTC_4, LTD_4, or LTE_4 (Graham and Koren, 1990). However, tryptase, a constituent of mast cells contained in the same granules as histamine, was found in elevated levels immediately after O_3 exposure, but not 18 h later (Koren et al., 1990). McBride et al. (1994) reported that asthmatic subjects are more sensitive to upper airway inflammation at O_3 concentrations that do not affect lung function. Nasal lavage and lung and nasal function were compared in 10 asthmatic and 8 nonasthmatic subjects exposed in a head dome to 0.12 and 0.24 ppm O_3 for 90 min during intermittent moderate exercise ($\dot{V}_E$ = 20 L/min). A significant increase in the number of PMNs in NL fluid was detected in the asthmatic subjects both immediately and 24 h after exposure to 0.24 ppm O_3. Total white blood count, a surrogate for PMN influx, was signficantly correlated with IL-8 in the NL fluid. No significant cellular changes were seen in nonasthmatic subjects, and no changes in lung or nasal function or biochemical mediators were found in either asthmatic or nonasthmatic subjects. These studies suggest that NL may serve as a sensitive and reliable tool to detect inflammation in the upper airways of humans exposed to xenobiotics.

7.2.4.7 Changes in Host Defense Capability Following Ozone Exposure

Concern about the effect of O_3 on human host defense capability derives from numerous animal studies demonstrating that acute exposure to as little as 0.08 ppm O_3 causes decrements in antibacterial host defenses and little, if any, effect on the course of acute viral infection (see Chapter 6, Section 6.2.3). A study of experimental rhinovirus infection in susceptible human volunteers failed to show any effect of 5 consecutive days of O_3 exposure on the clinical evolution or host response to a viral challenge (Henderson et al., 1988). In this study, 24 young males were inoculated with type 39 rhinovirus (1,000 TCID-50) administered as nose drops. Half were then exposed to 0.3 ppm O_3 (6 h/day) for 5 consecutive days while

undergoing intermittent light exercise, and half were exposed to clean air under the same regimen. There was no difference in rhinovirus titers in nasal secretions between the O_3-exposed and control groups, nor were there any differences in levels of interferon gamma or PMNs in NL fluid or in blood lymphocyte proliferative response to rhinovirus antigen. However, recent findings that rhinovirus can attach to the intracellular adhesion molecule (ICAM) receptor on respiratory tract epithelial cells (Greve et al., 1989) and that O_3 can up-regulate the ICAM receptor on nasal epithelial cells (Beck et al., 1994) suggest that more studies are needed to explore more fully the potential interaction between O_3 exposure and viral infectivity.

In a single study, human AM host defense capacity was measured in vitro in AMs removed from subjects exposed to 0.08 and 0.10 ppm O_3 for 6.6 h while undergoing moderate exercise. Alveolar macrophages from O_3-exposed subjects had significant decrements in complement-receptor-(but not antigen-antibody [Fc]-receptor)-mediated phagocytosis of *Candida albicans* (Devlin et al., 1991). These data show that acute in vivo exposure of humans to O_3 results in impairment of AM host defense capability, potentially resulting in decreased ability to phagocytose and kill inhaled microorganisms in vivo. Human AMs also have been exposed to O_3 in vitro to investigate whether changes in macrophage host defense functions are due to a direct effect of O_3 on AMs or secondary effects resulting from lung injury and inflammation. Becker et al. (1991) exposed AMs to 0.1 to 1.0 ppm O_3 in vitro for 1 h and showed a concentration-dependent decrease in phagocytosis of antibody-coated sheep erythrocytes; a small increase in PGE_2; and production of significantly lower levels of IL-1, IL-6, and TNF on stimulation with lipopolysaccharide when compared with air-exposed cells (Becker et al., 1991). Although the few studies in which animals have been exposed to virus in conjunction with O_3 exposure provide some evidence to suggest that O_3 impairs the immune system's ability to fight viral infections, there is insufficient human data to know whether O_3 exposure affects viral infectivity. However, there is potential cause for concern that O_3 may render humans and animals more susceptible to a subsequent bacterial challenge.

There are two studies that have investigated the effect of O_3 exposure on mucociliary clearance of inhaled particles, with conflicting results. In one study (Foster et al., 1987), seven male volunteers inhaled radiolabeled ferric oxide ($^{99m}Tc\text{-}Fe_2O_3$) particles and then were exposed to 0.2 and 0.4 ppm O_3 for 2 h while undergoing light IE. The investigators observed a concentration-dependent increase in rate of particle clearance 2 h after exposure, although increased clearance was confined primarily to the peripheral airways in subjects exposed to 0.2 ppm O_3. In the second study (Gerrity et al., 1993), 15 male or female subjects were exposed to 0.4 ppm O_3 for 1 h while undergoing CE (40 L/min); 2 h after exposure, subjects inhaled $^{99m}Tc\text{-}Fe_2O_3$ particles, and clearance was measured with a gamma camera for the next 3 h and on the next morning. There was no difference in the clearance rate of particles in air and O_3-exposed subjects. The discrepancy between these studies may be explained by differences in exposure protocol, time of particle inhalation, or time of clearance measurement, or by the presence of cough immediately following O_3 exposure, which may have accelerated clearance in the first study.

7.2.5 Extrapulmonary Effects of Ozone

It is still believed that O_3 reacts immediately on contact with respiratory system tissue and is not absorbed or transported to extrapulmonary sites to any significant degree (see Chapter 8). A number of laboratory animal studies presented in Chapter 6, however, suggest

that reaction products formed by the interaction of O_3 with respiratory system fluids or tissues may produce effects measured outside the respiratory tract—either in the blood, as changes in circulating blood lymphocytes, erythrocytes, and serum, or as changes in the structure or function of other organs, such as the parathyroid gland, the heart, the liver, and the central nervous system (see Chapter 6, Section 6.3). Very little is known, however, about the mechanisms by which O_3 could cause these extrapulmonary effects.

The results from human exposure studies discussed in the previous criteria document (U.S. Environmental Protection Agency, 1986) failed to demonstrate any consistent extrapulmonary effects (see Chapter 10, Section 10.6 of the 1986 document). Early studies on peripheral blood lymphocytes collected from human volunteers did not find any significant genotoxic or functional changes at O_3 exposures of 0.4 to 0.6 ppm for up to 4 h/day. Limited data on human subjects available at the time the 1986 criteria document was published also indicated that 0.5 ppm O_3 exposure for over 2 h caused transient changes in blood erythrocytes and sera (e.g., erythrocyte fragility and enzyme activities), but the physiological significance of these studies remains questionable. The conclusions drawn from these early studies raise doubt that cellular damage or altered function is occurring to circulating cells at O_3 exposures under 0.5 ppm.

Studies published since the publication of the previous criteria document (U.S. Environmental Protection Agency, 1986) on the potential extrapulmonary effects of in vivo O_3 exposure of human subjects have not been very definitive. Johnson et al. (1986) exposed 11 male nonsmokers to 0.5 ppm O_3 for 4 h on 2 consecutive days. When compared to air controls, O_3 exposure did not result in any significant change in the activity of blood plasma α-1-proteinase inhibitor. Schelegle et al. (1989) exposed 20 O_3-sensitive, healthy young men to 0.20 and 0.35 ppm O_3 with heavy exercise ($\dot{V}_E$ = 50 L/min). Plasma concentrations of $PGF_{2\alpha}$ were elevated after 40 and 80 min of exposure to the higher O_3 level (0.35 ppm). It is likely, however, that the elevation of this ecosanoid in the blood was due either to increased production or to decreased metabolism of $PGF_{2\alpha}$ in the lung.

The demonstration in the previous section (Section 7.2.4) of an array of inflammatory mediators and immune modulators released at the airway surface provides a possible mechanism for effects to occur elsewhere in the body.

7.2.6 Ozone Mixed with Other Pollutants

Although it is well known that polluted air contains a large number of chemical species, the most common approach to evaluating air pollution effects under laboratory conditions has been assessment of responses consequent to exposure to single pollutants. This has been the case for a variety of reasons, not the least of which is the problem inherent in adequately controlling the concentrations of multiple pollutants simultaneously. Further, atmospheric chemistry is very complicated, and it is difficult to adequately assess the exposure mixture as the number of constituent pollutants increases. Observed effects may be related to unknown reaction products, the monitored pollutants being only surrogates. Other problems inherent in mixture studies involve considerations such as whether pollutants are presented simultaneously or in sequential or overlapping patterns. Ideally, the selected pattern should at least approximate one that occurs in the ambient environment. In spite of these difficulties, information from mixture studies is important from the standpoint of attempts to better understand responses of humans to the complex mixture of ambient air.

The previous O_3 criteria document (U.S Environmental Protection Agency, 1986) evaluated the limited database of information available on mixtures of O_3 with one or more pollutants and concluded that pulmonary function changes were no more than additive and, in most cases, were attributable to O_3 alone. Several new studies have since appeared in which human subjects were exposed to mixtures of two or more pollutants or to individual pollutants sequentially (Table 7-13), extending the database for controlled studies. Epidemiological studies also have investigated mixtures of pollutants and have not found evidence suggestive of synergistic effects (see Section 7-4).

7.2.6.1 Ozone and Sulfur-Containing Pollutants

Horvath et al. (1987) compared the pulmonary function responses of male subjects (19 to 29 years of age) with normal baseline pulmonary function to four experimental conditions: (1) FA, (2) 0.25 ppm O_3, (3) 1,200 to 1,600 $\mu g/m^3$ H_2SO_4 aerosol, and (4) 0.25 ppm O_3 + 1,200 to 1,600 $\mu g/m^3$ H_2SO_4 aerosol. Exposures were completed in random sequence, a minimum of 1 week apart, and were conducted at 35 °C and 83% RH. Subjects alternated 20-min rest and exercise ($\dot{V}_E$ = 30 to 32 L/min) periods throughout the 2-h exposures. The results indicated that neither O_3 alone nor O_3 mixed with H_2SO_4 aerosol had significant effects on any pulmonary function, metabolic, or ventilatory parameter.

Koenig et al. (1990) evaluated sequential O_3 (0.12 ppm) and SO_2 (0.10 ppm) exposures in 13 allergic, asthmatic adolescents (12 to 18 years of age). Three subjects used no regular medications, the other 10 used one or more of beta-adrenergic agents, theophylline, and antihistamines. All subjects had a PC_2 for methacholine of 10 mg/mL or less. Subjects took their morning medication on experiment days if needed, but at least 4 h elapsed between any medication use and the start of the experiment. The subjects participated in three exposures at 22 °C and 75% RH, which were presented in random order and at least 1 week apart. The three exposures were (1) air + SO_2, (2) O_3 + O_3, and (3) O_3 + SO_2. The mouthpiece exposures were 1 h in duration, during which the subjects breathed one test gas for 45 min, followed by a second gas for the final 15 min. Subjects exercised at a $\dot{V}_E$ of about 30 L/min during the second and fourth 15-min segments of the exposure. Pulmonary functions were measured 2 to 3 and 7 to 8 min postexposure. Changes in FEV_1 and R_T were significantly greater following the O_3 + SO_2 exposure than following the other two exposures. Although the subject group was small, the results indicate that O_3 exposure may potentiate responses to SO_2 exposure in asthmatic adolescents. It should be noted that the SO_2 concentration (0.10 ppm) used in this study is a subthreshold level.

Linn et al. (1994) evaluated the pulmonary function and symptom responses of 15 atopic and normal subjects and 30 asthmatic subjects exposed to FA, 0.12 ppm O_3, 100 $\mu g/m^3$ respirable H_2SO_4 aerosol (MMAD = 0.5 μm), and a mixture of the two pollutants. The chamber exposures were 6.5 h in duration, during which the subjects walked on a treadmill ($\dot{V}_E$ ≈ 29 L/min) for 50 min of each hour. There was a 30-min lunch period following the third hour. Pulmonary function and symptom responses were measured preexposure and during the hourly 10-min breaks, and a methacholine bronchochallenge test was performed following each exposure. Relative to responses to the FA exposure, H_2SO_4

Table 7-13. Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] $\mu g/m^3$	Pollutant	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Peroxyacetyl Nitrate								
0.485 0.27	951 1,337	O_3 PAN	2 h IE $\dot{V}_E$ 25 L/min	T = 21 C WBGT	10 F	Healthy NS, 19 to 36 years old	Exposure to the mixture of PAN + O_3 induced decrements in FVC and FEV_1 averaging 10% greater than observed following exposure to O_3 alone.	Horvath et al. (1986)
0.45 0.30	882 1,485	O_3 PAN	2 h IE $\dot{V}_E$ 27 L/min	T 22 C RH 60%	3 M, 5 F	Healthy NS, mean age = 24 years	No differences between responses to exposure to O_3 alone and O_3 + PAN.	Drechsler-Parks et al. (1987b)
0.45 0.60 0.13	882 1,128 644	O_3 NO_2 PAN	2 h IE $\dot{V}_E$ 25 L/min	T = 24 C RH = 55 to 58%	16 M, 16 F	Healthy NS; 16 subjects, 19 to 26 years old; 16 subjects, 51 to 76 years old	No differences between responses to O_3 alone, O_3 + NO_2, O_3 + PAN, or O_3 + NO_2 + PAN.	Drechsler-Parks et al. (1989)
Nitrogen-Containing Pollutants								
0.12 0.30	235 564	O_3 NO_2	1 h (mouthpiece) IE $\dot{V}_E$ = 4 to 5 times resting	T = 22 C RH = 75%	5 M, 7 F	Healthy NS, 12 to 17 years old	No significant changes in any pulmonary function with O_3 alone or O_3 + NO_2.	Koenig et al. (1988)
0.20	392 500	O_3 HNO_3 H_2O	5 h IE (50 min/h exercise) $\dot{V}_E$ 40 L/min 2 h HNO_3 or H_2O fog or air, followed by 1-h break, followed by 3 h O_3	T = 20 C RH = 5%	6 M, 4 F	Healthy NS, minimum of 10% decrement in FEV_1 after 3 h exposure to 0.20 ppm O_3 with 50 min exercise/h	Exposure to HNO_3 or H_2O fog followed by O_3 induced smaller pulmonary function decrements than air followed by O_3.	Aris et al. (1991)
0.30 0.30	588 564 200	O_3 NO_2 H_2SO_4	2 h CE for 20 min $\dot{V}$ 25 L/min	T = 28 to 29 C RH = 50 to 60%	6 M	Healthy subjects, some smokers	Possible small decrease in SG_{aw}	Kagawa (1986)
0.15 0.15	294 282 200	O_3 NO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V}$ 25 L/min		6 M		Possible small decrease in SG_{aw}	
0.15 0.15 0.15	294 282 393 200	O_3 NO_2 SO_2 H_2SO_4	2 h, 60 min total exercise $\dot{V}$ 25 L/min		3 M		Possible small decrease in FEV_1	

Table 7-13 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] µg/m³	Pollutant	Exposure Duration and Activity	Exposure Conditions	Number and Gender of Subjects	Subject Characteristics[c]	Observed Effect(s)	Reference
Nitrogen-Containing Pollutants (cont'd)								
0.30 0.60	588 1,128	O_3 NO_2	1 h (mouthpiece) CE $\dot{V}_E$ ≥ 70 L/min for men $\dot{V}_E$ ≥ 50 L/min for women		20 M, 20 F	Healthy NS, 21.4 ± 1.5 (SD) years old for F, 22.7 ± 3.3 (SD) years old for M	No differences between responses to O_3 and NO_2 + O_3 for spirometric parameters. Increase in SR_{aw} with NO_2 + O_3 was significantly less than for O_3 alone.	Adams et al. (1987)
0.30 0.60	588 1,128	O_3 NO_2	2-h exposure to NO_2 or FA, followed 3 h later by 2-h exposure to O_3 IE $\dot{V}_E$ = 20 L/min/m² BSA	T = 21 °C RH = 40%	21 F	Healthy NS, 18 to 34 years old	No significant effect of NO_2 exposures on any measured parameter. Sequential exposure of NO_2 followed by O_3 induced small but significantly larger decrements in FEV_1 and $FEF_{25-75\%}$ than FA/O_3 sequence. Subjects had increased airway responsiveness to methacholine after both exposures, with significantly greater responsiveness after the NO_2/O_3 sequences than after the FA/O_3 sequence.	Hazucha et al. (1994)
Sulfur-Containing Pollutants								
0.12 0.10	235 262	O_3 SO_2	1 h (mouthpiece) IE $\dot{V}_E$ ≥ 30 L/min 45-min exposure to air or O_3, followed by 15-min exposure to O_3 or SO_2	T = 22 °C RH = 75%	8 M, 5 F	All allergic asthmatics, 12 to 18 years old, medications withheld for at least 4 h before exposures	Prior exposure to O_3 potentiated pulmonary function responses to SO_2; decrements in FEV_1 were ≥3, ≥2, and ≥8% for the air/O_3, O_3/O_3, and O_3/SO_2 exposures, respectively.	Koenig et al. (1990)
0.08 0.12 0.18	157 235 353 100 100	O_3 O_3 O_3 NaCl H_2SO_4	3-h exposure to aerosol, followed 24 h later by a 3-h exposure to O_3. IE (10 min per half hour) $\dot{V}_E$ = 4 times resting (30 to 364 min)	T = 21 °C RH ≥ 40%	Nonasthmatic, 16 M, 14 F Asthmatic 10 M, 20 F	NS, 18 to 45 years old NS, 21 to 42 years old	No significant changes in symptoms or lung function with any aerosol/O_3 combination in the healthy group. In asthmatics, H_2SO_4 preexposure enhanced the small decrements in FVC that occurred following exposure to 0.18 ppm O_3. Asthmatics had no significant changes on FEV_1 with any O_3 exposures, but symptoms were greater.	Utell et al. (1994)
0.12	235 100	O_3 H_2SO_4	6.5 h 2 consecutive days 50 min exercise/h $\dot{V}_E$ = 29 L/min	T = 21 °C RH = 50%	Nonasthmatic, 8 M, 7 F Asthmatic, 13 M, 17 F	NS, 22 to 41 years old NS, 18 to 50 years old	Exposure to O_3 or O_3 + H_2SO_4 induced significant decrements in forced expiratory function. Differences between O_3 and O_3 + H_2SO_4 were, at best, marginally significant. O_3 is the more important pollutant for inducing respiratory effects. A few asthmatic and nonasthmatic subjects were more responsive to O_3 + H_2SO_4 than to O_3 alone.	Linn et al. (1994)

Table 7-13 (cont'd). Ozone Mixed with Other Pollutants[a]

Concentration[b] ppm	Concentration[b] g/m	Pollutant	Exposure Duration and Activity	Exposure Conditions[c]	Number and Gender of Subjects	Subject Characteristics	Observed Effect(s)	Reference
Sulfur-Containing Pollutants (cont'd)								
0.12 0.30 0.05	235 564 70	O_3 NO_2 H_2SO_4 HNO_3	1.5 h with IE for 2 consecutive days; $\dot{V}_E$ 23.2 L/min	T = 22 C RH = 65%	Asthmatic adolescents; 22 completed study; 15 M, 7 F	NS, 12 to 19 years old	No significant pulmonary function changes following any exposure compared to response to clean air. Six additional subjects started the study, but dropped out due to uncomfortable symptoms.	Koenig et al. (1994)
0.25	490 1,200 to 1,600	O_3 H_2SO_4 aerosol	2 h IE; $\dot{V}_E$ = 30 to 32 L/min	T = 35 C RH = 83%	9 M	Healthy NS, 19 to 29 years old	No significant effects of exposure to O_3 alone or combined with H_2SO_4 aerosol.	Horvath et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Grouped by pollutant mixture.
[c]WBGT = 0.7 $T_{wet\ bulb}$ + 0.3 $T_{dry\ bulb\ or\ globe}$.

aerosol exposure alone induced no significant alteration in pulmonary function, symptoms, or bronchial reactivity to methacholine. Exposure to O_3 alone (FEV_1 decrement of about 100 mL compared to the FA response) or mixed with H_2SO_4 aerosol (FEV_1 decrement of about 189 mL compared to the FA exposure) induced significant decrements in forced expiratory function and increased bronchial reactivity. Both effects were greater on the first of 2 consecutive days of exposure. Group mean lung function and methacholine reactivity changes were somewhat larger following O_3 + H_2SO_4 aerosol compared to exposure to O_3 alone, but the differences were, at best, marginally significant and usually nonsignificant, depending on the function tested. However, there were a few individual subjects who showed significantly larger pulmonary function decrements following the exposure to O_3 + H_2SO_4 than following exposure to O_3 alone. The authors concluded that O_3 is more important than H_2SO_4 aerosol in inducing pulmonary dysfunction in normal, atopic, and asthmatic adults. There does, however, appear to be a more sensitive subpopulation that responds to O_3 + H_2SO_4 aerosol more strongly than the average adult.

Utell et al. (1994) reported on the pulmonary function and symptomology responses of 30 healthy adults (18 to 45 years of age) and 30 allergic asthmatics (21 to 42 years of age) who were exposed for 3 h to sodium chloride (NaCl) aerosol (100 $\mu g/m^3$) or H_2SO_4 aerosol (100 $\mu g/m^3$) and, 24 h later, to 0.08, 0.12, or 0.18 ppm O_3 for 3 h. The study was an incomplete block design, in that each subject completed chamber exposures to each of two O_3 concentrations following each of the aerosols (four of the possible six combinations per subject). Out of the total number of subjects, 20 healthy and 20 asthmatic subjects completed each of the possible exposure combinations. Subjects exercised for 10 min out of each half-hour of exposure ($\dot{V}_E$ = 4 times resting; 30 to 36 L/min). Environmental conditions averaged 21 $\pm$ 1 °C and 40 $\pm$ 5% RH. Ozone exposures were separated by at least 2 weeks. Healthy subjects had no significant pulmonary function response (2.1% or less) to O_3 exposure, regardless of the O_3 concentration or the aerosol preexposure. As a group, asthmatics had mean decrements in FVC of 5% or greater in only a few cases: 7.6% following the NaCl/0.08 ppm O_3 combination, 6.3% following the NaCl/0.12 ppm O_3 combination, and 6.5% following the H_2SO_4/0.18 ppm O_3 combination. No combination of aerosol and O_3 concentration induced a decrement in FEV_1 of 5% or greater. Although the statistical analysis indicates that exposure to H_2SO_4 aerosol significantly altered the pattern of response and recovery to O_3 exposure on the next day in asthmatics, the group mean data presented in the report show that functionally there is little difference between the responses to the various exposures or in the time course of recovery. The individual responses of the asthmatic subjects are reported to be more variable than those of the healthy subjects. Asthmatic subjects reported more respiratory symptoms than healthy subjects, but there was no dose-response relationship between O_3 concentration and symptom intensity for healthy or asthmatic subjects. The variability of the responses of the asthmatic subjects makes interpretation of these results difficult. Some of the asthmatic subjects were reported to experience exercise-induced bronchospasm, and, without FA control exposures, it is impossible to determine what, if any, portion of the asthmatic subjects' response is related to exercise-induced bronchospasm, compared to that related to O_3 exposure.

Kagawa (1986) exposed Japanese men to three mixtures: (1) O_3 (0.30 ppm) + NO_2 (0.30 ppm) + H_2SO_4 (200 $\mu g/m^3$), (2) O_3 (0.15 ppm) + NO_2 (0.15 ppm) + H_2SO_4 (200 $\mu g/m^3$), or (3) O_3 (0.15 ppm) + NO_2 (0.15 ppm) + SO_2 (0.15 ppm)+ H_2SO_4 (200 $\mu g/m^3$). Exposures were 2 h in duration, and subjects exercised for a total of 20 min during exposure 1 and for 60 min during exposures 2 and 3. Some of the subjects were

smokers. Reported symptoms were attributed to O_3 exposure, whereas small decrements in airway conductance (□10%) were observed following exposures to mixtures 1 and 2. Although the magnitude of the FEV_1 decrement is not stated, a possible decrease was observed after exposure 3. The responses observed with these mixed exposure conditions were no different than responses reported for exposures to similar concentrations of O_3, indicating no enhanced response due to the presence of the other pollutants in the mixtures.

7.2.6.2 Ozone and Nitrogen-Containing Pollutants

Adams et al. (1987) reported on the responses of 20 males and 20 females (18 to 30 years of age), all healthy nonsmokers, exposed to (1) FA, (2) 0.3 ppm O_3, (3) 0.6 ppm NO_2, and (4) 0.3 ppm O_3 + 0.6 ppm NO_2. Subjects were exposed via mouthpiece for 1 h, during which they exercised continuously at a $\dot{V}_E$ of about 70 L/min for males and 50 L/min for females. The exposures were presented in random order, a minimum of 5 days apart. There were no differences in any pulmonary function (FEV_1 decrement of about 22%) between the O_3 and NO_2 + O_3 exposures, except for SR_{aw}, which was lower following NO_2 + O_3 (+7.3% for females and □9.6% for males) than following O_3 alone (+15.3% for females and +4.0% for males).

Koenig et al. (1988) exposed 14 male and 10 female adolescents to FA, 0.30 ppm NO_2, 0.12 ppm O_3, and 0.30 ppm NO_2 + 0.12 ppm O_3. Twelve of the subjects were healthy normals, and the other 12 were allergic asthmatics. The asthmatics, except for one who took no regular medications, used one or more of beta-adrenergic agents, theophylline, and antihistamines. Asthmatic subjects took their morning medications if needed, but refrained from medication use for at least 4 h prior to the exposures. The mouthpiece exposures were 1 h in duration, during which the subjects exercised in 15-min periods (mean $\dot{V}_E = 32.8 \pm 6.0$ L/min), alternated with 15-min rest periods. No changes in any measure of pulmonary function were observed in normal or asthmatic subjects following O_3 or NO_2 + O_3 exposure.

Sequential exposure to 0.6 ppm NO_2 or FA for 2 h, followed 3 h later by a 2-h exposure to 0.3 ppm O_3 was investigated by Hazucha et al. (1994) in 21 healthy, nonsmoking females (18 to 34 years of age). Subjects alternated 15-min periods of exercise ($\dot{V}_E = 20$ L/min/m^2 BSA) and 15-min rest periods while in the exposure chamber, and rested in ambient air during the 3-h interexposure period. The 2 exposure days were separated by at least 2 weeks. Ambient conditions in the exposure chamber were 21 □C and 40% RH. Group mean decrements following the FA/O_3 exposure sequence were □10.8, □7.0, □10.2, and □14.9% for PEFR, FVC, FEV_1, and $FEF_{25\ 75\%}$, respectively. Following the NO_2/O_3 exposure sequence, the group mean decrements were □14.5, □8.5, □12.0, and □19.5%, respectively, for PEFR, FVC, FEV_1, and $FEF_{25\ 75\%}$. Although small, the differences in FEV_1 and $FEF_{25\ 75\%}$ between the FA/O_3 and NO_2/O_3 exposure sequences were statistically significant. There were no differences in the changes in SR_{aw} or symptomology between the two exposure sequences. The most striking finding of this study was that, although both exposure sequences increased airway responsiveness to methacholine, responsiveness was potentiated by the NO_2/O_3 exposure sequence compared to the FA/O_3 exposure sequence.

Aris et al. (1991) examined pulmonary function responses to a 3-h exposure to 0.2 ppm O_3 following a 2-h exposure to 0.54 mg/mL nitric acid (HNO_3; volume mean diameter = 6.0 ± 0.2 □m) or 0.55 mg/mL water (H_2O; volume mean diameter = 6.47 ± 0.4 □m) fog. This is a common pattern of pollutant exposure in coastal California areas. Subjects were 10 healthy adults 21 to 31 years of age; they were prescreened for a decrement of 10% or

greater in FEV_1 following 3 h of exposure to 0.2 ppm O_3, during which they exercised for 50 min of each hour ($\dot{V}_E$ = 40 L/min). Decrements in FEV_1 following the screening O_3 exposure ranged from 15 to 49%. The three exposures (FA + O_3, H_2O fog + O_3, and HNO_3 fog + O_3) were presented in random order, and were separated by a minimum of 2 weeks. The authors hypothesized that exposure to acidic fog, followed by O_3 exposure, would induce greater decrements in FVC and FEV_1 than H_2O fog or air followed by O_3 exposure. In fact, both HNO_3 and H_2O fog exposure seemed to ameliorate the effect of subsequent O_3 exposure on FEV_1 and FVC, although only the difference between the FEV_1 responses to FA + O_3 (28.5%) and H_2O fog + O_3 (18.5%) was significant. Group mean comparisons of methacholine responsiveness and O_3 responsiveness (defined as a minimum of a 10% decrement in FEV_1 following the prescreening O_3 exposure) suggest that the subjects classified as O_3 sensitive, on the average, had lower methacholine $PC_{1\ SRaw}$ doses. The individual data, however, do not always support this conclusion. Two of 10 O_3-sensitive subjects had methacholine $PC_{1\ SRaw}$ concentrations above the author's cut-off for airway hyperresponsiveness, and 3 of 10 O_3 nonsensitive subjects had hyperreactive airways based on the authors' criteria for methacholine $PC_{1\ SRaw}$.

Aris et al. (1993b) further examined pulmonary responses to combined O_3 and HNO_3 exposures. Ten healthy, nonsmoking adults, 19 to 41 years of age, were exposed to FA, 500 $\mu g/m^3$ of HNO_3 gas plus 0.2 ppm O_3, or to 0.2 ppm O_3 alone. The exposure protocol was 4 h in duration, with 50 min IE at 40 L/min alternating with 10-min rest periods each hour. Pulmonary function was measured during each rest period, whereas BAL, proximal airway lavage, and bronchial biopsies were performed 18 h after completion of each exposure. Mean FEV_1 and FVC decreased, and mean SR_{aw} and respiratory symptom scores increased across both the HNO_3 + O_3 and the O_3 exposures. The results indicated, however, that HNO_3 combined with O_3 did not exacerbate the pulmonary function decrements or respiratory symptoms caused by O_3 alone. Similarly, there were no statistically significant differences between the HNO_3 + O_3 and the O_3 exposures in the cellular or biochemical constituents in either the BAL or proximal airway lavage fluids or in the bronchial biopsy specimens. The authors concluded that HNO_3 does not potentiate the inflammatory response produced by O_3 in healthy individuals.

The objective of a study by Koenig et al. (1994) was to investigate possible interactions between oxidants (0.12 ppm O_3 + 0.30 ppm NO_2) and an H_2SO_4 aerosol (70 $\mu g/m^3$) with a mass median aerodynamic diameter (MMAD) of 0.6 μm ($\pm\sigma_g$ = 1.5). Twenty-two adolescent allergic asthmatics who also had exercise-induced bronchospasm and a positive response to a standardized methacholine bronchochallenge test completed all exposures. Subjects inhaled FA, O_3 + NO_2, O_3 + NO_2 + H_2SO_4, or O_3 + NO_2 + HNO_3 through a mouthpiece for 90 min on 2 consecutive days. Each pair of exposures was separated by at least 1 week. Subjects exercised ($\dot{V}_E$ about 10 times FVC) and rested in alternating 15-min periods. Pulmonary functions (FVC, FEV_1, $\dot{V}_{max5\ \%}$, $\dot{V}_{max75\%}$, and R_T) were measured before and after each exposure and on the day following the second consecutive exposure, at which time only pulmonary function was evaluated and a methacholine bronchochallenge was performed. Six additional subjects began the study, but dropped out before completion because of uncomfortable symptoms associated with the exposures; none dropped out following an FA exposure. There were no statistically significant changes in any measured parameter of pulmonary function following the three pollutant-containing exposures, compared to the FA exposure, contrary to expectations. (Also see Section 7.4 for related epidemiological studies.)

7.2.6.3 Ozone, Peroxyacetyl Nitrate, and More Complex Mixtures

Horvath et al. (1986) exposed 10 healthy young women (19 to 36 years of age) to (1) FA, (2) 0.48 ppm O_3, (3) 0.27 ppm PAN, and (4) 0.48 ppm O_3 + 0.27 ppm PAN. The chamber exposures were 2 h in duration, during which subjects alternated 20-min exercise periods ($\dot{V}_E$ = 25 L/min) and 15-min rest periods. Exposures were completed in random order and were at least 1 week apart. Exposure to PAN alone did not induce any significant changes in pulmonary function. Both O_3 and PAN + O_3 exposure induced significant decrements in FVC, FEV_1, and $FEF_{25\ 75\%}$; however, the decrements following the PAN + O_3 exposure were significantly larger (average of about 10%), suggesting interaction between PAN and O_3. It should be noted that typical peak ambient PAN concentrations are about 0.05 ppm. Symptom reports indicated that O_3 + PAN exposure induced greater subjective stress than did exposure to O_3 alone.

Drechsler-Parks et al. (1987b) exposed eight healthy young adults (mean age 24 years) to a mixture of 0.30 ppm PAN + 0.45 ppm O_3 on 5 consecutive days to evaluate possible attenuation. Subjects were reexposed to the PAN + O_3 mixture on the third and seventh days following the last consecutive day of exposure. Attenuation occurred with the same pattern and time sequence as has been reported for O_3 alone. The largest group mean decrements occurred following the second exposure, and the subjects became progressively less responsive with subsequent exposures. Two subjects failed to return to baseline values with 5 consecutive days of exposure. Pulmonary function changes after the follow-up exposures indicated that the attenuation response is relatively short lived, in that it began to abate within 3 to 7 days following the fifth consecutive day of exposure. These results are consistent with those of similar studies using exposure to O_3 alone (Horvath et al., 1981; Kulle et al., 1982), suggesting that PAN had no additional effect on attenuation to O_3. A greater number of symptoms was reported following all PAN + O_3 exposures than following exposure to O_3 alone.

Drechsler-Parks et al. (1989) studied 16 older men and women (51 to 76 years of age) and 16 young men and women (19 to 26 years of age) who each completed 2-h chamber exposures to FA, 0.45 ppm O_3, and mixtures of 0.45 ppm O_3 with 0.60 ppm NO_2 and/or 0.13 ppm PAN. Subjects alternated 20-min exercise ($\dot{V}_E$ about 25 L/min) and rest periods. Exposure to O_3 alone and in all combinations induced significant decrements in FVC (14 to 17%), FEV_1 (19 to 22%), and $FEF_{25\ 75\%}$ (28 to 30%) in the younger group. In the older group, these same three variables were significantly decreased only with NO_2 + O_3 exposure (7.3% for FVC, 8.4% for FEV_1, and 12% for $FEF_{25\ 75\%}$). Exposure of the older subjects to PAN + O_3 induced significant decrements only in FVC (4.2%) and FEV_1 (8.3%). The PAN + NO_2 + O_3 exposure induced a significant decrement only in FVC (6.4%) in the older subjects. All subjects reported more symptoms following the mixture exposures than following exposure to O_3 alone. These pulmonary function results following the exposure to O_3 + PAN are in contrast to those reported by Drechsler-Parks et al. (1984) and Horvath et al. (1986) on young adults exposed to 0.45 ppm O_3 + 0.30 ppm PAN. The results of both earlier studies suggested an interaction between O_3 and PAN, in that pulmonary function decrements following the mixture exposure were approximately 10% larger than those following exposure to O_3 alone, whereas there were no significant pulmonary function effects with exposure to PAN alone. A likely explanation for this discrepancy is that the PAN concentration used by Drechsler-Parks et al. (1989) was slightly less than half that used by Drechsler-Parks et al. (1984) and Horvath et al. (1986). Thus, if the additional effect of PAN is linear, an additional

effect of PAN + O_3 would be expected to be less than 5%, which probably would not be detected because it is within the variability of the pulmonary function measurements. In any case, ambient PAN concentrations are considerably less than 0.13 ppm. This indicates that, even if PAN and O_3 do interact in some way in their effects on pulmonary function, at typical ambient concentrations of O_3 and PAN, effects can be attributed to O_3 alone.

7.2.6.4 Summary

Information on interactive effects between O_3 and other pollutants remains sparse at this time. However, it is clear that O_3 is responsible for the largest share of observed effects when subjects are exposed to the mixtures of O_3 and other pollutants that have been studied to date. There is no evidence that simultaneous exposure of healthy individuals to ambient concentrations of O_3 plus NO_2, PAN, H_2SO_4, HNO_3, or SO_2 results in significant interaction. However, Aris et al. (1991) have reported that HNO_3 and H_2O fog exposure ameliorates the pulmonary function effects of a subsequent O_3 exposure. Koenig et al. (1990) found that preexposure to O_3 induced significant pulmonary function decrements in allergic asthmatic adolescents following a sequential SO_2 exposure. Both the O_3 and SO_2 concentrations were at subthreshold levels for the experimental design used.

Both studies that reported potentially significant effects have involved sequential exposure protocols, in contrast to the simultaneous exposure protocols, which generally have not shown effects beyond those that would be expected at the O_3 concentration used. It may be that certain preexposures predispose an individual to responses following a subsequent exposure; however, this question remains far from being resolved. Further, these results are related only to spirometry and plethysmography and may not be applicable to other possible endpoints.

7.3 Symptoms and Pulmonary Function in Controlled Studies of Ambient Air Exposures

Controlled O_3 exposure studies under a variety of different experimental conditions have generated a large amount of informative exposure-effects data. However, complete laboratory simulation of the pollutant mix present in ambient air is impossible on practical grounds. Thus, the exposure effects of one or several artificially generated pollutants (i.e., a simple mixture) on symptoms and lung function may not be comparable to those in ambient air where complex mixtures of pollutants likely exist. This section reviews two types of studies that utilize a mobile laboratory or a hypobaric chamber to investigate the acute effects of O_3 during exposures to ambient air or altitude, respectively. These studies can be designed to determine the independent effects of O_3 as well as possible interactions among many pollutants and other conditions present in typical ambient air.

7.3.1 Mobile Laboratory Studies

Quantitatively useful information on the effects of acute exposure to photochemical oxidants on symptoms and pulmonary function originated from field studies using a mobile laboratory, as presented in the previous criteria document (U.S. Environmental Protection Agency, 1986). These studies offer the advantage of studying the effects of ambient air on a local subject population by combining the experimental methods of both epidemiology and

controlled-exposure studies. Field studies using mobile exposure chambers involve subjects exposed to ambient air, FA without pollutants, or FA containing artificially generated concentrations of O_3 that are comparable to those measured in the ambient environment. The exposure air can also be conditioned to a desired temperature and humidity. As a result, measured health responses in ambient air can be compared to those found in more artificial or controlled conditions. The mobile laboratory shares many of the same limitations of stationary exposure laboratories (e.g., limited number of both subjects and artificially generated pollutants for testing). Ambient air studies in the mobile laboratory are dependent on ambient conditions, which can be unpredictable, uncontrollable, and not completely characterizable. Logistical problems (space, power, and locations with local interfering outdoor conditions) limit access to many ambient pollution sites of interest.

As summarized in Table 7-14, investigators at the Rancho Los Amigos Medical Center in California used a mobile laboratory and demonstrated that respiratory effects in Los Angeles residents are related to O_3 concentration and level of exercise (Linn et al., 1980, 1983b; Avol et al., 1983, 1984, 1985a,b,c, 1987). Such effects include pulmonary function decrements at O_3 concentrations of 0.144 ppm in healthy exercising adolescents (Avol et al., 1985a,b) and increased respiratory symptoms and pulmonary function decrements at 0.153 ppm in heavily exercising athletes (Avol et al., 1984, 1985c) and at 0.174 ppm in lightly exercising normal and asthmatic subjects (Linn et al., 1980, 1983b). The observed effects were typically mild, and generally no substantial differences were seen between asthmatic and nonasthmatic subjects. Postexposure pulmonary function decrements appeared to last several hours longer in the asthmatics, but no statistical test was reported for this difference (Avol et al., 1983; Linn et al., 1983b). The medication status of the asthmatic subjects during the studies was not reported, although medications were temporarily withheld prior to exposures. The subjects' clinical severity typically was mild, based on their baseline lung function and exercise capability. Many of the normal subjects with a history of allergy appeared to be more responsive to O_3 than "nonallergic" normal subjects (Linn et al., 1980, 1983b), although a standardized evaluation of atopic status was not performed. Direct comparative studies of exercising athletes (Avol et al., 1984, 1985c) with chamber exposures to oxidant-polluted ambient air (mean O_3 concentration of 0.15 ppm) and purified air containing a controlled concentration of generated O_3 at 0.16 ppm showed no significant differences in lung function and symptoms, suggesting that coexisting ambient pollutants had minimal contribution to the measured responses under the typical summer ambient conditions in Southern California. Effects of copollutants in other regions of the country remain to be investigated with the mobile laboratory. These field studies emphasize the importance of adequate characterization of subjects and the ambient air, exercise levels, duration of exposure, and individual variations in sensitivity in interpreting observed exposure effects. Although these factors need to be investigated over a wider range of experimental conditions, the results from these field studies are, so far, consistent with those from controlled human exposure studies. Short-term respiratory effects of summer ambient oxidant pollution in Southern California are predominantly, if not entirely, caused by ambient O_3 in typical healthy or asthmatic residents, according to mobile laboratory studies (Avol et al., 1984, 1985c). Overall, the symptoms and decrements in lung function were generally modest and, while statistically significant in some cases, were probably not clinically significant.

Table 7-14. Acute Effects of Ozone in Ambient Air in Field Studies with a Mobile Laboratory[a]

Mean Ozone Concentration[b] 6ppm	Mean Ozone Concentration[b] g/m³	Ambient Temperature[c] (C)	Exposure Duration	Activity Level ($\dot{V}_E$)	Number of Subjects	Observed Effect(s)	Reference
0.113 ± .033	221 ± 65	33 ± 1	1 h	CE (22 L/min)	66 healthy children, 8 to 11 years old	No significant changes in forced expiratory function and respiratory symptoms after exposure to 0.113 ppm O_3 in ambient air.	Avol et al. (1987)
0.144 ± .043	282 ± 84	32 ± 1	1 h	CE (32 L/min)	59 healthy adolescents, 12 to 15 years old	Small significant decreases in FVC (2.1%), $FEV_{0.75}$ (4.0%), FEV_1 (4.2%), and PEFR (4.4%) relative to control with no recovery during a 1-h postexposure rest; no significant increases in symptoms.	Avol et al. (1985a,b)
0.153 ± .025	300 ± 49	32 ± 2	1 h	CE (53 L/min)	50 healthy adults (competitive bicyclists)	Mild increases in lower respiratory symptom scores and significant decreases in FEV_1 (5.3%) and FVC; mean changes in ambient air were not statistically different from those in purified air containing 0.16 ppm O_3.	Avol et al. (1984, 1985c)
0.156 ± .055	306 ± 107	33 ± 4	1 h	CE (38 L/min)	48 healthy adults, 50 asthmatic adults	No significant changes for total symptom score or forced expiratory performance in normals or asthmatics; however, FEV_1 remained low or decreased further (3%) 3 h after ambient air exposure in asthmatics.	Linn et al. (1983b) Avol et al. (1983)
0.165 ± .059	323 ± 115	33 ± 3	1 h	CE (42 L/min)	60 "healthy" adults (7 were asthmatic)	Small significant decreases in FEV_1 (3.3%) and FVC with no recovery during a 1-h postexposure rest; TLC decreased and N_2 increased slightly.	Linn et al. (1983b) Avol et al. (1983)

Table 7-14 (cont'd). Acute Effects of Ozone in Ambient Air in Field Studies with a Mobile Laboratory[a]

Mean Ozone Concentration[b] ppm	Mean Ozone Concentration[b] μg/m³	Ambient Temperature[c] (°C)	Exposure Duration	Activity Level ($\dot{V}_E$)	Number of Subjects	Observed Effect(s)	Reference
0.174 ± .068	341 ± 133	33 ± 2	2 h	IE (2 times resting) at 15-min intervals	34 "healthy" adults, 30 asthmatic adults	Increased symptom scores and small significant decreases in FEV_1 (2.4%), FVC, PEFR, and TLC in both asthmatic and healthy subjects; however, 25/34 healthy subjects were allergic and "atypically" reactive to polluted ambient air.	Linn et al. (1980, 1983b)

[a]See Appendix A for abbreviations and acronyms.
[b]Ranked by lowest level of O_3 in ambient air, presented as the mean ± SD.
[c]Mean ± SD.

7.3.2 High-Altitude Studies

Symptoms and pulmonary function resulting from exposure to O_3 in commercial aircraft flying at high altitudes and in altitude-simulation studies were reviewed in the previous criteria document (U.S. Environmental Protection Agency, 1986). Attention has focused on the health effects in flight crew, specifically flight attendants because of their physical activities at altitude and exposure patterns to peak levels of cabin O_3. The most quantitatively useful information was based on a series of hypobaric studies of normal nonsmoking subjects who were exposed to 1,829 m (6,000 ft) and O_3 at concentrations of 0.2 and 0.3 ppm for 3 or 4 h (Lategola et al., 1980a,b). Increased symptoms and pulmonary function decrements occurred at 0.3 ppm but not at 0.2 ppm under light exercise conditions. However, the exposure conditions did not reflect higher (peak) O_3 concentrations reported to occur in certain aircraft at high altitudes or the higher cabin altitudes attained by new-generation commercial aircraft.

No reports have appeared subsequently in the literature that specifically study the health effects of aircraft cabin O_3. However, O_3 levels were reported to be very low (average concentration 0.01 to 0.02 ppm) during 92 randomly selected smoking and nonsmoking flights in 1989 (Nagda et al., 1991). None of the flights exceeded the time-weighted average standard of 0.10 ppm (during any 3-h interval) promulgated by the U.S. Federal Aviation Administration, perhaps related to the use of O_3-scrubbing catalytic filters (Melton, 1990). However, in-flight O_3 exposure is possible because catalytic filters are not necessarily in continuous use during flight.

7.4 Field and Epidemiology Studies

7.4.1 Acute Effects of Ozone Exposure

7.4.1.1 Introduction

Field and epidemiology studies addressing the acute effects of O_3 on lung function decrements and increased morbidity and mortality in human populations involve those combinations of environmental conditions and copollutant and activity levels present under real-world conditions of O_3 exposure. This real-world relevance is an advantage over animal or human chamber studies. Thus, results of such studies are essential components of an understanding of overall effects of O_3. However, the conditions under which epidemiologic studies are carried out cannot be controlled in the same way that they can in experimental studies. Parameters that may be difficult or impossible to estimate or control outside the laboratory include actual O_3 exposures, levels of temperature, RH, allergens, correlated pollutants other than O_3, and breathing rates and activity patterns of subjects. Variations in these factors can be important sources of variability in data and results and may, under certain conditions, lead to biases (e.g., confounding) in results. These and other issues of importance in the interpretation of epidemiology study results are discussed in the sections below.

The limitations of epidemiologic studies of O_3 health effects noted above were highlighted in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986), which reached the conclusion that, because of such factors, epidemiologic studies on the acute effects of O_3 on lung function available at that time did not provide information that is quantitatively useful in the standard-setting process. Since publication of the 1986 O_3 criteria document, however, results have become available from a substantial number of well-conducted, individual-level studies and aggregate-level, time-series studies. New statistical

techniques also have become available to deal with confounders that were not appropriately considered when the data were first analyzed. In the following sections, the more recent studies and reanalyses of older studies will be evaluated collectively.

7.4.1.2 Individual-Level Studies

The studies discussed in this section fall into three main categories: (1) summer camp studies, (2) exercise studies, and (3) daily life studies. Summer camp studies involve collection of sequential (usually daily) data on lung function, respiratory symptoms, and environmental conditions over the course of 1 or 2-week attendance at camps. Exercise studies are unique in that lung function and respiratory symptoms are measured before and after each of a series of discrete exercise events in the presence of ambient air pollution. Daily life studies measure lung function, respiratory symptoms, and exacerbation of existing respiratory diseases, along with environmental variables at regular intervals in the course of normal daily activities of a population. These include studies of healthy adults and school-aged children as well as studies of individuals with preexisting disease (e.g., asthma). Medication use also may be monitored in asthmatics. Studies of this kind that focus on exacerbation of asthma symptoms usually have been referred to as panel studies.

The important differences among the three study types relate primarily to issues of exposure assessment. Because subjects usually are out-of-doors or in well-ventilated cabins, exposure estimation errors are minimized in camp and exercise studies. In contrast, larger exposure estimation errors may occur in daily life studies. Camp and daily life studies enable assessment of the effects of cumulative O_3 exposures, whereas exercise studies limit attention to rather brief exposures. Exercise studies offer the potential of assessing individual $\dot{V}_E$ values and O_3 concentrations during the relevant exposure period, whereas such assessments are more difficult in camp and daily life studies.

Although the study designs differ in some ways, the central design feature of all of these study types is the collection of repeated measurements on individuals. This feature is exploited in data analysis by having each subject serve as his or her own control. For continuous outcomes such as lung function, subject-specific linear regressions are usually performed with lung function (or change in lung function) as the outcome variable and O_3 or other environmental factors as the explanatory variable. The regression slope is a measure of individual lung function response to O_3. The mean slope across individuals often is used as a measure of the average population response. A more statistically valid approach involves computing the mean slope with weighting proportional to the inverse variances of the individual slopes. An alternative approach has been to use analysis of covariance methods to fit a population-pooled slope and separate, subject-specific y-intercepts. To date, no studies have used nonlinear (e.g., quadratic) models in relating lung function decrements to O_3 exposures, which in chamber studies have been shown to better describe the functional relationship between O_3 exposures and lung function decrements (see Chapter 9, Section 9.3.4).

Issues in the Interpretation of Individual Level Studies

The most basic question affecting the interpretation of acute O_3 epidemiology studies is whether (and if so, to what extent) the associations observed between O_3 and decreased pulmonary function are causally related to O_3 and not merely due to confounding by some other factor (e.g., temperature, allergens, time trends in spirometry, or other pollutants).

By definition, a confounder is an unmeasured or unaccounted-for variable that has an effect on the measured outcome and also is correlated with O_3 concentrations. Variables that satisfy only one of these two conditions are not confounders. For example, a variable that affects lung function but is independent of O_3 would add variation to lung function measurements but would not confound an O_3/lung function analysis, and a variable that correlates with O_3 but does not directly affect lung function (in the range of measurements) would not confound an analysis of O_3 effects. Other variables might modify the effect of O_3 on lung function, thereby increasing or decreasing the O_3 effect under the conditions of study. Epidemiologists refer to this latter phenomenon as "effect modification". The presence of effect modification does not bias the results of a study, but can provide insights into the range of effect magnitudes (e.g., slope of lung function decrement on O_3 level) that occur under varying environmental conditions.

Ambient air temperature often exhibits a moderate to high correlation over time with O_3 in acute epidemiology studies due, in part, to the dependence of O_3 formation rate on light intensity. Among the studies reviewed in this section, correlations ranging from −0.06 to 0.90 (mean = 0.51) have been reported. Correlations between O_3 and RH, when reported, have been in the range −0.4 to −0.6. Several human chamber studies have examined the possible direct effects of temperature and RH on lung function independent of O_3, with somewhat mixed results (Stacy et al., 1982; Folinsbee et al., 1985; Eschenbacher et al., 1992). Two studies reported increases in FEV_1 at high temperature (30 and 37 °C) and 60% RH (Stacy et al., 1982; Eschenbacher et al., 1992), whereas the other reported no effect on FEV_1 at 35 °C, and a decrease at 40 °C (Folinsbee et al., 1985). Referring to results of acute O_3 epidemiology studies, Eschenbacher and colleagues (1992) concluded, based on their own results, that "the associations found between ambient O_3 and daily changes in ventilatory function cannot be attributed to the heat and humidity stress often associated with high O_3 concentrations." Temperatures observed in the epidemiology studies reviewed in the present section primarily have been below 30 °C, with occasional peaks as high as 35 °C. It should be noted that subjects studied epidemiologically usually will have had an opportunity to acclimate to ambient temperatures prior to or soon after the start of the study. In any event, given the laboratory findings, a significant confounding role for temperature in these studies seems unlikely. The possibility that changes in ambient temperature may introduce biases in measured lung volumes (e.g., through inaccurate correction of volumes to body temperature) is an issue that deserves further study.

Exposure to specific allergens can influence lung function in individuals who have diseases characterized by IgE-mediated, Fc interactions (i.e., atopy) and may also affect individuals who have an atopic tendency (e.g., as assessed by positive prick skin test or serum levels of total IgE) without diagnosed clinical disease. Raizenne et al. (1989) detected positive reactions to one or more allergens by skin prick in 49% of 96 young nonasthmatic females enrolled in a summer camp study. Few data are available on the correlation between O_3 and allergen levels during acute epidemiology studies. However, because both variables to some are extent influenced by weather patterns, some correlation seems likely. Thus, a possible confounding role of airborne allergens in such studies cannot be ruled out. Because of the specific nature of individual antigen sensitization and uncertainty regarding the full set of relevant allergens in a given setting, attempts to measure and to control statistically for allergen levels on a group level in epidemiology studies may not be very effective.

The potential effects of time trends in spirometry due to training effects are also of concern. There have been several recent studies that have looked at time trends in serial lung function measurements (mainly FEV_1 and PEFR) independent of air pollution effects (Raizenne et al., 1989; Avol et al., 1990; Hoek and Brunekreef, 1992). In each case, average FEV_1 measurements have been observed to decline steadily over the first few measurements and then to stabilize or recover slightly to a flat pattern. Average FVC measurements follow a similar pattern. In contrast, PEFR often has been observed to increase steadily over successive measurements. Similar patterns have been observed in studies with intervals between lung function measurements ranging from 12 h to 1 week. The consistency of these observations across studies suggests that they represent real phenomena that should be recognized in designing and analyzing studies involving repeated lung function measurements. However, time trends will result in confounding of O_3 effects only if, by chance, the trend correlates with temporal variations in O_3 concentrations. Such chance correlations could be either positive or negative and, if present, would have a larger impact (i.e., produce an undesirable degree of confounding) on studies in which all subjects begin the study simultaneously and have few follow-up measurements. Studies that focus on daily changes in lung function may be less impacted by this phenomenon.

It is also important to consider the roles of other pollutants as possible confounders or effect modifiers. In the studies to be reviewed in this section, most copollutants (e.g., SO_2, NO_2, sulfate, and acid aerosols) were present at levels well below those that have produced lung function decrements in healthy subjects following short-term exposures in chamber studies (see Section 7.2.6). In contrast, an extensive and growing database is available from chamber studies documenting the independent acute effects of ambient-level O_3 on lung function (see Section 7.2.1). Although direct lung function effects of other pollutants at typical ambient concentrations seem unlikely, it has been suggested that the effects of O_3 may be potentiated by coexposure or previous exposure to other pollutants, most notably acid aerosols (Spektor et al., 1988b). Some data from animal studies suggest interactive effects of O_3 and acid exposures for certain pulmonary outcomes (see Chapter 6). However, to date, analyses directed towards this phenomenon in field studies of human lung function (via analysis of the relationship between acid aerosol levels and residuals from regressions of lung function on O_3) have proven negative (Spektor et al., 1988a,b). That is, after controlling for the influence of O_3, no significant association between acid aerosol peaks and lung function decrements has been observed. Acid aerosol episodes, which often occur coincident with high O_3 levels in the summer in the northeastern United States, may extend for several hours or days. However, the possible potentiating effects of prolonged acid peaks on O_3 effects are still poorly understood. Some recent epidemiological studies (Pope et al., 1991; Pope and Dockery, 1992; Koenig et al., 1993; Roemer et al., 1993) have reported significant associations between lung function decrements and ambient particulate matter (PM) concentrations. Although no supporting evidence for lung function effects due to ambient-level particle exposure is yet available from human or laboratory animal controlled studies, the possibility of some confounding by particles in the studies reviewed here cannot be ruled out.

In epidemiologic studies, activity levels are difficult to control and to measure, although this varies with study type (see below). Chamber studies have shown clearly that lung O_3 doses and associated functional effects increase as a function of physical activity level (Hazucha, 1987). Epidemiologic study designs often have been chosen that result in relatively

high subject activity levels (exercise studies and camp studies), but generally the studies have been carried out without quantitative information on $\dot{V}_E$ distributions across subjects and time.

Variations in activity levels will introduce variability in the relationship between personal exposure and personal dose. If this variability occurs primarily between subjects, it will result in differing O_3 doses to people exposed to the same O_3 level and yield differences in response that may be misinterpreted as O_3 sensitivity variations. If variability in activity levels occurs over time for a given subject, it will add error to the functional relationship linking lung function and O_3 exposure. In either case, the influence of activity level variability is to add dose estimation error (or misclassification). Estimates of $\dot{V}_E$ based on heart rate measurements can be derived using subject-specific calibrations under representative ranges of exercise levels and types (Samet et al., 1993; Raizenne and Spengler, 1989). However, the utility of such $\dot{V}_E$ estimates for reducing dose-misclassification errors in acute O_3 epidemiology studies has not yet been demonstrated (Kinney, 1986; Spektor et al., 1988b; Raizenne and Spengler, 1989). This is partly due to the logistical difficulties associated with collecting accurate data and also may be due to the fact that, for a given subject, $\dot{V}_E$ variations across days are usually small in comparison to O_3 concentration variations. The same issues arise in the context of O_3 exposure misclassification in "daily life" studies (see below), where outdoor O_3 concentrations are used to estimate exposures of subjects who spend substantial amounts of time indoors during the period over which lung function measurements take place.

Camp Studies of Lung Function in Children

Summer camp studies provide the most extensive and reliable information on the acute pulmonary effects of O_3 under natural conditions. Camp studies involve the collection of sequential (usually daily) data on lung function on each of a large number of children, along with concurrent measurements of O_3 exposures and other environmental factors over the course of a single-week or multiweek summer camp. Data analyses usually consist of estimating the linear association between lung function and environmental variables on an individual basis (allowing each subject to serve as his/her own control) and then testing the mean population association for statistical significance. As noted, summer camps offer the significant advantage that subject exposures are especially well estimated because they are based on on-site, outdoor O_3 monitoring. In addition, these studies assess the pulmonary effects of natural diurnal patterns of O_3 exposures, which often involve broad daytime peaks.

Since the last O_3 criteria document (U.S. Environmental Protection Agency, 1986), eight camp studies have been reported. Design characteristics and results are summarized in Table 7-15. Six of these studies have focused solely on normal (i.e., nonasthmatic) children, one focused on asthmatics exclusively (Thurston et al., 1995), and one used both normal and asthmatic children (Raizenne et al., 1987). Although methods and results varied somewhat across studies, this group of studies collectively provides substantial evidence for associations between ambient O_3 exposures, together with other pollutants, and acute decrements in lung function. Interpretation of these associations as causal is supported by evidence of biological plausibility. For example, the well-documented direct effects of O_3 on lung function in human chamber studies; the evidence, also from chamber studies, indicating a lack of direct effects of other collinear environmental factors (e.g., temperature and acid aerosols) at the levels at which these factors occur in the camp studies; exposure-response relationships; and consistency across studies all provide strong support. Camp studies involving asthmatic

children generally have yielded lung function/O_3 associations that are similar in absolute magnitude to those observed in nonasthmatics (Raizenne et al., 1987;

Table 7-15. Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Hourly O_3 ranged from □10 to 110 ppb. SO_2, NO_x, O_3, $SO_4^=$, H_2SO_4, pH, PM_{10}, $PM_{2.5}$, RH, temperature, barometric pressure, and wind speed and direction also were measured.	Effects of pollutants and other environmental variables on symptoms and lung function were examined in children attending a summer camp at Lake Couchiching, about 100 km north of Toronto, ON. Study was conducted June 30 through July 8, 1983; n = 52, 23 nonasthmatics (11 males, 12 females) and 29 asthmatics (16 males, 13 females), avg. age = 12.1 years. Symptom questionnaire and function tests given twice daily to each child between 7:30 and 9:30 a.m. and 4:30 and 6:30 p.m. Children's activity levels not estimated.	Strongest association between lung function and environmental variables was in nonasthmatics, with FVC decrements significantly correlated ($p < 0.01$) with lagged-avg SO_4, $PM_{2.5}$, and temperature. Unlagged PEFR significantly correlated with 1 h O_3. Also, significant association of temperature with all lung function indices in nonasthmatics, but not in asthmatics. Coefficient of variation stable across morning and evening tests.	Raizenne et al. (1987)[b]
1-h O_3 ranged from < 10 to 143 ppb; max 1-h O_3 > 100 ppb on 14 days of total study (6 weeks). For other pollutants and variables measured, see Raizenne et al. (1987) because same protocol used here as in that study.	(a) Effects of pollutants and other environmental variables on lung function were examined in girls attending one of three 2-week Girl Guide camp sessions on the north shore of Lake Erie. Cohort (n = 104) screened by MC and skin-prick tests for 10 common respiratory allergens; five asthmatics withdrawn from the study (n = 99). Lung function tests administered twice daily. Children's activity levels not estimated.	(a) Associations between aerometric data and lung function measurements were not reported by pollutant in this reference. Aggregate analysis for full study not reported. Lung function changes reported for 5 episode days only. FEV_1 decrements statistically significant on 2 episode days for methacholine- nonresponsive subjects.	Raizenne et al. (1987, 1989)[b]
	(b) Subset of 12 girls (7 MC+, 5 MC-) studied pre- and postexercise on 1 low-pollution (control) day and 1 peak-pollution day (episode, 1 h O_3 > 139 ppb, $SO_4^=$ > 80 □g/m³).	(b) Group mean FVC increased postexercise in the n = 12 subset by 40 mL, 71 mL in MC□ and 17 mL in MC+. Pollution effect not statistically significant.	
Continuous 1-h O_3, SO_2, NO_2, and acid aerosols (as H_2SO_4); 1-h O_3 range = 40-143 ppb; max 12-h acid particle concentration = 28 □g/m³ in one episode; FP $SO_4^=$ 100 □g/m³ for peak hour.	Time-activity model used to evaluate likely cumulative (6 h) O_3 and H_2SO_4 exposures/doses experienced by children in above Lake Erie Girl Guide camp study, summer 1986. See Raizenne et al. (1987, 1989) for protocol and related information. Dosimetry model was developed for relating heart rate (from a 12-min, graded, cycle ergometer test) to ventilation and then to O_3 and H_2SO_4 concentration. Also, five randomly selected children wore portable heart-rate monitors, providing data for use in the dosimetric model.	Application of the dosimetry model used to estimate individual 6-h cumulative doses for O_3 and H_2SO_4 exposures on 1 control and 1 episode day indicated negative trend in lung function (PEFR) as cumulative dose increased for both O_3 and H_2SO_4, although slopes for each did not differ significantly from zero ($p > 0.10$).	Raizenne and Spengler (1989)[b]

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Max 1 h O_3 ranged from 40 to □100 ppb, with max 1 h > 80 ppb on 9 of 27 days of O_3 recorded. O_3, SO_4, H_2SO_4, PM_{15}, $PM_{2.5}$, temperature, humidity, and wind speed and direction measured. Levels not reported for SO_2, pH, NO_3, and NH_4^+.	Effects of pollutants and other environmental variables on respiratory functions in 91 children (53 boys, 38 girls; ages 8-15) attending 2 to 4 weeks of summer camp at Fairview Lake, NJ. Subsets were n = 37 for all 4 weeks, n = 34 for first 2 weeks only, n = 20 for last 2 weeks only. Symptom questionnaire; FVC, FEV_1, MMEF, and PERF (by spirometry) were measured once each test day (most of days in camp) sometime between 11:00 a.m. and 6:30 p.m. All children had validated spirometric data for □7 days of their 2- or 4-week camp stay. Activity levels of the children were not estimated. Respiratory health status determined by parental questionnaire only. Children slept in screened-in shelters but otherwise were exposed to ambient air 24 h/day. Average regression slopes for respiratory function vs. max 1-h O_3 concentration reported for the full cohort, for boys and girls separately, and for subsets in attendance for all 4 weeks and for respective 2-week sessions. Regressions also repeated for data below 80 and 60 ppb 1-h O_3, and for data with THI < 78 □F.	Average regression slopes (±SE) were □1.03 ± 0.24 and □1.42 ± 0.17 mL/ppb for FVC and FEV_1, respectively; and □6.78 ± 0.73 and □2.48 ± 0.26 mL/s/ppb for PEFR and MMEF, respectively. Most slopes of regression significant at $p < 0.05$ (differences from zero). Not clear if slopes for data subsets significantly different from each other (e.g., function vs. O_3 < 60 ppb and function vs. O_3 < 80 ppb). No formal analysis performed for possible concentration threshold.	Spektor et al. (1988a)[b]

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Maximal 1 h O_3 concentrations ranged from approximately 40 to 150 ppb over the course of the study. 12-h average aerosol acidity measurements ranged from near 0 to 18.6 $\mu g/m^3$ (H_2SO_4 equivalent). Temperature and RH measured, but levels not reported. THI reached a maximum of 81 °F. All environmental measurements were made on site.	Effects of O_3 and other environmental variables on lung function studied in a group of 46 children (13 girls, 33 boys; ages 8-14) at a 4-week, 1988 summer camp in southwestern New Jersey (Fairview Lake). Same location used in previous camp study by same investigators. Subjects had no history of lung diseases or atopy. Two lung function measurement periods each day (a.m. and p.m.) along with collection of respiratory symptom data. Data collected during or after periods of rain were excluded from analysis. Results for FVC, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$, and PEFR reported. Linear day-of-study trends were examined for lung function. Subject-specific linear regressions were performed relating lung function in a.m., p.m., and p.m. − a.m. differences to O_3 averaged over various periods. Average slopes across subjects were tested for significant differences from zero. Regressions were repeated after excluding days with O_3 at or above 120 ppb. Regression residuals were tested for correlation with THI and H^+ concentrations.	No significant linear day-of-study effect seen for any of the lung function variables tested, but the linear model may not have been optimal for testing this effect. In a subset of 35 subjects with at least 2 consecutive days of lung function measurements, mean regression slopes of a.m. lung function variables on previous-day mean or 1-h maximum O_3 were all significantly negative (e.g., mean slope of FEV_1 on 1-h maximum O_3 was −0.50 ± 0.12 mL/ppb). These results suggest a possible carry-over effect from previous-day O_3 exposures. In the full set of 46 subjects, regressions of p.m. lung function on previous-hour O_3, maximum 1-h O_3 for same day, or average O_3 for day were significantly negative in most cases (e.g., mean slope of FEV_1 on previous-hour O_3 was −1.60 ± 0.30 mL/ppb). All regressions of the p.m. − a.m. lung function differences on intervening O_3 concentrations were significantly negative (e.g., mean slope of FEV_1 on mean O_3 between a.m. and p.m. measurements was −0.63 [±0.09] mL/ppb). No correlation seen between regression residuals and THI or H^+ concentrations, indicating there was no remaining effect of these variables with lung function after accounting for O_3. However, no models were fit that included O_3 and these variables simultaneously, nor were interaction effects tested for. The strong and consistent associations between lung function decrements and O_3 concentrations in this study contrast with results reported from studies in Canada and California at similar levels of O_3.	Spektor et al. (1991) Spektor and Lippmann (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h O_3 preceding lung function measurements ranged from 25 to 245 ppb. Pollutants measured on site included O_3; NO_2 (range: 0 to 40 ppb), SO_2 (range: 1 to 8 ppb); and fine (mean = 23.9 $\mu g/m^3$), coarse (mean = 36.6 $\mu g/m^3$), and total (mean = 59 $\mu g/m^3$) PM_{10} mass. Temperature averaged 21.5 °C (range: 13.5 to 25.5 °C) and RH averaged 43.3%.	Effects of O_3 and other environmental factors on lung function examined in 43 children (24 female, 19 male; ages 7-13) attending 1 of 3 sequential weeks (three subjects stayed an additional week) of summer camp in the San Bernardino Mountains of California. Camp was at 5,710 ft above sea level. Lung function measured by spirometry up to three times daily on each subject; analytical measures included FVC, FEV_1, and PEFR. No report of respiratory data derived from questionnaires. Subject activity levels prior to lung function testing were not characterized. Campers slept in well-ventilated cabins. Subjects came mostly from homes in the Los Angeles Basin, and thus were likely to have been exposed to high O_3 levels prior to camp. Simple linear regression models were fit on an individual basis (subject-specific slopes) and by pooling across individuals (common population slope) to determine the linear relations between the three lung function variables and various O_3 metrics (1-h average preceding hour of spirometry, 1-h average 2 h previous to hour of spirometry, or 6-h average preceding spirometry). The common slope model was repeated separately for morning, noon, and evening lung-function measurements, and separately for data with 1-h O_3 levels above and below 120 ppb. Multiple regression models were fit that included O_3 along with temperature, RH, and coarse and fine PM mass.	The population-pooled regression slopes (±SE) of FVC and FEV_1 on previous hour O_3 were −0.40 (±0.10) and −0.38 (±0.09) mL/ppb, respectively ($p < 0.0001$ in both cases); for PEFR, the regression slope was −0.13 (±0.36) mL/s/ppb (not significant). Similar, though slightly more negative, slopes were obtained using 2-h and 6-h average O_3. Interpretation of differences across the three O_3 metrics is substantially hampered by the high correlations among them ($r \approx 0.90$). When temperature, RH, and coarse and fine PM mass were included with O_3 in multiple regression models, the O_3 slopes increased in absolute magnitude to −0.68 (±0.16) and −0.76 (±0.15) mL/ppb for FVC and FEV_1, respectively, and to −1.91 (±0.63) for PEFR. Technical problems with the temperature sensor in the first week of the study did not appear to influence these results. Data were split on the basis of whether or not the maximum 1-h O_3 concentration in the 6 h preceding spirometry was above 120 ppb. Regression slopes relating lung function and previous 1-, 3-, and 6-h average O_3 were more negative in the high concentration stratum. This result is consistent with the nonlinear (e.g., quadratic) relationships between lung function and O_3 exposure observed in chamber studies. Because levels of pollutants other than O_3 were quite low (NO_2 and SO_2), and/or were uncorrelated with O_3 levels (PM), the regression results reported from this well-conducted study are likely to represent real influences of O_3 on lung function.	Higgins et al. (1990); Gross et al. (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Daily maximum O_3 concentrations ranged from approximately 60 to 160 ppb (derived visually from figure presented in paper). Other pollutants measured on site included SO_2, NO_2, CO, total hydrocarbons, and size-segregated PM mass. Aside from O_3, all gaseous pollutant levels reported to be very low (data not presented). 24-h TSP concentrations ranged from 18 to 54 $\mu g/m^3$. Airborne allergen data collected. Temperature ranged from 10 to 15 °C at night and from 25 to 35 °C during day. RH ranged from 30 to 45% at night and from 5 to 20% during day.	Effects of O_3 and other environmental variables on lung function examined in 293 children (139 girls, 154 boys; ages 8-17) attending one of six 1-week camp sessions at a summer camp located in the mountains near Idyllwild, CA, 190 km southeast of Los Angeles (altitude: 1,570 m). Lung function measured twice daily on each camper (a.m.: 0730 to 0930; p.m.: 1600 to 1930). Analyses presented for FVC, FEV_1, PEFR, and $FEF_{25-75\%}$. Symptom questionnaires completed prior to each test. Used repeated measures analysis of variance model to test for day-of-study and a.m./p.m. effects on lung function independent of pollution concentrations. Linear regressions of morning, afternoon, and p.m. − a.m. difference of lung function on O_3 were performed with simultaneous control of day and a.m./p.m. effects. Upper and lower quartiles of distribution of individual FEV_1/O_3 regression slopes were examined with respect to subject characteristics. Changes in FEV_1 over several days analyzed in relation to intervening integrated O_3 concentrations.	Significant day-of-study effect observed for FVC and FEV_1 characterized by steady drop over first few days of measurement, followed by partial reversal later in week. For PEFR, p.m. measurements were significantly higher than a.m. measurements. Controlling for day and a.m./p.m. effects, the authors reported that no consistent O_3 effects on lung function were observed. The a.m. lung-function measurements had a significant positive correlation with O_3 averaged over the previous 1, 8, or 24 h. The p.m. measurements reported to have no correlation with O_3. The p.m. − a.m. lung function differences were negatively correlated with previous 8-h average O_3 concentrations, but not with previous 1-h O_3 concentrations. No quantitative results reported for the above lung function/O_3 findings. There were no discernable differences between subjects in the upper and lower quartiles of the distribution of individual regression slopes of a.m. − p.m. FEV_1 difference on previous 1 h O_3. Regressions of change in FEV_1 over several days (four separate intervals ranging from approximately 8 h to approximately 80 h) with integrated O_3 concentrations yielded negative slopes ranging from −0.41 to −1.46 mL/ppb, one of which was statistically significant. The time-trends in FVC and FEV_1 measurements observed in this study are qualitatively consistent with those seen in some other summer camp studies. The lack of consistent negative slopes relating lung function with O_3 concentrations contrasts with other, eastern U.S., summer camp studies at similar O_3 levels.	Avol et al. (1990) Avol et al. (1991)

Table 7-15 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Camp Studies[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
O_3 data collected at a site 8 mi from camps. Daily 1-h O_3 maxima ranged from approximately 40 ppb to approximately 200 ppb. 12-h aerosol acidity concentrations ranged between 14 and 360 neq/m^3. Temperature and RH data obtained from a nearby site.	Report of data collected during two simultaneous summer camps located 2 mi apart in central New Jersey in 1988. 34 subjects were studied, including 20 camp counselors (ages 14-35) and 14 campers (ages 9-13). Study spanned 19 days. Spirometry and respiratory symptom data collected each afternoon. Analysis of FVC, FEV_1, and PEFR in relation to O_3 and temperature using linear regression within camps and subject types (i.e., counselors vs. campers).	Regressions of lung function on 1-h and 8-h average O_3 within several subject subsets yielded inconsistent results, with some mean slopes apparently significantly positive, and one negative mean slope, highlighted by authors, of borderline significance ($p < 0.10$).	Berry et al. (1991)
Daily 1-h maximum O_3 concentrations ranged from 70 to 160 ppb in 1991 and from 10 to 63 ppb in 1992. On-site measurements also made for acid aerosols (approximately 20 to 110 nmoles/m^3 in 1991 and 15 to 55 nmoles/m^3 in 1992) and temperature (between 21 and 32 °C over 2 years).	Effects of O_3 and acid aerosols on peak flow, respiratory symptoms, and medication usage in asthmatic children evaluated at two 1-week summer camps (June of 1991 and 1992) in the Connecticut River Valley. Fifty-two and 55 subjects were studied in 1991 and 1992, respectively, ranging in age from 7-13. Peak flow measured twice daily (approximately 9:00 a.m. and 5:00 p.m.). Combining data from the two studies, individual regressions of daily change in FEV_1 on O_3 or H^+ concentrations were performed.	In subjects without asthma exacerbations during the camps, statistically significant, negative mean slopes were found relating ΔPEFR and O_3 or H^+ concentrations. The correlation between these two pollutants was not reported. The mean slopes were −2.3 (±0.7) mL/s/ppb for O_3, and −1.2 (±0.6) mL/s/nmol/m^3 for H^+. In the case of O_3, a scatter-plot with ΔPEFR demonstrated an apparently linear trend. In contrast, the H^+ regression results appeared to be driven entirely by one data point.	Thurston et al. (1995)

[a]See Appendix A for abbreviations and acronyms.
[b]Cited in U.S. Environmental Protection Agency (1992).

Thurston et al., 1995); however, the health significance of a given drop in FEV_1 may be greater for those with preexisting, compromised respiratory function.

Although similar study designs have been employed in most of the camp studies summarized in Table 7-15, differences in analytical methods have made quantitative comparisons between studies difficult to interpret. In particular, it has not been clear to what extent differences in results across studies may be due to differences in study characteristics (e.g., O_3 effect potentiation by other pollutants and activity levels) as opposed to differences in data analysis methods.

For better comparison in this document, data from six of the camp studies summarized in Table 7-15 were reanalyzed using uniform analytical methods. For each study, afternoon lung function data (FEV_1) were regressed on concurrent 1-h O_3 concentrations using an analysis of covariance model that included subject-specific intercepts and a single, pooled O_3 slope. Although intersubject variation in responses to O_3 would be expected on the basis of controlled chamber study results (see Section 7.2), a common-slope model was chosen for this analysis because emphasis was placed on estimating the average response in each study population. The study-specific slopes computed with this model ranged from −0.19 to −1.29 mL/ppb across the six studies (Table 7-16). All but one of these slopes were statistically significant ($p < 0.02$). When data for all six studies were pooled, a slope of −0.5 mL/ppb was observed. The slope from the 1988 Fairview Lake, NJ, study (−1.29 mL/ppb) was greater in absolute magnitude than the slopes from the other studies (which ranged from −0.19 to −0.84 mL/ppb). Overall, however, these pooled results indicate a quantitative consistency among studies that is not as readily apparent in the absence of the combined analysis.

Table 7-16. Slopes from Regressions of Forced Expiratory Volume in One Second on Ozone for Six Camp Studies[a]

Study Name	Slope ± SE (mL/ppb)[b]	p-Value	Reference
Fairview Lake, 1984	−0.50 ± 0.16	0.002	Spektor et al. (1988a)
Fairview Lake, 1988	−1.29 ± 0.27	0.0001	Spektor et al. (1991) Spektor and Lippmann (1991)
Lake Couchiching	−0.19 ± 0.44	0.66	Raizenne et al. (1987)
Lake Erie	−0.29 ± 0.10	0.003	Raizenne et al. (1987, 1989)
San Bernardino Mountains	−0.84 ± 0.20	0.0001	Higgins et al. (1990) Gross et al. (1991)
Pine Springs Ranch	−0.32 ± 0.13	0.013	Avol et al. (1990, 1991)
All studies	−0.50 ± 0.07	< 0.0001	

[a]For each study, data were analyzed in one regression model that included a pooled O_3 slope and separate subject-specific intercepts. See Appendix A for abbreviations and acronyms.
[b]Slope is the weighted mean of six study-specific slopes. The SE is the weighted SE of mean slope.

It is not clear why the 1988 New Jersey study yielded a larger slope than the other studies. Possible explanations include greater subject activity levels (resulting in higher O_3 doses at a given exposure level), potentiation of the O_3 effect by other pollutants (such as acid aerosols), the relative absence of O_3 tolerance in the New Jersey study, or confounding by

airborne allergens. There are no firm data on activity levels across the six studies. Thus, whereas this factor surely contributes to the random variability within and between studies, it is not known whether activity levels were substantially and systematically higher in the 1988 New Jersey study. Potentiation of the O_3 effects on lung function in asthmatics by acid aerosols has been demonstrated in a chamber study in which O_3 exposure was administered 1 day following a 3-h exposure to 100 $\mu g/m^3$ H_2SO_4 (Utell et al., 1994). Although the relevance of these data to the nonasthmatic subjects who experienced much lower acid levels at northeastern summer-camps is not clear, they do demonstrate that potentiation can occur between these pollutants. However, this factor alone cannot explain the observed differences across camp results, because a camp study in southern Ontario (Raizenne et al., 1989), which yielded relatively low FEV_1 slopes on O_3, experienced sulfate aerosol levels that were comparable to those seen in New Jersey. Similarly, whereas tolerance due to prior exposures to high O_3 levels has been suggested as an explanation for the smaller slopes seen in the California studies, a lower subject activity level has been suggested to explain the smaller slopes in southern Ontario. Data have not been reported on comparative levels of airborne allergens during the various camp studies. None of the subjects in the 1988 New Jersey study reported a history of asthma or atopy, minimizing the likelihood of confounding by airborne allergens. However, given the lack of allergen data and the potential for substantial numbers of "silent hyper-responders" (Raizenne et al., 1989), this possibility cannot be completely discounted. Thus, no one factor seems adequate to explain the differences in results across studies. Quite possibly, these differences reflect the combined influence of several of the factors discussed above. Indeed, given the many possible sources of camp-to-camp variability, it is surprising that results are as consistent as they are across six studies by three investigative groups.

Several investigators have reported regression results for 1-h average O_3 and for longer averaging times (e.g., 6 to 8 h) (Higgins et al., 1990; Avol et al., 1990, 1991; Spektor et al., 1988a, 1991). In general, similar results have been obtained regardless of the averaging time. Attempts to draw conclusions regarding the relative importance of short-term peaks and longer term averages from such analyses have been hampered by the high degree of correlation between 1-h and multihour averages. Until better analytical methods are found for dealing with this problem, comparative results will remain difficult to interpret.

Lung Function in Exercising Subjects

This subsection discusses studies involving lung function measurement immediately before and after a series of discrete outdoor exercise activities in the presence of air pollution. This design is similar in principle to the ambient chamber studies conducted in the early 1980s (see Section 7.3), in which subjects exercised under a specified protocol in a chamber ventilated with ambient air. Here, however, there is typically less control imposed over exercise duration and intensity, and less assessment of achieved $\dot{V}_E$. Compensating to some extent for this diminished control is the relative ease of collecting numerous repeated measurements at varying ambient O_3 levels for the same subjects, improving the precision of concentration-response estimation. In contrast to camp studies, duration of relevant O_3 exposure is assumed to be known, as it is defined by the length of each exercise event.

Results from five exercise studies (Selwyn et al., 1985; Spektor et al., 1988b; Hoek and Brunekreef, 1992; Hoek et al., 1993a; Braun-Fahrlander et al., 1994; Brunekreef et al., 1994) are summarized in Table 7-17. One of the studies (Selwyn et al., 1985) was discussed

in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) but is reviewed again here because of its apparent consistency with the more recent study of Spektor et al. (1988b).

Certain design variations across studies are worth noting. In the Houston study, each of 24 recreational runners performed spirometry before and after a series of approximately 28 runs on a track from late spring to early fall (Selwyn et al., 1985). Each run was 3 mi long, and each subject attempted to maintain a similar heart rate across all runs. Minute ventilation was not assessed. In the study carried out in Tuxedo, NY, adult runners and walkers were allowed to choose their own exercise level and duration, but again were encouraged to maintain a steady heart rate for the duration of the study (Spektor et al., 1988b). Minute ventilation of each subject while running was estimated by measurement of $\dot{V}_E$ during a treadmill test that achieved a heart rate typical of that subject's experience while running. In the study of 128 Swiss school children (Braun-Fahrlander et al., 1994), 10-min exercise periods on a cycle ergometer were utilized on four to six occasions over a 6-mo period.

Table 7-17. Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h O_3 concentration ranged from 21 to 124 ppb, max THI = 78 °C; max acidic aerosol (as H_2SO_4) = 9 µg/m^3 during study. SO_2, NO_x, PM_{15}, $PM_{2.5}$, $SO_4^=$, NO_3, NH_4^+, temperature, and RH measured but not reported.	Effects of O_3 on respiratory function and symptoms examined in 30 nonsmoking adults (2 of 10 non-Caucasian females) exercising almost daily outdoors (Tuxedo, NY) for 15 to 55 min (average ca. 30 min) from July to early August 1985. Pre- and postexercise lung function measured, and questionnaire answered postexercise. Pulse rate, calibrated to $\dot{V}_E$ indoors, taken postexercise. Exercise regimen self-selected but constant for each subject over the course of study. Dosimetry estimated and linear regressions done for pulmonary function changes vs. (1) mean O_3 concentration during exercise and (2) inhaled O_3 dose. Persistence of effects tested by linear regressions of before-exercise lung function on previous-day O_3 during exercise. Subjects screened only by questionnaire; two with previous history of asthma but asymptomatic.	Significant ($p < 0.01$) decrements in FVC, FEV_1, PEFR, $FEF_{25-75\%}$, and FEV_1/FVC associated with O_3. For example, the mean slope of ΔFEV_1 on O_3 across all subjects was −1.35 mL/ppb (±0.35). No persistence of effects seen. No symptoms reported by subjects. Mean decrements showed unexpected inverse relationship with calculated $\dot{V}_E$ levels, as indicated by regressing pulmonary function changes and postexercise function against inhaled O_3 during exercise. $\dot{V}_E$ ranges given, but not group or subset means. Subjects not screened for atopy. Exercise done in Sterling Forest, wooded research park, on paved roads or trails.	Spektor et al. (1988b)
15-min peak O_3 measured during runs averaged 47 ppb (range: 4 to 135 ppb). Ambient T averaged 29.4 °C (range: 18.0 to 37.8 °C). RH averaged 62.6% (range: 37.0 to 88.0%). Levels of other pollutants were low, median values were SO_2, 3 ppb; NO_2, 6 ppb; FP, 10 µg/m^3. Median of subject-specific correlations of O_3 and RH correlated was -0.42.	Effects of O_3 on lung-function change during running outdoors were examined in 24 conditioned, recreational runners (6 women, 18 men, ages 29-47) at a track 30 mi southeast of Houston, TX, from May to October, 1981. All runs were 3 mi in length, and each subject performed at a near-constant heart rate for the duration of the study. An average of 28 runs completed by each subject during the study. Spirometry carried out before and after each run, with analysis of FVC, FEV_1, $FEF_{25-75\%}$, and $FEF_{0.2-1.2L}$. Change in each lung function variable was regressed, for each subject, on 15-min maximum O_3 measured during the run. The mean slope across subjects was tested for significance. The regression was repeated with temperature and RH in the model.	Mean slope of FEV_1 on O_3 alone was −0.4 mL/ppb ($p = 0.03$). In regressions that included temperature and RH, the O_3 slope dropped to −0.07 (not significant). Although temperature reached high levels during the study, a substantial direct effect of temperature or RH on lung function, relative to that of O_3, seems unlikely. A possible potentiating role of high temperature and RH on $\dot{V}_E$, and corresponding O_3 dose, cannot be ruled out. Lung function effect observed in simple O_3 model seem likely to be a valid reflection of O_3 effects under varying environmental conditions.	Selwyn et al. (1985)

In contrast to these studies, early investigation of O_3 effects in The Netherlands involved lower and more variable exercise levels, without any specific attempt to control exercise intensity (Hoek and Brunekreef, 1992; Hoek et al., 1993a). Here, children engaged in sports training and skills development activities that were characterized by the investigators as low to moderate in intensity. Lung function change after exercise was assessed using peak flow meters. Later studies in The Netherlands investigated the effects of heavy exercise levels of variable duration in amateur cyclists, but lung function was evaluated by spirometry (Brunekreef et al., 1994).

Although the designs varied somewhat, O_3 exposure levels were similar in most of the studies: in Houston, 15-min peaks while running varied from 4 to 135 ppb; in Tuxedo, 1-h O_3 levels ranged from 21 to 124 ppb; and in The Netherlands, 1-h maxima on study days ranged from 10 to 120 ppb. The Swiss study observed O_3 levels between 20 and 80 ppb during the exercise period.

The studies in adults (Selwyn et al., 1985; Spektor et al., 1988b; Brunekreef et al., 1994) involving fairly intense exercise yielded statistically significant mean slopes of ΔFEV_1 (i.e., FEV_1 after exercise minus FEV_1 before exercise) regressed on O_3 levels measured during exercise, whereas the studies in children did not. The mean slope observed in the Tuxedo study across all subjects was Δ1.35 mL/ppb ($\pm$ 0.35), but was reduced to Δ0.55 mL/ppb ($\pm$ 0.45) in the group of 10 runners who achieved the highest $\dot{V}_E$ values ($>$100 L/min) during exercise. The mean slope reported from the Houston study was similar to the latter number, Δ0.4 mL/ppb ($\pm$ 0.16). The large effect level observed in the Tuxedo study led Spektor et al. (1988b) to speculate that O_3 effects may have been potentiated by other pollutants such as acid aerosols; however, this phenomenon was not demonstrated analytically from the available acid monitoring data. In the Houston study, the O_3 effect became small and nonsignificant when temperature and RH were added to the model. Effects of temperature and O_3 on lung function were highly correlated and hard to separate in the Dutch amateur cyclists (Brunekreef et al., 1994), although adjustment for humidity did not change the findings. Given the available knowledge base on the independent effects of O_3 and temperature on lung function, it seems reasonable to interpret the results from these studies as demonstrating acute effects of low concentrations of ambient O_3 on lung function with moderate to heavy exercise. The predominantly negative findings

Table 7-17 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
1-h maximum O_3 concentrations during study ranged from 50 to 240 $\mu g/m^3$ (25 to 120 ppb). The highest 4-h average $PM_{2.5}$ level was 70 $\mu g/m^3$, the highest 4-h average sulfate concentration was 21 $\mu g/m^3$, the highest 24-h average NO_2 concentration was 51 $\mu g/m^3$. Temperature data were collected but levels were not reported.	The relationship between lung function change and O_3 exposures during outdoor exercise examined in a population of 83 children (43 girls, 40 boys; ages not given) in Wageningen, The Netherlands. Study covered the period from late May to mid-July, 1989. Lung function assessed using hand-held peak-flow meters before and after various outdoor, sports-training exercises lasting approximately 1 h. Change in PEFR regressed on O_3, $O_3 \times$ exercise duration, and temperature for each subject; and distribution of slopes were examined. Postexercise PEFR analyzed in relation to same and previous day 1-h O_3 maximum, and temperature. Analyses repeated in subsets of subjects with varying levels of correlation between O_3 and temperature during their series of exercise events.	For 55 children with at least four sets of before and after exercise peak flow measurements, the mean slope of the PEFR change on O_3 during exercise was 0.035 ($\pm$0.030) mL/s/$\mu g/m^3$. For 65 subjects with at least four postexercise measurements, the mean slope of PEFR on previous-hour O_3 was 0.080 ($\pm$0.023), which is statistically significant, but in the nonplausible direction. Adjustment for temperature resulted in negative mean slopes, but these are difficult to interpret because of the high statistical correlation between same-day O_3 and temperature ($r = 0.86$). Exercise events were of low intensity as compared with chamber studies and with the Tuxedo runners study (Spektor et al., 1988b). Significant exposures may have occurred prior to the exercise period. H^+ levels were low (< 5 $\mu g/m^3$) as measured simultaneously at three other nonurban sites in The Netherlands. The possibility of a physical effect of temperature on mini-Wright peak flow meter measurements was noted by authors.	Hoek and Brunekreef (1992) Hoek et al. (1993a)
1-h maximum O_3 concentrations during the exercise period ranged from 0.02 to 0.08 ppm. The highest pollutant levels measured during the study period were 0.13 ppm for 1-h mean O_3 and 70 $\mu g/m^3$ for the mean NO_2. No measurements of particulates were available.	The acute effects of ambient O_3 on lung function were examined in 128 Swiss children, aged 9 to 11 years, after 10 min of outdoor exercise on a cycle ergometer (60 W). Study covered the period from May through October 1989. Changes in lung function were regressed on current O_3 concentration, with or without adjustment for temperature, RH, and other factors.	Elevated O_3 levels were significantly associated with decreased peak flows (PEFR), but not FVC or FEV_1, after exercise. The average adjusted regression slope for PEFR was -1.14 mL/s/ppm. This corresponds to an average decrease in PEFR of -7.8 and -11.7 at 0.08 and 0.12 ppm O_3, respectively. The significant association for PEFR, but not FVC or FEV_1, is not consistent with other studies. The low O_3 levels and short exercise period raise a question of plausibility regarding the results.	Braun-Fahrlander et al. (1994)

Table 7-17 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Lung Function in Exercising Subjects[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
During exercise, the maximum hourly O_3 concentration averaged 87 $\mu g/m^3$ (0.04 ppm), with a range of 26 to 195 $\mu g/m^3$ (0.01 to 0.10 ppm). Temperature averaged 17.9 °C, with a range of 7.1 to 30.2 °C. NO_2 and SO_2 concentrations were low; 24-h averages were 26.0 and 7.5 $\mu g/m^3$, respectively. No measurements of PM_{10} were made.	The relationship between lung-function change and O_3 exposure was investigated in 23 amateur cyclists, 18 to 37 years of age, during training sessions and races between June 4 and August 18, 1981, in The Netherlands. Lung function was measured with spirometry 30 min before and between 10 and 60 min after cycling in rural locations. Acute respiratory symptoms were recorded in a diary before and after exercise. The difference between pre- and post-exercise lung function was regressed on the mean O_3 concentration during exercise. Time trend, pollen, ambient temperature, and absolute humidity were taken into account as potential confounders. Regression slopes were pooled, and mean and median slopes were calculated. The effect of O_3 during exercise on mean symptom scores was determined by a logistic regression model; all coefficients were converted to estimated odds ratios.	Lung function was negatively related to O_3 concentration during exercise; effects were stronger in midsummer than in the late summer. Mean regression coefficients were −1.16 ± 0.33, −0.52 ± 0.26, −2.96 ± 1.06, and 0.44 ± 0.46 mL/s/$\mu g/m^3$ for FVC, FEV_1, PEF, and $FEF_{25-75\%}$, respectively. For all but $FEF_{25-75\%}$, the mean coefficients were significantly different from zero. Adjustments for air humidity resulted in slightly more negative coefficients for FEV_1 and PEF. Acute respiratory symptoms of shortness of breath, chest tightness, and wheeze were positively related to O_3.	Brunekreef et al. (1994)

[a]See Appendix A for abbreviations and acronyms.

of the studies in children are more difficult to interpret, but may be related to the low exercise intensities achieved, low exposures, and, perhaps, associated O_3 tolerance that occurred prior to the exercise period under study or to some subtle effect of confounders on the peak flow measurements.

Lung Function in Daily Life Studies

This set of studies is characterized by the assessment of lung function, respiratory symptoms, and environmental factor associations in the course of people's daily lives. This section discusses only the lung function data from these studies. For logistical reasons, studies of this kind usually have involved either spirometry conducted at regular intervals (every 1 to 3 weeks) in schools (Kinney et al., 1989; Castillejos et al., 1992; Hoek et al., 1993b) or self-administered peak flow measurements in subjects of various ages over various periods (Vedal et al., 1987; Krzyzanowski et al., 1989). Although daily life studies have the worthwhile goal of characterizing air pollution effects on respiratory health in the real world, they suffer from significant exposure assessment uncertainties owing to the use of outdoor O_3 monitoring, the incomplete and variable penetration of O_3 indoors, and the preponderance of time spent indoors by study subjects. This problem is probably less severe for the studies involving schoolchildren, who often spend substantial time outdoors after school, when O_3 levels may be elevated. Indeed, three of the school-based studies have found statistically significant associations between lung function and previous-day O_3 levels (Castillejos et al., 1992; Kinney et al., 1989; Hoek et al., 1993b). Another difficulty in interpreting the results of these studies is the possible role of seasonal factors (e.g., pollens, epidemics of respiratory infection, changes in activity patterns) as potential confounders of the analyses.

In addition to these general limitations inherent in the study design, several of the studies summarized in Table 7-18 have other problems that limit their utility for assessing O_3 effects on lung function. The study of Vedal et al. (1987), although well conducted, took place from September through May, a period when O_3 levels generally are low, and other potential respiratory insults may dominate. The statistical significance of results from a study carried out in Tucson, AZ, is difficult to interpret because of the multiple statistical tests performed (Krzyzanowski et al., 1989).

The remaining studies, although subject to the general criticisms noted previously, provide suggestive evidence that ambient O_3 may play a role in short-term lung function declines among children engaged in their normal daily routines (Kinney et al., 1989; Castillejos et al., 1992; Hoek et al., 1993b). The Mexico City study of Castillejos et al. (1992) is especially noteworthy because of the novel observation of FEV_1 and $FEF_{25\ 75\%}$ decrements that were strongly related to O_3 levels averaged over 24 to 168 h previous to spirometry, but not to previous-hour O_3 levels. The strength of these associations (measured by the ratio of the regression slope to its standard error) increased steadily as averaging time increased. Ozone levels observed throughout this 6-mo study were high by U.S. standards; 1-h average O_3 concentrations in the hour preceding lung function measurements ranged from 14 to 287 ppb, with a mean of 99 ppb. The authors suggested these results may reflect an inflammatory response in the airways rather than the well-known acute physiological response. However, further studies will be necessary to test this hypothesis.

Panel Studies of Symptom Prevalence

Many field and epidemiological studies reviewed both in the last criteria document (U.S. Environmental Protection Agency, 1986) and in the previous section of this document

Table 7-18. Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Max O_3 (1-h) concentrations ranged from 3 to 63 ppb. Other ambient pollutants measured were NO_2, TSP, IP, RSP, FP, SO_2, and FP $SO_4^=$.	Lung function measured by spirometry for 154 children ages 10-12 years (90 males, 64 females) in Kingston and Harriman, TN. Spirometry done between 10 a.m. and 1 p.m. on up to 6 days at least 1 week apart during February to April 1981. Child-specific linear regression models of FVC, $FEV_{0.75}$, MMEF, and $\dot{V}_{max75\%}$ fit on 1-h O_3 max and 24-h FP and FP $SO_4^=$. Means ± SD of distributions of estimated child-specific slopes computed and tested for significance by *t*-test.	Significantly negative mean slopes on O_3 for all lung function variables. For example, mean slope of $FEV_{0.75}$ on O_3 was −0.99 mL/ppb (±0.36). Among regressions on FP and FP $SO_4^=$, only one statistically significant mean slope (i.e., positive mean slope of MMEF on FP). Results insensitive to outlier audits and inconclusive for sensitivity variation. Association between fitted slopes and individual characteristics not significant. Low O_3 levels raise plausibility questions.	Kinney (1986)[b] Kinney et al. (1989)[b]
1-h average O_3 concentrations in hour preceding spirometry ranged from 14 to 287 ppb, with mean of 99 ppb. No other pollutants measured. Temperature ranged from 3.9 to 27.8 °C. RH ranged from 18.9 to 92.3%.	Effects of O_3 on lung function examined during regular school hours in a group of 148 children (65 girls, 83 boys; ages 7-9) from three schools in Mexico City. Spirometry and symptom data (cough/phlegm) collected between 0800 and 1400 hours every 2 weeks over the period January through June 1988. To account for lung growth over the study period, residuals from lung function prediction equations were used in analyses. Analyses limited to 143 subjects with at least seven valid measurements. Schools were not air conditioned, and windows were usually open. Schools and subject residences all within 5 km of O_3 monitoring site. Associations between O_3 and lung function (FVC, FEV_1, and $FEF_{25-75\%}$) examined by computing the weighted mean of subject-specific regression slopes relating these variables. Various O_3 averaging times (from 1 h to 168 h) were tested. After analyzing population as a whole, regressions were repeated in subject subsets defined by sex, report of chronic symptoms, and maternal smoking. Overall regressions repeated with temperature and RH in model with O_3.	Only FVC had a statistically significant negative mean slope in relation to previous hour O_3 concentration (−0.059 ± 0.23 mL/ppb). This slope is approximately one order of magnitude lower than those observed in some camp studies. Both FEV_1 and $FEF_{25-75\%}$ had significant negative associations with O_3 averaged over the previous 24, 48, and 168 h. For example, the mean slope of FEV_1 on 48-h average O_3 was −0.592 ± 0.109 mL/ppb. The authors speculated that the FVC result reflects the acute, reversible effects of O_3 on one's ability to take a deep breath, whereas the FEV_1 and $FEF_{25-75\%}$ observations may reflect inflammatory effects of more prolonged O_3 exposures. It should be noted that both FVC and FEV_1 had significant negative slopes on 1-h maximum O_3 measured in the previous 24 h. Adjustment for temperature and RH diminished somewhat the associations between lung function and O_3. Associations between lung function decrements and O_3 exposure often appeared larger in children with chronic respiratory symptoms than in those without, and in children of mothers who were current smokers; however, these results were not statistically confirmed.	Castillejos et al. (1992)

Table 7-18 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Daily maximum 1-h O_3 concentrations on days prior to lung-function testing ranged from 7 to 206 $\mu g/m^3$ (3.5 to 103 ppb). Levels of other pollutants measured (SO_2, NO_2, PM_{10}, and aerosol H^+) were reported to be low during study. Ambient temperature (range: 5 to 31 °C) and some pollen data also were collected.	Associations between morning lung function and previous day O_3 examined during school in 533 children (ages 7-11) from seven schools in three towns in The Netherlands. Towns were selected without local pollution sources and with low levels of pollutants other than O_3. Study spanned the period from March through July, with lung-function measurements collected every 2-3 weeks. An overall time trend was fit to the lung- function data to account for lung growth. Data on FVC, FEV_1, PEFR, and $FEF_{25-75\%}$ analyzed in relation to previous-day 1-h maximum O_3 concentrations using subject-specific linear regressions followed by analysis of mean slopes. Intersubject variations in responsiveness to O_3 were tested via an F-test. The influence of chronic respiratory symptoms and other subject characteristics (e.g., age, sex) on O_3 responsiveness was examined. Models that included other pollutants were also considered.	Negative, usually statistically significant mean slopes seen for lung function regressed on previous-day 1-h maximum O_3 for the seven individual schools. Over all 533 subjects, mean regression slopes for FVC and FEV_1 were -0.20 ± 0.05 and -0.21 ± 0.04 mL/$\mu g/m^3$, respectively; and for PEFR and $FEF_{25-75\%}$ were -0.72 ± 0.22 and -0.45 ± 0.12 mL/s/$\mu g/m^3$, respectively. These coefficients may be doubled to convert to slopes in terms of parts per billion. The authors report that adding SO_2, NO_2, or PM_{10} did not materially change the O_3 slopes. There was evidence for inter-subject variation in O_3 responsiveness, but this variation was not statistically related to available subject characteristics data. Temperature data not included in models, perhaps due to high correlation with O_3. The lung function/O_3 relationships noted above are qualitatively similar to those reported in the 1988 Fairview Lake camp study and the Mexico City school children's study.	Hoek and Brunekreef (1992) Hoek et al. (1993b)
1-h maximum O_3 concentrations on PEFR measurement days ranged from 20 to 103 ppb. No other pollutants assessed, but ambient temperature data included.	Relationship between daily peak flow measurements and ambient O_3 concentrations in a population sample of 732 subjects (both adults and children) over 2-week periods during normal daily activities in Tucson, AZ. Peak flow assessed using hand-held peak-flow meters up to four times per day. PEFR measurements on initial 2 days for each subject dropped to avoid possible learning effects, leaving a series of up to 12 measurement days per subject. Population-pooled regression slopes computed for PEFR on 1- and 8-h average O_3 for children (ages $\leq$ 15) and adults (ages > 15), controlling for residual auto-correlations. Outcome measures included PEFR diurnal variability and afternoon PEFR levels. Besides O_3, potential explanatory variables included temperature, average time outdoors, acute respiratory infections, asthma, and environmental tobacco smoke exposure.	Significant positive associations observed between O_3 concentrations and PEFR diurnal variability; the effect magnitude was greatest in asthmatic subjects. In children only, noon PEFR was suppressed on days with higher O_3 levels. The uncertain relationship between central site O_3 levels and personal exposures in this southwestern community was not addressed. Although the statistical models employed were appropriate and well chosen, it appears that a substantial amount of exploratory data analysis was performed prior to selection of results to present in the paper, leading to uncertainties regarding the statistical validity of the hypothesis tests presented.	Krzyzanowski et al. (1989)

Table 7-18 (cont'd). Acute Effects of Photochemical Oxidant Pollution: Daily Life Studies of Lung Function and Respiratory Symptoms[a]

Pollutants/Environmental Variables	Study Description	Results and Comments	Reference
Means and range of max daily 1-h values: O_3 mean = 32.4 g/m^3, range = 0-129 g/m^3; SO_2 mean = 51.2 g/m^3, range = 18-176 g/m^3; NO_2 mean = 40.5 g/m^3, range = 12-79 g/m^3; CoH mean = 0.38 CoH units, range = 0.1-1.3 CoH units; temperature mean = 1.3 C, range = 22 to +22 C.	Follow-up study (September 1980 through April 1981) of pollutant-respiratory symptom relationships in subsets of children from 1979 Chestnut Ridge cross-sectional study of more than 4,000 elementary school children. Subsamples selected from six schools in study area with consistently higher levels of air pollution during previous 4 years. Subsamples (three) stratified by reported symptoms. One or more of following measures taken for 144 children: diaries, symptom questionnaire, spirometry. Telephone follow-up each 2 weeks on diaries, spirometry done at school, pollutants (including O_3) measured at one monitor (data from 17 monitors for SO_2 generally reflected in data at single monitor). Diary panel study covered 8 mo; successive PEFR spirometry studies of 9 weeks each done in respective groups of the three subsamples.	Relationships of maximum hourly SO_2, NO_2, O_3, and CoH and minimum temperature for each 24-h period to daily upper and lower respiratory illness, wheeze, and PEFR were evaluated using multiple regression models adjusted for illness occurrence or levels of PEFR on preceding day. No air pollutant was strongly associated with respiratory illness or with PEFR. Authors concluded that this study can best be interpreted as showing no acute effects of studied pollutants on respiratory symptoms or PEFR in children at levels lower than the current NAAQS, but also noted that conclusion must be tempered by relatively low levels of pollutants encountered and possibility of exposure misclassification.	Vedal et al. (1987)

[a]See Appendix A for abbreviations and acronyms.
[b]Cited in U.S. Environmental Protection Agency (1992).

reported results that indicated associations between ambient oxidant exposures and various measures of respiratory effects (e.g., irritative respiratory symptoms and acute pulmonary function decrements) in children and adults. The aggregation of individual studies provides reasonably good evidence for an association between ambient photochemical oxidants and acute respiratory effects and a database that is generally coherent, consistent, and biologically plausible. In addition, other studies of irritative symptoms in children and adults also were reported in the 1986 document. For example, Hammer et al. (1974) reported qualitative associations between ambient oxidant levels and symptoms such as eye and throat irritation, chest discomfort, cough, and headache at total oxidant levels greater than 0.15 ppm in young adults (nursing students). Wayne et al. (1967) reported a high correlation ($R^2 = 0.89$) between ambient total oxidant levels (1 h prior to competition) and impaired exercise performance (running time) in high school students during cross-country track meets in Los Angeles, CA. Symptoms were not measured, but Wayne speculated that chest discomfort from oxidant inhalation impaired exercise performance. Although results such as these are consistent with evidence from controlled human exposure studies, precise characterization of ambient pollutants and environmental conditions and rigorous statistical analyses were lacking in the studies. Thus, the primarily qualitative data from these and other studies were not satisfactory to provide quantitative conclusions about the relationship of ambient O_3 concentrations and acute respiratory illness.

Schwartz (1992), Schwartz and Zeger (1990), and Schwartz et al. (1988) reanalyzed the original diary data of student nurses reported earlier by Hammer et al. (1974) (Table 7-19). The nurses were told that the diaries were part of a prospective study of viral infections. Logistic regression models including time series analyses were used to control for autocorrelation effects that are frequently present in time series data. The reanalysis for daily prevalence rates of symptoms (Schwartz et al., 1988) confirmed that ambient oxidants were significantly associated with cough and eye discomfort. However, earlier reported associations between oxidants and headache or chest discomfort were not confirmed. Cough was the one symptom that showed an apparent threshold near 0.20 ppm total oxidants, which approximates the threshold value reported by Hammer et al. (1974). Further reanalysis of the diary data (Hammer et al., 1974) by Schwartz (1992) and by Schwartz and Zeger (1990) for the effects of air pollutants on the risk of new episodes of respiratory and other symptoms and on their durations revealed interesting findings. The mean plus or minus standard deviation (SD) level of oxidants was 0.102 ± 0.074 ppm. In logistic regression models, an increase in oxidant concentration by one SD (0.074 ppm) was associated with a 17% increased risk of chest discomfort and a 20% increased risk of eye irritation. These associations were highly significant ($p < 0.001$). In addition, photochemical oxidants were significantly ($p < 0.0001$) associated with the duration of episodes of cough, phlegm, and sore throat.

Krupnick et al. (1990) and Ostro et al. (1993) reanalyzed daily health data from over 5,000 children and adults living in the Los Angeles area during a 6-mo period (September 1978 to March 1979) (Table 7-19). The original study was reported by Flesh et al. (1982). The presence or absence of daily respiratory symptoms associated with daily exposure to ambient O_3 and other air pollutants was analyzed in a pooled, cross-sectional, time-series model. Krupnick et al. (1990) reported statistically significant effects of O_3 levels on daily reported respiratory symptoms in healthy nonsmoking adults, but not among smokers, children, and patients with chronic respiratory disease. Ostro et al. (1993) evaluated the daily reports of 321 nonsmoking adults and, using a logistic regression model,

Table 7-19. Acute Effects of Photochemical Oxidant Pollution: Symptom Prevalence[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Total oxidant, CO, SO_2, NO, and NO_2 measured; total oxidant concentrations reached episodic levels (maximum 1-h/day < 0.4 to 0.5 ppm); mean daily temperature was 71.8 °C.	Reanalysis of daily diary study of student nurses working and living at schools in Los Angeles (see U.S. Environmental Protection Agency, 1986, for details of Hammer et al., 1974). This series of papers reexamines the nurses' data using logistic regression models and time- series methods to account for serial correlation (autocorrelation) of symptoms on successive days. The effects of total oxidants on daily prevalence rates of symptoms, risks of developing new symptoms (episodes), and duration of episodes were analyzed.	Associations found between total oxidants and prevalence of cough and eye irritation, confirming part of findings of original study. Association with cough only at oxidant concentrations above approximately 0.20 ppm. Previously reported associations between oxidants and chest discomfort and headache (Hammer et al., 1974) not confirmed. Oxidants associated with increased risk (incidence) of chest discomfort and eye irritation and duration of episodes of cough, phlegm, and sore throat. Duration of symptoms showed concentration-response relationships even below 0.12 ppm. Findings suggest different effects of oxidants on symptom characteristics. Lack of daily particulate measurements, small number of subjects, and heterogeneous individual responses restrict quantitative interpretation of results. Lung function was not measured.	Schwartz (1992) Schwartz and Zeger (1990) Schwartz et al. (1988)
Daily O_3, NO_2, SO_2, and CoH and every sixth-day sulfates measured at one site (Azusa). 1-h daily maximum O_3 0.1 ppm, 7-h average O_3 0.07 ppm; sulfates 8.43 $\mu g/m^3$; maximum temperature was 22.4 °C.	Reanalysis of daily diaries completed during 181-day survey period (September 1978 to March 1979) by 756 children and 572 adults (Krupnick et al., 1990) and 321 nonsmoking adults (Ostro et al., 1993) living in Glendora, Covina, or Azusa, CA (see Flesh et al., 1982 for details). Presence or absence of 19 (upper and lower) respiratory and two nonrespiratory symptoms recorded daily. Presence or absence of symptoms analyzed in a pooled cross-sectional time-series model. Nonpollution factors, including sex, gas stove use, day of study, and a chronic disease indicator were included in final regression models used to measure effects of ambient air pollution. Logistic regression analyses for entire sample to determine effect of each pollutant on health endpoints. Lagged effects of each pollutant and effects in individuals (n = 74) without air conditioners and those with preexisting respiratory infection were analyzed.	Logistic regression model indicated significant associations between incidence of lower respiratory symptoms and healthy nonsmoking adults (but not among smokers, children, or patients with chronic respiratory disease); 1-h daily maximum O_3 levels (OR = 1.22, 95% CI of 1.11-1.34, for a 0.1 ppm change), 7-h average O_3 level (OR = 1.32, 95% CI of 1.14-1.52), and ambient sulfates (OR = 1.30, 95% CI of 1.09-1.54, for a 10 $\mu g/m^3$ change). CoH was significantly related to daily symptoms in children. Gas stove in the home was associated with lower respiratory tract symptoms (OR = 1.23, 95% CI of 1.03-1.47), as were the effects of O_3 in subgroups without residential air conditioner (OR = 1.24) and with preexisting respiratory infection (OR = 1.24). All the above increased risks were statistically significant ($p < 0.05$). Interpretation of results limited by selection of sample; undersampling of young adults; aggregation of symptoms of all severity levels into one measure; possible reporting bias; and absence of indoor exposure, aeroallergen, and lung function data.	Krupnick et al. (1990) Ostro et al. (1993)

[a]See Appendix A for abbreviations and acronyms.

found a statistically significant association between the incidence of lower respiratory tract symptoms and 1-h daily maximum and 7-h average O_3 levels (22 and 32% increased risk, respectively, with 0.1-ppm increase in O_3) and ambient sulfates (30% increased risk with a 10-$\mu g/m^3$ change). The lower respiratory tract effects of O_3 were greater in the subgroups with gas stoves, without residential air conditioners, and with preexisting respiratory infection. Interpretation of the results is limited by the selection of the sample for analysis; undersampling of young adults; aggregation of symptoms of all severity levels into one measure; possible reporting bias; and absence of indoor exposure, outdoor aeroallergen, and lung function data.

The results from the above panel studies suggest a modest but biologically plausible relationship between short-term exposure to ambient oxidants/O_3 and respiratory symptoms. The interpretation of these recent reanalyses is limited by several factors. Heterogeneous individual responses occur, and analyses of grouped data possibly may miss susceptible subgroups. The lack of specific measurements of O_3 and other pollutants (especially particles) and of personal exposure or risk variables (e.g., time-activity data) weaken the assessment of confounders and effect modifiers. In addition, the overall data analysis pertains to small and very selected samples that have uncertain representativeness to the general population.

Aggravation of Existing Respiratory Diseases

Prior epidemiological data on the effects of ambient O_3 levels in subjects with existing respiratory disease have been difficult to interpret due to methodological limitations (U.S. Environmental Protection Agency, 1986). Exacerbation of asthma and other health endpoints subsequently has been evaluated, and more recent studies have observed possible increases in symptom aggravation or changes in lung function of asthmatic subjects in relation to increased O_3 or total oxidant levels, as well as interactions between O_3 concentrations and temperature. However, no consistent pattern of findings for aggravation of symptoms or lung function changes has been reported for patients with other types of chronic lung disease. Some of the major issues in interpreting results from studies of respiratory exacerbations have been inadequate sample size and characterization of the study subjects, lack of information on the possible effects of medications, the absence of records for all days on which symptoms could have occurred, inadequate interpretation of the clinical significance of measured changes, the role of confounders and effect modifiers (e.g., temperature, humidity, particles, aeroallergens), and personal or group characterization of indoor-outdoor exposures. For example, Whittemore and Korn (1980) and Holguin et al. (1985) found small increases in the probability of asthma attacks associated with previous attacks, decreased temperature, and incremental increases in oxidant and O_3 concentrations. Lebowitz et al. (1982, 1983, 1985) and Lebowitz (1984) reported effects in asthmatics, such as decreased PEFR and increased respiratory symptoms, that were related to the interaction of O_3 and temperature. None of these studies adequately assessed possible effect modification by other pollutants, particularly inhalable particles, which may have independent effects.

Epidemiological studies published since the 1986 criteria document (U.S. Environmental Protection Agency, 1986) have attempted to control for many methodological issues (e.g., with [1] better estimates of exposure to pollutants [as well as O_3] and environmental variables that can confound or modify responses, [2] serial measurements of pulmonary function for determining correlations with pollutants and other environmental variables, [3] better biomedical characterization of cohorts, and [4] more robust analytical

approaches that control for autocorrelation of environmental variables and health responses). Recent studies generally have provided further evidence that supports a relationship between ambient O_3/oxidant concentrations and respiratory morbidity in asthmatic subjects (Table 7-20).

Gong (1987) studied the relationship between air quality and respiratory status of 83 asthmatic subjects living in a high-oxidant area of Los Angeles County. The study covered February to December 1983, but data analyses were limited to a 230-day period (April 15 through November 30) because of staggered entry of subjects into the study and the high frequency of missing or incomplete data encountered in the earlier part of the study period. Regression and correlation analyses between O_3 and average symptom scores, asthma medication index (AMI), and day and night PEFR across subjects showed weak, nonsignificant relationships. These daily outcome variables were compared across days with maximum 1-h-average O_3 in three ranges: <0.12 ppm, 0.12 to 0.19 ppm, and >0.20 ppm; "no statistical or clinical significance was detected." Individual exposures and activity patterns were not estimated in these two analyses. Multiple regression analyses also indicated the lack of a significant overall relationship between O_3 (and their independent variables) and respiratory status, despite the use of lagged variables and the inclusion of other pollutants, meteorological variables, aeroallergens, and AMI. Total suspended particles directly affected PEFR, but the relationship was not consistent in the analysis. Aeroallergens showed significantly negative relationships to respiratory variables, but only the effect of certain molds was considered clinically relevant. Temperature and humidity showed no significant effect on the respiratory variables on this study.

Although there was no significant overall effect of O_3 on respiratory variables in the 83 asthmatic subjects, multiple regression analysis of subjects whose O_3 coefficients on various days were in the top quartile for dependent variables (respiratory measures) showed significant and consistent effects of O_3 on Day t and the previous day (Day t $\square$ 1). Multiple regression testing of subsets for associations of symptom score or day or night PEFR on the same-day O_3 and previous-day values of the same responses showed highly significant O_3 coefficients for all three respiratory measures.

The clinical significance of responses in symptom scores and day and night peak flow was evaluated for all subjects by individual regression analyses. No subject had evidence of significant worsening of symptoms attributable to O_3 during the study. Adult subjects with high scores in fatigue, hyperventilation, dyspnea, congestion, and rapid breathing in the Asthma Symptom Checklist had more negative slope coefficients for O_3 than subjects with low-to-moderate scores on the checklist. "Responders" (statistically identified by multiple regression analysis) scored consistently higher in the factors representing fatigue, hyperventilation, and rapid breathing. The higher scores of these responders, however, "were apparently not associated with differences in ambient O_3 concentrations since the test scores were similar during relatively low (first test) and high (second test) O_3 days. The significance of the psychological results is unclear at this time" (Gong, 1987).

Lebowitz et al. (1987) performed a time series analysis to evaluate daily respiratory responses to outdoor and indoor air pollutant and aeroallergen exposures in potentially sensitive adults living in a dry climate (Tucson, AZ). Daily symptoms and PEFR were recorded in well-characterized groups of asthmatics, allergic subjects, patients with chronic airways obstruction, and asymptomatic healthy controls (total sample size of 204) over 2 years. Daily diaries included acute symptoms, medication use, and doctors' visits.

Table 7-20. Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Air pollutant measurements for April to November 1983 used in statistical analyses. Daily maximum of NO_x, SO_2, CO, THC less than California standards or NAAQS. $SO_4^{=}$ > 25 $\mu g/m^3$ on 4 days; TSP > 100 $\mu g/m^3$ on 78% of days with data. Daily maximum 1-h average O_3 concentrations (from continuous monitoring) = 0.01-0.11 ppm on 103 days, 0.12-0.19 ppm on 65 days, 0.2-0.34 ppm on 60 days, and 0.35-0.38 ppm on 3 days. Outdoor aeroallergens sampled with Roto-Rod: spores, pollens, grasses, molds, miscellaneous debris; all generally low except for group of common molds (rusts, smuts, mushroom) present in thousands per square centimeter on sampler. Mean (±SD) daily temperature at 1 p.m. during 200 days: 26 ± 11 °C, range 13-41 °C; 128 days with ≥ 24 °C.	Effects of pollutants and other environmental variables on respiratory symptoms and PEFR evaluated in 11-mo population study of asthmatics living in high-O_3 area (Glendora) of Los Angeles County, CA. Detailed questionnaires given at outset on medical/occupational histories and personal factors, including general activity patterns; psychological testing (Asthma Symptom Checklist, State-Trait Anxiety Inventory, etc.) also given, once during good air period and once during smoggy period. Lung function (spirometry) and bronchodilator responses measured at outset in all subjects. Daily diaries (checked 2×/week), mini-Wright peak flow meters, and Nebulizer Chronolog attached to metered-dose inhaler used to record symptoms, day and night PEFR, and medication use, respectively. Multiple regression analyses for overall group; then subsets (two groups of "responders") analyzed separately and compared with rest of cohort.	Eight of 91 subjects completing study (of 109 recruited) showed no variability in asthma status during the 230-day study. Respiratory status of final study population (n = 83 with generally mild or stable asthma), as a whole, not related, either clinically or statistically, to maximum 1-h average O_3 on Days t, t-1, t-2, or t-3 for any respiratory variable even when adjusting for medication use, symptoms, and PEFR on Day t-1. Subset analyses showed association of O_3 with symptoms and with day and night PEFR in subjects in top quartile for respiratory measures, but association did not follow a consistent relationship with ambient O_3 concentrations. $\dot{V}_E$ levels during outdoor time not estimated. Outcomes not related to time outdoors vs. indoors or to outdoor time on "clean" vs. "smoggy" days. Subsets ("responders") differed from rest of cohort mainly in scores of Asthma Symptom Checklist for factors representing fatigue, hyperventilation, and rapid breathing, but there was no difference in responders between clean and smoggy periods. Aeroallergens from maple, oak, beech, and elm trees showed significant (and clinically relevant) relationships to respiratory variables. Exposure assessment limited by outdoors-only monitoring and lack of time-activity data.	Gong (1987) Gong et al. (1985)
Hourly outdoor O_3, CO, NO_2, and TSP measured from three stations. Hourly maxima used for O_3 and CO; daily CO and NO_2 derived as weighted measures for each cluster sampling site, and daily values were used. Sample of homes monitored inside and outside for particulates and gases and evaluated for housing characteristics (e.g., gas stove usage). Meteorological variables measured daily. Temperature data not reported.	Effects of outdoor and indoor air pollutants and aeroallergens evaluated in a 2-year study of 22 subjects with asthma, 33 with airway obstructive disease, 30 atopics, and 14 normals living in an arid environment (Tucson, AZ). Subjects part of a community population sample of 117 families (see U.S. Environmental Protection Agency, 1986, for details of Lebowitz et al., 1982, 1983, 1985; Lebowitz, 1984) and had well-characterized symptoms, medication use, lung function, methacholine response (in a subsample), and immunological status. Daily diaries (acute symptoms, medication use, and doctors' visits) and daily PEFR (2×/day) performed for 3 mo, 2-4×/study period. Duration of time spent outdoors recorded. Spectral time series analyses used to evaluate each respiratory response variable for periodic tendencies and covariance (dependent and independent) functions as processes in time in the different groups.	Asthmatics had greatest number of respiratory complaints, which were related to the presence of gas stoves, active smoking, humidity, and temperature. O_3 was associated with peak flow and temperature (late spring), wheeze (Day t-3 with humidity), and productive cough (Day t-2). O_3 (Day t-3) was related to productive cough during the summer in allergic subjects. Outdoor gases and meteorological variables significantly related to symptoms and PEFR, both independently and as effect modifiers. No significant O_3 effect in patients with obstructive disease or in normals. Small number of subjects and study days and lack of indoor NO_2 and PM_{10} measurements, measured pollutant values, and effect estimates limit quantitative interpretation of study.	Lebowitz et al. (1987)

Table 7-20 (cont'd). Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Outdoor O_3 levels measured hourly by three stations and maximum 1- and 8-h average values were used to represent O_3 levels for all subjects on a given day. For each day of the study, the mean of the maximum 8-h O_3 average for the 4 preceding days was calculated to be an index of cumulative exposure. PM_{10} was measured daily at one station. Mean ± SD of maximum 1-h O_3 concentrations was 0.055 ± 0.014 ppm (range: 0.015-0.092 ppm), moving average maximum 8-h O_3 levels were 0.046 ± 0.013 ppm (0.09-0.082 ppm). Maximum daily outdoor temperature was 87 °F (30 °C) per person-day, maximum PM_{10} was 187 μg/m³ (mean 42 μg/m³).	Temporal effect of ambient O_3 concentration on PEFR during 30-mo study period in 287 children (13% physician-diagnosed asthmatics) and 523 nonsmoking adults (9% asthmatics) in the Tucson community population sample. Mini-Wright peak flow meters used four or fewer times per day but only for 2-week periods, and only one meter was assigned/household. Children's tests were supervised by adult, and initial 2 days of observation were eliminated from analysis. Symptoms from daily diaries were also used in analysis. Random-effects longitudinal model was used for analyses to account for autocorrelation of PEFR values. Multifactorial ANCOVA was used to analyze day-to-day changes in daily average PEFR and symptom prevalence rates (the dependent variables) in relation to 8-h O_3 values on the same day and previous days (lags of 0 and 1).	Analyzed PEFR data limited to at least 12 measurements for at least 6 days in 78% of children and 74% of adults. Noon PEFR in nonasthmatic and asthmatic children was lower with higher 1-h maximum O_3 levels: 11.9 L/min/0.1 ppm O_3 ($p < 0.05$) and 31.0 L/min/0.1 ppm O_3 ($p < 0.1$), respectively. Effect of 8-h O_3 mean on evening PEFR seen only in asthmatic children, possibly reflecting a cumulative O_3 response during course of day. Among adults, evening PEFR was decreased in asthmatics who spent more time outdoors on days with higher O_3 concentrations (C × T effect). The ANCOVA model showed significant interactive effects of O_3 × temperature × PM_{10} on daily average PEFR. Daily rates of allergic-irritant symptoms increased with the maximum 8-h O_3 average (>0.056 ppm) on the previous day and increased more with interactions of O_3 × temperature × PM_{10}. Missing PEFR data, possible overestimation of outdoor O_3 exposure, large variability of responses in asthmatics, medication use on days with high O_3 levels, relatively low O_3 levels, and uncertain effects of indoor and outdoor allergens and respiratory infections limit interpretation.	Lebowitz et al. (1991) Krzyzanowski et al. (1992)

Table 7-20 (cont'd). Aggravation of Existing Respiratory Diseases by Photochemical Oxidant Pollution[a]

Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
Hourly O_3, twice daily (9:00 a.m. and 9:00 p.m.) acidic aerosols (sulfates, SO_4, and H^+), and pollen counts were measured on site. Hourly temperature, RH, and O_3 measured from nearby monitors. In 1991, pollution levels increased daily until Day 5, when maximum 1-h O_3 reached 0.154 ppm and daytime H^+ and sulfate levels were 245 nm/m³ and 26.7 μg/m³, respectively. In 1992, air quality was better (e.g., the highest daily 1-h maximum O_3 was 0.063 ppm). Temperature data not reported.	Effects of ambient summertime haze air pollution on asthmatic children (ages 7-13) attending 1-week asthma camp in Connecticut River Valley were evaluated during June 1991 (n = 50) and 1992 (n = 55). PEFR and symptoms (2×/day) and number of as-needed (p.r.n.) inhaled bronchodilator treatments given by on-site physician during each study day were recorded. Correlations between health outcomes and air pollutants were performed.	In 1991, daily total number of p.r.n. treatments highly correlated ($r > 0.80$) with maximum O_3, SO_4, daytime H^+, and maximum temperature, but only SO_4 ($r = 0.97$) and H^+ ($r = 0.985$) were significant ($p < 0.05$) and remained so after temperature was included in the analysis. Daily pollen counts were not associated with treatments p.r.n. Afternoon chest symptoms (cough, phlegm, and wheeze) and changes in morning-afternoon PEFR values (excluding children given medication) were significantly correlated ($p < 0.05$) with O_3 and H^+, respectively. Scheduled medications did not apparently provide a protective effect ($X^2 = 3.25$, $p = 0.067$), although the failure to achieve statistical significance is not unexpected given the small sample size. In 1992, change in PEFR (magnitude not reported), chest symptoms, and the fewer daily exacerbations (maximum 27 vs. 37 in 1991) were not significantly correlated with pollution, pollen, or temperature. Only sore throat, runny nose, and eye irritation were correlated with pollen counts. Although the data are only in preliminary form, the 1991 results appear consistent with an effect of summertime haze air pollution on PEFR, chest symptoms, and asthma exacerbations. The 1992 results are consistent with less health effects owing to cleaner ambient conditions. Small number of subjects and study days and lack of results for other pollutants limit the interpretation of the studies.	Thurston et al. (1995)

[a]See Appendix A for abbreviations and acronyms.

A sample of homes was evaluated for environmental characteristics and was monitored indoors and outdoors at the home for gases and particles, in addition to regional stationary outdoor monitors. Asthmatics showed the most respiratory responses. Outdoor O_3 levels were significantly ($p < 0.05$) related to wheeze, productive cough, and peak flow (late spring) in the asthmatic group. Statistical interactions between O_3 and smoking, presence of a gas stove, maximum temperature, and minimum humidity ($R^2 = 0.49$) were found. The other groups did not demonstrate an O_3 effect, except for the atopic group, which had increased summertime productive cough related to O_3 levels. Thus, these results indicate an O_3 effect on asthmatics and that statistical interactions between O_3 and other environmental factors are significantly related to symptoms and peak flow. On the other hand, the results are largely descriptive and qualitative without adequate effect estimators.

A subsequent analysis of the same community population sample in Tucson (Lebowitz et al., 1991; Krzyzanowski et al., 1992) evaluated the temporal relationship between PEFR and ambient O_3 in 287 children and 523 nonsmoking adults. During part of the study period, ambient particles with a MMAD of 10 μm or less (PM_1) were collected daily at one monitoring station. A random-effects longitudinal model and multifactorial analysis of covariance were used for analyses. During the study period, the maximum ambient O_3 concentrations were relatively low (i.e., the 1-h maximum never exceeded 0.092 ppm). In children, noon peak flows were decreased on days when there was a high O_3 concentration. Children with physician-confirmed asthma experienced the greatest decrease in noon peak flow. Evening peak flow also was significantly related to O_3 in children, especially asthmatic children, suggesting a cumulative O_3 response during the course of the day. Among adults, evening peak flows were decreased in asthmatics who spent more time outdoors on days when O_3 levels were high. After adjustment for covariates, significant statistical interactions of 8-h O_3 levels with PM_1 and temperature on daily PEFR were found. There was a significant increase in allergic-irritant symptom rates related to prolonged exposure to O_3 (maximum 8-h average on the previous day and the interactions of O_3, temperature, and humidity). The study had some methodologic problems (e.g., missing daily PEFR data in many subjects, lack of information about specific hours spent outdoors, medication usage, and relatively low O_3 levels during the study period). Nonetheless, the data analyses, control of confounders, and overall exposure assessment strengthen the conclusions of the study: the respiratory response to O_3 is acute, occurs more often in asthmatics, and increases as temperature and PM_1 increase.

The respiratory effects of ambient O_3 and other coexisting pollutants were evaluated during a 1-week asthma camp in the Connecticut River Valley in June of 1991 and 1992 (Thurston et al., 1995). Each child (age 7 to 13 years) participated in the same daily activities all week. Peak flow and symptoms were recorded twice a day, as well as the number of as-needed (p.r.n.) treatments of inhaled bronchodilator administered by an on-site physician during each day (each representing an exacerbation of asthma). Hourly measurements of O_3 and twice daily samples of acidic aerosols (sulfates [SO_4] and hydrogen ions [H^+]) were collected. The results indicate a strong association between the ambient air pollution mix and the occurrence of asthmatic exacerbations in children. During 1991, pollution levels progressively increased until Day 5, when the 1-h maximum O_3 concentration reached 0.154 ppm, and the daytime (9:00 a.m. to 9:00 p.m.) H^+ and SO_4 concentrations were 254 nm/m^3 and 26.7 μg/m^3, respectively. The correlations of the daily total number of p.r.n. treatments required with daily maximum O_3, daytime SO_4 and H^+, and maximum temperature were all high ($r > 0.8$), but only SO_4 ($r = 0.97$) and H^+ ($r = 0.98$) were significant ($p < 0.05$) given

the small number of days involved. Afternoon symptoms (cough, phlegm, and wheeze) and morning-afternoon change in PEFR (without medication) were significantly correlated ($p < 0.05$) with O_3 and H^+, respectively. During 1992, the local air quality was better (e.g., the daily 1-h maximum O_3 concentration was only 0.063 ppm). There were fewer asthmatic exacerbations (maximum of 27 versus 37 in 1991), and they were not significantly correlated with pollution, pollen, or temperature. Pollutants were not significantly correlated with symptoms or PEFR. Overall, the 1991 data indicate a coherence in the associations of summertime haze air pollution with peak flow, chest symptoms, and asthma exacerbations in children. The lack of correlation during 1992 likely was due to the improved air quality and indirectly supports the results of the previous year. An adequate interpretation of these preliminary results is limited by the small number of subjects and study days and the lack of results for other pollutants. These camp studies remain to be reported in more detail.

The above epidemiological studies have generally supported a direct association between ambient O_3/oxidant concentrations and acute respiratory morbidity in asthmatics. The recent studies have strengthened their conclusions by improvements or new approaches in the estimations of O_3 exposure, confounders, and effect modifiers; characterization of the subjects and serial measurements of their responses; and analytical approaches. Thus, the aggregate results can be viewed as biologically and temporally plausible, consistent, and coherent to some extent; however, some methodological problems persist. The studies share certain deficiencies such as small numbers of subjects (which may reduce statistical power) and the lack of time-activity measurements and significant data about individual responses and their distribution. The independent effect of ambient O_3, as estimated by statistical models in epidemiological studies, is difficult, at best, to clearly differentiate from those of copollutants because O_3 (or another pollutant such as H^+) may be acting only as an indicator of the toxic potency of the ambient mixture of pollutants. This, in combination with measurement error and uncontrolled associations with other factors, complicates analytical findings about the relationships among components of an ambient mixture and may not accurately disentangle the effects of O_3 in a biologically appropriate fashion.

7.4.1.3 Aggregate Population Time Series Studies

Aggregate population, or "ecological", time series studies are epidemiological investigations in which the associations between air pollution and human health outcomes are evaluated over time in the population as a whole (e.g., with respect to deaths per day in a given city) and for which outcomes and exposures are not matched for the individuals within the population. Indeed, aggregate population time series studies of extreme air pollution episodes have provided some of the clearest evidence of the adverse effects of air pollution on humans. For example, during the historic December 1952 London Fog episode, in which extremely high sulfur oxide and PM air pollution levels were experienced, total mortality in Greater London rose from roughly 300 to 900 deaths/day, and acute respiratory hospital admissions rose from 175 to 460/day (United Kingdom Ministry of Health, 1954). At more routine levels of air pollution, any effects of air pollution are necessarily less obvious, and, as shall be discussed below, methodological issues exist as to the proper analysis and interpretation of such aggregate population time series data.

The previous criteria document (U.S. Environmental Protection Agency, 1986) discussed several methodological issues with regard to the epidemiological studies of O_3 and photochemical oxidants available at that time. Limitations identified included interferences by

or interactions with other pollutants and meteorological factors in the ambient environment; lack of comprehensive exposure issue assessments, such as individual activity patterns and evaluation of pollutant monitor appropriateness; difficulty in identifying the responsible oxidant species; and inadequate characterization of the study population. However, most of these criticisms are not relevant to time series studies. For example, because the same population is being followed from day to day, the study population acts as its own control, obviating the need for a detailed population characterization. Also, central site monitoring data can be useful in these studies for two reasons: (1) although O_3 concentrations can vary spatially within an airshed, they usually are highly correlated across sites over time, so that correlational time series studies are not as dependent on detailed exposure assessments as are, for example, cross-sectional studies; and (2) if the ultimate use of these studies is to be included as criteria for ambient standards, the attainment of which is evaluated at central monitoring stations, then these are the data most relevant for analysis. However, the usually high correlation of the 1-h daily maximum O_3 concentration with other averaging times (e.g., an 8-h average daily maximum) inhibits the ability of such time series studies to discriminate the most biologically relevant O_3 averaging time.

Of the concerns raised by the previous criteria document regarding epidemiological studies in general, the most relevant to time-series studies is the potential for other serially correlated environmental factors (e.g., temperature or other pollutants) to confound the unique identification of O_3 as a critical causal factor in any environmental health effects identified via time series analyses of aggregate population data. As discussed by Thurston and Kinney (1995), either upward or downward bias in the O_3 effect estimate can result if the model is misspecified. In the case of underspecification, if another environmental factor that is both serially correlated with O_3 over time and also may be causally related with the effect under consideration (e.g., temperature stress effects on mortality) is excluded from the analysis, then O_3 may "pick up" that environmental factor's effect in the model, biasing the O_3 coefficient upward. Conversely, the inclusion of variables in the model that are correlated with O_3 concentrations but are unlikely to be causally related to the health outcome (e.g., the inverse of wind speed) results in model overspecification, which may bias the O_3 coefficient downward. Only variables that are biologically plausible should be included in a time-series model, and intercorrelations of the model coefficients should be low if model specification bias is to be minimized.

One aspect of evaluating time series epidemiologic studies of the health effects of air pollution that was not raised directly by the previous criteria document but which can be crucial to proper interpretation is the statistical question of how each study has addressed the potentially confounding influences of long-wave (e.g., seasonal) variations in the health outcome data. The seasonality of morbidity and mortality was mentioned explicitly in Hippocrates' treatise on "Airs, Waters, and Places" and has been studied over the years (Hechter and Goldsmith, 1961). In respiratory diseases such as asthma, this seasonality of admissions is very common, due in part to the multifactorial nature of these diseases. For example, spring and fall increases in pollen and winter influenza epidemics superimpose long-wave cycles on the day-to-day variations in respiratory hospital admission rates. Such long-wave cycles need to be addressed as part of any time series analysis for two reasons: (1) they result in strong autocorrelations that violate the underlying assumptions of most statistical approaches used to analyze such data; and (2) their inclusion can lead to misleading conclusions (i.e., confounding), in that the long-wave relationships would likely obscure the

acute (i.e., short-wave) effects being evaluated. The need to address seasonal cycles in respiratory disease time series data in order to avoid spurious long-wave dominated correlations has long been recognized (e.g., Ipsen et al., 1969) but too often has been ignored or inadequately addressed in the published literature. Autocorrelation, although often contributed to by seasonal cycles in the data, can be introduced by other causes as well. For example, Lipfert (1993) noted the need for hospitalization studies to take into account both weekly and seasonal temporal patterns in the data. Thus, an important criterion for the evaluation of aggregate population time series studies of the acute morbidity and mortality effects of O_3 is whether or not the authors have appropriately addressed all long-wave periodicities in the data as part of their analysis.

There are a variety of statistical approaches available to address all long-wave confounding in time series analyses, each having advantages and disadvantages. The primary goal in invoking such procedures is to eliminate the long-wave autocorrelation "noise" in the data without inadvertently removing any O_3-related health effects "signal" at the same time. In particular, steps that address autocorrelation in the model but also remove or explain short-wave variance in the health outcome variable of interest (e.g., by applying prefilters to the series that affect periodicities down to a few days or by analyzing the residuals from prior regressions of the outcome variable on "control" variables that are correlated with O_3, such as temperature) carry with them the risk of also removing short-wave associations of interest before the actual analysis has begun. Furthermore, although there are standard regression diagnostics available to determine whether autocorrelation remains a significant problem (e.g., the Durbin-Watson statistic), no such check exists to determine whether the autocorrelation removal methods also have inadvertently removed an O_3-health effects association of interest. Thus, although steps must be taken in time series analyses to address the potentially large biases resulting from long-wave (e.g., seasonal) autocorrelations, care must be taken not to also remove the signal of interest when dealing with the autocorrelation problem.

Emergency Room Visits and Hospital Admissions

Many investigators have evaluated the associations between hospital emergency room visits or hospital admissions and air pollution. Hospital admissions are far more common (as counts per day) than, for example, mortality, thereby providing greater statistical reliability and avoiding the distributional complications that may be presented by low counts. Also, admission to the hospital is a well defined endpoint, having the desirable feature that every patient must have been seen by a physician and deemed sick enough to require hospitalization. Emergency room (ER) visits provide larger daily counts, but are not necessarily as severe an endpoint. In a well-designed study in Quebec, Canada, hospital admission diagnosis at discharge was found to be very reliable, with the study confirming the classification of respiratory admissions in general 92% of the time, and asthma admissions 95% of the time (Delfino et al., 1993). Similarly, a study by Martinez et al. (1993) of respiratory emergency room admissions in Barcelona, Spain, during 1985 to 1989 concluded that identification of asthma admissions was highly reliable, as was the discrimination of asthma and COPD diagnoses. Daily series of hospital admissions thus represent an especially useful research resource for the investigation of the human health consequences of O_3 exposure.

Hospital admission and ER visit studies that have considered O_3 associations are summarized in Table 7-21. In the previous criteria documents (U.S. Environmental Protection Agency, 1978, 1986), such studies were found to give inconsistent results for

Table 7-21. Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.11 to 0.28 avg max 1 h during low and high periods, respectively	Oxidant	Comparison of admissions to Los Angeles County Hospital for respiratory and cardiac conditions during smog and smog-free periods from August to November 1954.	No consistent relationship between admissions and high-smog periods; however, statistical analyses were not reported. Clear seasonal trends in admissions (increasing from summer to winter) not addressed.	California Dept. of Public Health (1955[b], 1956[b], 1957[b])
0.12 avg concentration 6 a.m. - 1 p.m.	Oxidant	Respiratory and cardiovascular admissions to Los Angeles County Hospital for residents living within 8 mi of downtown Los Angeles between August and December 1954.	Inconclusive results; partial correlation coefficients between total oxidants and admissions were variable. Method of patient selection was not given. Other pollutants were not considered. Seasonal trend not addressed.	Brant and Hill (1964)[b] Brant (1965)
(Not reported)	Oxidant	Admissions of Blue Cross patients to Los Angeles hospitals with > 100 beds between March and October 1961; daily average concentrations of oxidant, O_3, CO, SO_2, NO_2, NO, and PM by Los Angeles air pollution control districts.	Correlation coefficients between admissions for allergies, eye inflammation, and acute upper and lower respiratory infections and all pollutants were statistically significant; correlations between cardiovascular and other respiratory diseases were significant for oxidant, O_3, and SO_2; significant positive correlations were noted with length of hospital stay for SO_2, NO_2, and NO_x. Correlations were not significant for temperature and RH or for pollutants with other disease categories. Reported seasonal variations in admissions and pollution not addressed.	Sterling et al. (1966[b], 1967[b])
(Not reported)	Oxidant	Admissions for all adults and children with acute respiratory illness in four Hamilton, Ontario hospitals during the 12 mo from July 1, 1970, to June 30, 1971; city-avg pollution monitoring for O_x (KI), SO_2, PM, CoH, CO, NO_x, HC, temperature, wind direction and velocity, RH, and pollen.	Correlation found between admissions and an air pollution index for SO_2 and CoH; negative correlation between temperature and admissions; and nonsignificant negative correlations found with concentrations of O_x (KI). However, clear, long-wave trends (e.g., seasonality) not addressed.	Levy et al. (1977)[c]
(Not reported)	O	Emergency room visits for cardiac and respiratory disease in two major hospitals in the city of Chicago from April 1977 to April 1978; 1-h concentrations of O_3, SO_2, NO_2, NO, and CO from an EPA site close to the hospital, 24-h concentrations of TSP, SO_2, and NO_2 from the Chicago Air Sampling Network.	No significant association between admissions for any disease groups and O_3, CO, or TSP; SO_2 and NO accounted for part of the variation of ER visits for respiratory and cardiovascular admissions. However, the analysis has a lack of control for confounding, possible unaddressed seasonality in admissions (time series not shown), and model overspecification (e.g., use of wind speed).	Namekata et al. (1979)[c]
0.07 and 0.39 avg max 1 h during low and high periods, respectively	O_3	ER visits and hospital admissions for children with asthma symptoms during periods of high and low air pollution in Los Angeles from August 1979 to January 1980; daily maximum hourly concentrations of O_3, SO_2, NO, NO_2, HC, and CoH; weekly maximum hourly concentrations of $SO_4^=$ and TSP; biweekly allergens and daily meteorological variables from regional monitoring stations.	Asthma positively correlated with CoH, HC, NO_2, and allergens on same day and negatively correlated with O_3 and SO_2; asthma positively correlated with NO_2 on Days 2 and 3 after exposure; correlations were stronger on Day 2 for most variables; nonsignificant correlation for $SO_4^=$ and TSP. Monthly admissions and pollution data indicate strong seasonality, which is not accounted for. This results in (seasonally driven) positive correlations with CoH, HC, and NO_2 and negative correlations with O_3.	Richards et al. (1981)[c]

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.03 and 0.11 avg max 1 h for low and high areas, respectively	Oxidant	Daily hospital ER admissions in four Southern California communities during 1974 and 1975. Max hourly average concentrations of oxidant, NO_2, NO, CO, SO_2, CoH; 24-h average concentrations of PM and $SO_4^=$; and daily meteorological conditions from monitoring sites 8 km from the hospitals.	Admissions significantly associated with oxidant and temperature in all locations. Long-term trends and day-of-week effects appropriately controlled, but not seasonality. Path-analysis-guided regression used to discriminate among the intercorrelated pollutant and meteorological factors, indicating O_3 to be most important only at the highest O_3 site. However, lack of catchment area population figures and inadequate seasonality adjustments prevent quantitative use of results.	Goldsmith et al. (1983)[c]
0.03 to 0.12 avg of max 1-h/day for 15 stations	O_3	Admissions to 79 acute-care hospitals in Southern Ontario for the months of January, February, July, and August in 1974 and 1976 to 1983. Hourly average concentrations of particulate (CoH), O_3, SO_2, NO_2, and daily temperature from 15 air sampling stations within the region.	Excess respiratory admissions most strongly associated (p 0.001) with O_3, sulfate, and temperature during July and August with 24- and 48-h lag. No such associations exist for nonrespiratory (control) diseases. Seasonality minimized by selection of narrow study period, and day-of-week effects controlled. $SO_4^=$ and O_3 highly intercorrelated ($r = 0.65$), making effect discrimination difficult. A lack of independent regression coefficients prevents quantitative application of results.	Bates and Sizto (1983, 1987, 1989) Bates (1985)
0.025 to 0.075 3-mo avg of monthly means from all city sites	O_3	Analysis of quarterly hospital admission rates for childhood asthma in Hong Kong during 1983-1987 ($n = 19$). Quarterly means of SO_2, NO_2, NO, O_3, TSP, and RSP considered.	Concludes that asthma is negatively correlated with SO_2, but not with O_3. However, analysis uses quarterly means and lacks seasonality controls.	Tseng and Li (1990)
0.001 to 0.085 mean 1-h max O_3 (avg of 11 sites)	O_3	Analysis of emergency room visits, by cause, to acute care hospitals in the Vancouver, BC, area July 1984 to October 1986. SO_2, NO_2, O_3, SO_4, and temperature considered.	Summer (May to October) total emergency (but not respiratory) visits significantly correlated with temperature and O_3. Day-of-week effects addressed. Seasonality reduced by study period selection, but opposing within season cycles in asthma visits and O_3 not addressed, which may have weakened reported O_3-respiratory visit relationship. Also, O_3 levels much lower than in previously studied in Southern Ontario.	Bates et al. (1990)
0 to 0.13 avg of max 1 h/day for two stations	O_3	ER admissions for COPD in Barcelona, Spain, during 1985 to 1986. 24-h avg SO_2 and BS city-wide averages. 1-h max SO_2, CO, NO_2, and O_3 obtained from two stations.	A weak but statistically significant association found between COPD admissions and levels of SO_2, BS, and CO, after accounting for seasonality and autocorrelation and during season-specific analyses. However, O_3 was eliminated from the analysis based on its seasonally driven negative correlation with admissions (prior to long-wave controls). Thus, no conclusions regarding O_3 can be made from this work.	Sunyer et al. (1991)
0 to 0.04 daily mean	O	Hospital admissions for asthma in Helsinki, Finland, from 1987 to 1989; 24 h average SO_2, NO_2, TSP, and O_3 city-wide averages.	After accounting for daily minimum temperature, NO_2 and O_3 were significantly correlated on the same day as admissions, whereas O_3 was most significant on the prior day ($p = 0.006$). However, long-wave peaks (e.g., in April for asthma) were not addressed and autocorrelation was not assessed.	Ponka (1991)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.06 to 0.13 mean of 1000 to 1500 hours O_3 (0.12 1-h max was exceeded on 42 of 226 total study days, whereas 0.08 was exceeded on 102 days)	O_3	ER visits for asthma, bronchitis, and finger wounds (a nonrespiratory control) at nine hospitals in central New Jersey were analyzed for the period May to August 1988 and 1989. Daily values of O_3 and SO_2 obtained from nearest of five monitoring sites. Barometric pressure, temperature, RH, and visibility (as an index of sulfate) obtained from a Newark measurement station.	Bivariate correlations indicated asthma visits to be strongly negatively correlated with temperature and weakly negatively correlated with O_3, suggesting a seasonality influence, despite limitation to the O_3 season. However, simultaneous regression of asthma visits on all environmental variables yielded significant (positive) O_3 and (negative) temperature coefficients only, suggesting that temperature acted as a long-wave control variable, revealing the short-wave O_3 relationship with asthma. Day-of-week effects on visits found unimportant. No environmental associations seen with bronchitis or control cases (finger cuts).	Cody et al. (1992)
0.00 to 0.05 avg of daily means from 22 stations	O_3	Admissions to 79 acute-care hospitals in Southern Ontario for the months of January, February, July, and August in 1979 to 1985. Hourly average O_3, SO_2, NO_2, temperature, RH, wind speed, barometric pressure, and daily average TSP and $SO_4^=$.	An elaborate reanalysis of the Bates and Sizto (1989) data set augmented to 1985. Long-wave influences controlled using time period subsets and AR modeling. Despite possible overspecification of models (e.g., use of wind speed) and AR filtering of the short wave, results confirm Bates and Sizto's overall conclusions regarding significant O_3 associations. Response to air pollution estimated to be 19 to 24% of summer respiratory admissions, although the exact contribution by O_3 to the total was not estimated.	Lipfert and Hammerstrom (1992)
0.01 to 0.05 3-mo avg of daily means from all city sites	O_3	Age-specific quarterly asthmatic hospital discharge rates in Hong Kong from 1983 to 1989 examined in relation to quarterly mean levels of TSP, RSP, NO_2, NO_x, and O_3 (n = 27).	Concludes that asthma morbidity is correlated with particles, but not O_3. However, analysis uses quarterly means and lacks seasonality controls.	Tseng et al. (1992)
0.03 to 0.21 1-h daily max at central site in each area	O_3	Daily emergency admissions to acute care hospitals for asthma, total respiratory, and control disease categories in the New York City, Albany, and Buffalo, NY, metropolitan areas from June to August 1988 and 1989; daily 1-h maximum O_3 and temperature and daily average sulfate and acid aerosols (H^+) considered.	Significant positive associations found for O_3, $SO_4^=$, and H^+ with asthma and total respiratory admissions, but not for control categories. Long-wave and day-of-week effects removed, and temperature effects controlled. Strongest O_3 associations in higher pollution year (1988) and in more urban population centers (Buffalo and New York, NY).	Thurston et al. (1992)
0.01 to 0.16 1-h daily max at central site	O_3	Daily admissions to 22 acute care hospitals in Toronto, Ontario, for asthma, total respiratory, and control disease categories during July and August 1986, 1987, and 1988; daily 1-h maximum O_3, SO_2, NO_2, temperature, and daytime (9:00 a.m. to 5:00 p.m.) $SO_4^=$ and H^+ considered.	Significant positive correlations found for O_3, H^+, $SO_4^=$, PM_{10}, and TSP with asthma and for total respiratory admissions, but not for SO_2 or NO_2, and not with control admissions. Long-wave and day-of-week effects removed. Multivariate regressions and sensitivity analyses suggested that O_3 was the pollutant of primary importance, but H^+ may potentiate O_3 effects. Except for H^+, all PM metrics considered became nonsignificant when entered into regressions simultaneously with O_3. Ozone significant even after dropping days >0.12 ppm.	Thurston et al. (1994)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.01 to 0.15 1-h daily max	O_3	Daily emergency respiratory admissions to 168 acute care hospitals in Ontario, Canada, during May to August 1983 to 1988 were related to daily levels of O_3 and SO_4 at the nearest of 22 and 9 monitoring sites, respectively. Admissions broken into 0 to 1, 2 to 34, 35 to 64, and 65+ age groups, and by geographical subregion.	Ozone and $SO_4^=$ positively and significantly associated with admissions for asthma and COPD in all age groups. Associations consistent across regions. Seasonal and day of week effects addressed prior to analysis. Analyses also controlled for individual hospital influences. No pollutant associations found for nonrespiratory control admissions. Simultaneous regressions suggest O_3 to be more important than $SO_4^=$.	Burnett et al. (1994)
0.01 to 0.11 1-h daily max averaged over seven sites in Montreal	O_3	Daily urgent hospital admissions to 31 hospitals in Montreal, Canada, during May-October and August-July from 1984-1988 related to daily levels of O_3, SO_4, PM_{10}, temperature, and humidity. Admissions broken into asthma, nonasthma respiratory, total respiratory, and a nonrespiratory "control" group of admissions categories.	Ozone and temperature positively and significantly correlated with total respiratory admissions during the July-August period, but not with control admissions categories. However, O_3 and T are both nonsignificant when entered simultaneously.	Delfino et al. (1994a)
0.02 to 0.16 1-h avg daily max 0.01 to 0.12 8-h avg daily max	O_3	Daily numbers of emergency asthma visits by patients 1 to 16 years old to an inner city hospital in Atlanta, GA, from June to August 1990 were related to daily levels of O_3, SO_2, PM_{10}, pollen, and T.	Hospital visits were found to be significantly higher on days when the previous day's 1-h max O_3 exceeded 0.11. No relationship was found below 0.11, or with 8-h avg daily maximum O_3. Day-of-week effects were accounted for. Seasonality effect reduced by study period selection, but probable long-wave seasonal cycles superimposed on the day-to-day fluctuations were not directly addressed, which probably weakened the reported O_3-admissions associations.	White et al. (1994)
0.01 to 0.04 (10th to 90th percentile) 24-h daily avg	O_3	Daily respiratory admissions by patients □ 65 years of age in Birmingham, AL, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point. COPD and pneumonia admissions examined. Multiple O_3 monitoring sites averaged, but the numbers of sites varied over time.	COPD and pneumonia admissions positively correlated with O_3 and PM_{10} over time. A 50 ppb increase in 24 h average O_3 was associated with RR = 1.14 for pneumonia (95% CI = 0.94 to 1.38) and RR = 1.17 for COPD (95% CI = 0.86 to 1.60). Seasonal fluctuations addressed using 48 monthly dummy variables. Auto-regression methods employed to reduce autocorrelation. Day-of-week effects not addressed.	Schwartz (1994a)
0.01 to 0.04 24-h daily avg (10th to 90th percentile) 0.02 to 0.09 1-h avg daily max (10th to 90th percentile)	O_3	Daily respiratory admissions for patients □ 65 years of age in Detroit, MI, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point.	Pneumonia and COPD respiratory admissions were found to be significantly associated with both PM_{10} and O_3, even after eliminating noncompliance days. Monthly dummy variables were employed to account for seasonal variations, but day of week effects were not addressed. Asthma admissions were not associated with pollution, but this was attributed to the very low counts in this category for the elderly.	Schwartz (1994b)

Table 7-21 (cont'd). Hospital Admissions/Visits in Relation to Photochemical Oxidant Pollution: Time Series Studies[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.01 to 0.04 (10th to 90th percentile) 24-h daily avg	O_3	Daily respiratory admissions for patients 65 years of age in Minneapolis-St. Paul, MN, from 1986 to 1989 were related to daily levels of O_3, PM_{10}, temperature, and dew point.	Pneumonia respiratory admissions were significantly associated with O_3 and PM_{10}. No O_3-COPD association was found. The pneumonia RR associated with a 50-ppb increase in 24-h average O_3 was RR = 1.22 (95% CI = 1.02 to 1.47). Excluding days with 1-h max O_3 above 120 ppb did not alter results. Various methods, including the use of monthly dummy variables, were used to control for seasonality effects, all yielding similar results.	Schwartz (1994c)
0.053 (±0.005) Mean (±SD) 10 a.m. to 3 p.m. avg	O_3	ER visits for asthma at central New Jersey hospitals from May to August 1986 to 1989 related to daily levels of O_3 and temperature. Other environmental variables considered include RH, sulfates, NO_2, SO_2, and visibility.	Asthma visits were significantly associated with O_3 and a.m. temperature, but not with other environmental variables considered. O_3 coefficient implies a 44% mean effect, but unaddressed temperature effects may be a confounding factor. An analysis limited to July and August reduces this concern, yielding a 16% mean effect by O_3.	Weisel et al. (1995) Weisel (1994)

[a]See Appendix A for abbreviations and acronyms.
[b]Reviewed in U.S. Environmental Protection Agency (1978).
[c]Reviewed in U.S. Environmental Protection Agency (1986).

reasons that were not apparent. A common weakness of many of those studies, however, was a failure to control adequately for seasonal differences in hospital usage and O_3 concentration. Therefore, each of the updated critiques in this table now includes an evaluation of how the data were (or were not) controlled for long-wave influences (e.g., seasonality). With this factor taken into account, the older studies' varying results are now more understandable. In a number of these studies, documented long-wave periodicities in the data were ignored, resulting in nonsignificant associations (i.e., California Department of Public Health, 1955, 1956, 1957; Brant and Hill, 1964; Brant, 1965; Levy et al., 1977; Namekata et al., 1979) or even significant negative correlations between O_3 and hospital visits and admissions (Richards et al., 1981) as a result of the generally higher respiratory admission rates in the colder months, when O_3 levels are at their lowest. Two studies that did not control for seasonality still reported significant positive correlations between hospital admissions and oxidants (Sterling et al., 1966, 1967; Goldsmith et al., 1983), although Sterling et al. excluded the winter months from the analysis and Goldsmith et al. did detrend the data. Also, unlike any of the previously cited studies, both of these analyses controlled for day-of-week effects on hospital admission rates (e.g., due to consistently lower admissions on weekends), an important factor in hospital admissions variations that also must be addressed (see Sterling et al., 1966). Moreover, the one previously reviewed study that adequately controlled for both long-wave and day-of-week influences (Bates and Sizto, 1983, 1987, 1989) reported very significant associations ($p < 0.001$) between O_3 levels and summertime (July and August) respiratory hospital admissions. However, other intercorrelated environmental variables (e.g., acidic sulfates) also may have been cofactors in this association (Bates and Sizto, 1987). Overall, a review of these older studies suggests that, if the data are analyzed using newer statistical techniques, a significant association may be found between elevated ambient O_3 concentrations and acute increases in daily respiratory hospital admissions.

Since the last criteria document (U.S. Environmental Protection Agency, 1986), a number of new ER visit and hospital admissions studies have been completed, a few of which share some of the same statistical flaws found in many of the older studies. For example, Tseng and Li (1990) and Tseng et al. (1992) failed to control for the seasonality of admissions and pollutants in their statistical analyses of quarterly hospital admissions in Hong Kong, causing them to report no associations with O_3, but a significant (and likely spurious) negative correlation of age-specific asthma admissions with quarterly mean SO_2 in the first of these papers and a significant (and also likely spurious) positive association with TSP in the second paper, but no association with O_3. Sunyer et al. (1991) failed to consider seasonality in their initial evaluation of an O_3 relationship with COPD hospital admissions in Barcelona, Spain; causing them to eliminate O_3 from consideration in the study and any evaluation of possible health effects. Also, Bates et al. (1990), using a largely descriptive approach, characterized the seasonal periodicities of Vancouver, BC, respiratory ER visits. Their subanalysis of the warm season (May through October) included a dominant fall asthma peak, which would obscure any summertime O_3 associations, and therefore, little can be inferred from this data analysis about the existence or nonexistence of an acute relationship between O_3 and Vancouver hospital visits for respiratory causes. Ponka (1991) showed significant O_3 associations with asthma hospital admissions in Helsinki, Finland, over a 3-year period. The model also included temperature, but did not address directly the noted long-wave variations in both admissions and pollution. Thus, whether a study has adequately addressed statistical confounding by the prominent long-wave cycles in respiratory hospital admissions

series, which are clearly dominated by other causes (e.g., spring pollen, fall respiratory infection, winter influenza seasons), continues to be a crucial criterion in evaluating the usefulness of a study's results.

Fortunately, there are also a number of new studies that have addressed both long-wave and day-of-week influences in their analyses. Cody et al. (1992) did not control directly for seasonality, but they did narrow their analysis of central New Jersey hospital ER visits to the high O_3 season (May through August). Even so, their initial correlational analysis yielded negative associations between hospital visits and both temperature and O_3, which suggests that within-season long-wave effects existed (e.g., generally higher asthma visits in May, at the end of the pollen season, when O_3 and temperature are lower on average than in July or August). However, the authors did conduct subsequent regressions of respiratory visits on both temperature and O_3 simultaneously, yielding a significant positive coefficient for O_3 and a negative coefficient for temperature, which suggests that the inclusion of temperature may have indirectly accounted for the long-wave cycle, allowing the positive short wave O_3-visit relationship to be seen. Day-of-week influences were considered, but found to be unimportant for these ER visit data. No such pollution-hospital visit relationship was found for finger cut (i.e., control disease) visits.

Weisel et al. (1995) examined central New Jersey hospital ER visits for asthma (mean = 5.4/day) during the high O_3 season (May through August) for 1986 through 1990. Using a stepwise regression analysis, a significant positive coefficient for O_3 and a negative coefficient for morning temperature was found. Other environmental factors considered, including rate of temperature change, RH, every-sixth-day sulfates, NO_2, SO_2, and visibility (an index of fine particles [FPs]), were not found to be correlated with asthma visits. This study did not directly address long-wave confounding, instead following the same approach as Cody et al. (1992) in using temperature to indirectly control for such seasonal confounding and diminishing autocorrelation to nonsignificance (DW = 2). However, it is not clear to what extent O_3 may be inadvertently picking up short-wave temperature effects not modeled by this specification. These are likely to be opposite to the seasonal effect apparently being captured by the temperature variable (as indicated by its negative coefficient). The highest O_3 coefficient was found on the lowest O_3 year, which is consistent with unaddressed confounding. Thus, the O_3 effects reported (which imply an overall O_3 mean effect equal to 44% of all asthma visits) should be viewed as maximum effects estimates, possibly contributed to by colinear high temperature influences. Indeed, limiting the analysis to July and August of each year (thereby reducing long-wave confounding) resulted in a less negative temperature coefficient and an overall O_3 coefficient one-third of that for May through August (Weisel, 1994), implying approximately a 16% mean effect, which is more consistent with published hospital admissions study results. A covariance analysis presented indicates an average 28% increase in the number of hospital ER visits for asthma on high-O_3 days (above 0.06 ppm) versus low-O_3 days (below 0.06 ppm) after controlling for temperature, but seasonal cycles were again not directly accounted for in the analysis. Overall, these results are consistent with an O_3 effect on asthma morbidity.

Thurston et al. (1992) analyzed unscheduled (emergency) admissions to acute care hospitals in three New York State metropolitan areas during the summers of 1988 and 1989. Environmental variables considered included daily 1-h maximum O_3 and 24-h average SO_4 and acid aerosol (H^+) concentrations, as well as daily maximum temperature recorded at central sites in each community. Long-wave periodicities in the data were reduced by selecting a June

through August study period. However, because of remaining within-season long-wave cycles in the data series (i.e., day-to-day fluctuations superimposed on an annual cycle in admissions), data were prefiltered using sine and cosine waves with annual periodicities. Day-of-week effects also were controlled via regression. These adjustments resulted in nonsignificant autocorrelations in the data series and also improved the pollution correlations with admissions. For example, in New York City, the same-day O_3-asthma correlation rose from a nonsignificant $r = 0.04$ in the raw data to a significant $r = 0.24$ after prefiltering. This shows the importance of addressing long-wave cycles in such data, even when these data come from a single season. In contrast, correlations between the pollution data and hospital admissions for nonrespiratory control diseases were nonsignificant both before and after prefiltering. The strongest O_3-respiratory admissions associations were found during the period of high pollution in the summer of 1988 and in the most urbanized communities considered (i.e., Buffalo and New York City). After controlling for temperature effects via simultaneous regression, the summer haze pollutants (i.e., $SO_4^{=}$, H^+, O_3) remained significantly related to total respiratory and asthma admissions. However, these pollutants' high intercorrelation prevented the clear discrimination of a single pollutant as the causal agent. Depending on the index pollutant, the admission category, and the city considered, it was found that summer haze pollutants accounted for approximately 5 to 20% of June through August total respiratory and asthma admissions, on average, and that these admissions increased approximately 30% above average on the highest pollution days.

Lipfert and Hammerstrom (1992) reanalyzed the Bates and Sizto (1989) hospital admissions data set for 79 acute-care hospitals in southern Ontario, incorporating more elaborate statistical methods and extending the data set through 1985. Long-wave influences were once again reduced by using the short study periods previously employed by Bates and Sizto (e.g., July and August only for summer), as well as by employing prewhitening and autoregressive procedures to the data. Day-of-week effects also were controlled. In addition, the models were specified much more extensively, to include a variety of new meteorological variables that may have caused some confounding with the pollutant variables (e.g., wind speed correlated at $r = -0.55$ with NO_2). Despite possible model overspecification (e.g., the inclusion of wind speed), summer haze pollutants (i.e., O_3, SO_4, SO_2) were still found to have significant effects on hospital admissions in southern Ontario. In contrast, pollution associations with hospital admissions for accidental causes became nonsignificant in these models. Although air pollution concentrations were generally within U.S. air quality standards, the pollutant mean effect accounted for 19 to 24% of all summer respiratory admissions, although the "responsible" pollutants could not be selected by the authors with certainty.

Burnett et al. (1994) also employed the Ontario acute care hospital database to analyze the effects of air pollution on hospital admissions, but their analysis considered all of Ontario and analyzed the data from each individual hospital, rather than aggregating the counts by region. Slow moving temporal cycles, including seasonal and yearly effects, were removed (via an 19-day, moving-average-equivalent, high-pass filter), and day-of-week effects were controlled prior to the analysis. Poisson regression techniques were employed because of the low daily admission counts at individual hospitals. Ozone displayed a positive association with respiratory admissions in 91% of the 168 hospitals, and 5% of summertime (May through August) respiratory admissions (mean = 107/day) were attributed to O_3 (mean = 50 ppb). Positive associations were found in all age groups (0 to 1, 2 to 34, 35 to 64, and 65+). A

parallel analysis of nonrespiratory admissions showed no such associations, which indicates the association specificity. Ozone was found to be a stronger predictor of admissions than SO_4, which accounted for an additional 1% of summertime respiratory admissions. Temperature had no effect on the pollution-respiratory admission relationship.

Thurston et al. (1994) focused their analysis of respiratory hospital admissions in the Toronto metropolitan area during the summers (July through August) of 1986 to 1988, when they directly monitored for strong particulate acidity (H^+) pollution on a daily basis at several sites in that city. Long-wave cycles, and their associated autocorrelations, were removed by first fitting sine and cosine series having annual periodicity (as well as day-of-week dummy variables) to the data via regression, and analyzing the resulting residuals. Strong and significant positive associations with asthma and respiratory admissions were found for both O_3 and H^+, and somewhat weaker significant associations with $SO_4^=$, $PM_{2.5}$, PM_1, and TSP, as measured at a central site in downtown Toronto. No such associations were found for SO_2 or NO_2, nor for any pollutant with nonrespiratory control admissions. Temperature was only weakly correlated with respiratory admissions and became nonsignificant when entered in regressions with air pollution indices. Simultaneous regressions and sensitivity analyses indicated that O_3 was the summertime haze constituent of greatest importance to respiratory and asthma admissions, although elevated H^+ was suggested as a possible potentiator of this effect. During multipollutant, simultaneous regressions on admissions, O_3 was consistently the most significant. Of the particle metrics, only H^+ remained statistically significant when entered into the admissions regressions simultaneously with O_3. Sensitivity analyses also showed that dropping all days above the current U.S. O_3 standard of 0.12 ppm (2 of a total 117 days) did not significantly change the O_3 coefficients. The simultaneous O_3, H^+, and temperature model indicated that 21 $\pm$ 8% of all respiratory admissions during the three summers were associated with O_3 air pollution, on average, and that admissions rose an estimated 37 $\pm$ 15% above that otherwise expected on the highest O_3 day (0.159 ppm). Moreover, despite differing health care systems, the Toronto regression results for the summer of 1988 were remarkably consistent with previously reported results for that same summer in Buffalo, NY, (see Table 7-22).

Delfino et al. (1994a) studied daily urgent hospital admissions for respiratory and other illnesses at 31 hospitals in Montreal, Canada during the warm periods of the year between 1984 and 1988. Respiratory admissions were considered as a whole and split into asthma and nonasthma categories, using definitions compatible with those previously used by Bates and Sitzo (1987) and by Thurston et al. (1994). Both 1-h and 8-h maximum O_3 concentrations were considered in the analyses, as well as weather variables (temperature and relative humidity) and PM measurements, although 83% (five out of every six) PM measurements were not directly measured but, instead, were estimated from other environmental variables including visibility, temperature, and O_3 concentration on those missing PM days (Delfino et al., 1994b). Seasonal cycles were addressed by applying a 19-day moving average high-pass filter to the health and environmental data before analysis for associations. Day-of-the-week and autocorrelation effects also were addressed, when present. For the months of July and August, during the study period, a significant association was found between all respiratory admissions and both 8-h daily maximum O_3 (p 0.01) and 1-h daily maximum O_3 (p 0.03) 4 days prior to admission, despite the fact that no day exceeded 0.12-ppm 1-h daily maximum O_3 (90th percentile = 118 g/m^3 or 0.06 ppm O_3). Of the significant bivariate environmental-admission associations found, the association with 8-h

maximum O_3 was the highest reported ($r = 0.15$), tied only by the 4-day lag in temperature. However, the addition into the regression of temperature on the

Table 7-22. Comparison of Regressions of Daily Summertime Respiratory Admissions on Ozone and Temperature in Toronto, Ontario, and Buffalo, New York, for the Summer of 1988[a]

City and Year	Respiratory Admissions Category	Temperature, Pollutant Model Specification	Pollutant Regression Coefficient (Admissions/ppb/10^6 persons ± SE)	Pollutant Mean Effect (% ± SE)	Max/Mean Pollutant Relative Risk ± SE
Toronto (pop. = 2.4×10^6) 1988 summer	Total respiratory (mean = 14.1/day)	T(LG2), O_3 (LG1)[b]	0.022 ± 0.010[c]	26.4 ± 11.8	1.34 ± 0.15
Toronto (pop. = 2.4×10^6) 1988 summer	Total asthma (mean = 9.5/day)	T(LG2), O_3 (LG1)	0.014 ± 0.008[c]	25.3 ± 14.9	1.32 ± 0.19
Buffalo (pop. = 2.0×10^6) 1988 summer	Total respiratory (mean = 25.0/day)	T(LG2), O_3 (LG2)	0.030 ± 0.016[c]	18.4 ± 9.9	1.25 ± 0.09
Buffalo (pop. = 2.0×10^6) 1988 summer	Total asthma (mean = 7.1/day)	T(LG2), O_3 (LG3)	0.012 ± 0.004[d]	23.9 ± 10.1	1.25 ± 0.14

[a]See Appendix A for abbreviations and acronyms.
[b]LG = lag between exposure and admission, in days.
[c]$p < 0.05$ (one-way test).
[d]$p < 0.01$ (one-way test).

Source: Thurston et al. (1994).

same day as the O_3 reduced both the O_3 and the temperature associations to nonsignificance. It should be noted that the authors also found a significant association between asthma admissions and their estimated PM_1 variable during May through October; however, because both temperature and O_3 were used to estimate these observations, it is difficult to interpret this association separately from that for O_3 and temperature. No significant correlations were found between O_3 and nonrespiratory, control admissions (e.g., appendicitis). The authors conclude that their findings "should be regarded as a reflection of the potential public health burden of respiratory disease attributable to photochemical air pollutants."

White et al. (1994) reported daily emergency room visit records from June through August 1990 at a large inner city hospital in Atlanta, GA. Daily counts of visits for asthma or reactive airway disease by patients 1 to 16 years of age (mean = 6.6/day) were related to daily levels of O_3, SO_2, PM_1 , pollen, and temperature. Seasonality likely was reduced by the study period selection, although no effort was made to address possible within-season long-wave cycles in the data. Day-of-week and temperature effects were controlled as part of a Poisson model employed to address the small admission numbers at a single hospital. This model yielded a 1.42 admissions rate ratio ($p = 0.057$, 95% CI = 0.99 to 2.0) for the number of asthma visits following days with O_3 levels equal to or exceeding a 1-h maximum of 0.11 ppm, which is consistent with the relative risk values reported by Thurston et al. (1992, 1994). No admissions relationship with O_3 was seen below 0.11 ppm or with 8-h average O_3.

In a study of Birmingham, AL, data, Schwartz (1994a) separately examined O_3 and PM_1 influences on hospital admissions by the elderly for pneumonia (mean = 5.9/day) and COPD (mean = 2.2/day) causes from 1986 to 1989. Other potentially confounding pollutants (e.g., SO_2 and NO_2) were not considered, nor was any control admission category analyzed. Poisson regression analyses were employed controlling for time trends, seasonal fluctuations, and weather, but day-of-week effects (which can be a large influence on such admissions) were not addressed. Weather was controlled by including dummy variables for seven (unspecified) temperature and dew point range categories in the regression. Seasonal fluctuations were controlled through the use of 48 monthly dummy variables, which raises the concern that within-month long-wave confounding may have remained. However, autoregressive models were reportedly used whenever serial correlation was found in model residuals. Base model results (excluding winter months) yielded a 2-day lag relative risk (RR) estimate of 1.14 for pneumonia admissions from a 50 ppb increase in 24-h average O_3 (95% confidence interval, CI = 0.94 to 1.38). Excluding days exceeding 120 ppb yielded similar results (RR = 1.12, CI = 0.92 to 1.37). For COPD, the basic model yielded a RR = 1.17 (CI = 0.86 to 1.60), whereas excluding days above 120 ppb similarly gave RR = 1.18 (CI = 0.86 to 1.62). No models considered O_3 and PM_1 simultaneously. Two other comparative models (the inclusion of sine/cosine cycles of various periodicities up to 2 years in the regression and the analysis of deviations from a nonparametric smoothing of admission counts) were tested for PM_1 , but not for O_3, so the model sensitivity of the O_3 effect was not tested. Overall, even after excluding days exceeding the standard, this work indicated a fairly consistent O_3 effect across respiratory categories that approached, but did not reach, statistical significance.

Schwartz (1994b) analyzed O_3 and PM_1 air pollution relationships with daily hospital admissions of persons 65 years or older in the Detroit, MI, metropolitan statistical area from 1986 to 1989. Daily counts for pneumonia (mean = 15.7/day), asthma (mean = 0.75/day), and all other COPDs (mean = 5.8/day) were regressed on the pollution variables and various seasonal, trend, and temperature dummy variables, using Poisson

modeling. However, day-of-week effects were not addressed. Ozone was analyzed with respect to both its daily 24-h average and 1-h maximum. Autoregressive analyses and residuals plots indicated no remaining autocorrelation in the model. Both O_3 and PM_1 were significant in simultaneous pollutant models for pneumonia and COPD but not for asthma (which was ascribed to the low daily counts for this category). These simultaneous coefficients were reportedly similar to those from the single pollutant models, although the correlations of the coefficients were not provided. Dropping all days exceeding the 1-h maximum O_3 standard did not change the size of the O_3 coefficients, which remained significant ($p < 0.01$). Based on the regression coefficients and data presented, it can be estimated that the mean effect for O_3 (11.6%) was double that for PM_1 (5.7%) in the pneumonia model, but comparable for COPD (12.2% for O_3 versus 10.2% for PM_1). On an absolute scale, these results imply that O_3 was associated with 1.7 ($\pm$0.2) respiratory admissions by the elderly/day/100 ppb (as a 1-h maximum) per million persons in the Detroit metropolitan area. This estimate does not include admissions by persons less than 65 years of age (which likely would have included higher asthma admissions, for example), so that the total respiratory admissions associated with O_3 in the entire population likely would be higher than estimated from this work.

Schwartz (1994c) evaluated the associations of both PM_1 and O_3 with respiratory hospital admissions by the elderly in Minneapolis-St. Paul, MN, from 1986 to 1989. Due to small counts, Poisson modeling methods were employed. Various modeling approaches were employed to address weather influences, including (1) the use of annual, monthly, temperature, and dew point dummy variables; (2) a stepwise spline approach to fit data dependence on time, temperature, and dew point (an indicator of the water content of the air); and (3) a generalized additive model using nonparametric smooth functions of time, temperature, and dew point temperature. Autoregressive methods were employed to eliminate autocorrelations, when significant. However, these various complex statistical manipulations were not sufficiently documented to permit critical review of these methods or replication of results (e.g., dummy variable ranges were not provided and statistical packages were not referenced). Although no association was found for COPD in the elderly, O_3 did make a significant independent contribution to hospital admissions by the elderly for pneumonia (mean = 6.0/day), even after controlling for weather and PM_1 . Although all models gave similar results, the best data fit (as measured by analysis of deviance) and strongest O_3 association was reported for the stepwise spline model, which yielded a pneumonia admissions relative risk of 1.22 (95% CI = 1.02 to 1.47) for a 50 ppb increase in the 1-day lag of the 24-h average of O_3. The use of 1-h daily maximum O_3 in these analyses reportedly yielded less significant associations with admissions. However, eliminating days with either PM_1 above 150 $\mu g/m^3$ or a 1-h maximum O_3 above 120 ppb from the analysis did not alter results significantly.

Table 7-23 intercompares the O_3-respiratory hospital admissions effect estimates for the various studies providing sufficient information to allow the derivation of such pollutant-specific estimates. The estimates are presented in two ways: (1) as an absolute number of daily admissions per 100-ppb increase in 1-h O_3 concentration per million persons, total population, and (2) as a percent increase in the daily admission rate of the relevant admissions category, presented as a relative risk per 100-ppb increase in 1-h O_3 concentration. A reference increment of 100-ppb O_3 is employed here because this is

Table 7-23. Summary of Effect Estimates for Ozone in Recent Studies of Respiratory Hospital Admissions[a]

Location	Reference	Respiratory Admission Category	Effect Size (± SE) [Admissions/100 ppb O_3/day/10^6 persons]	Relative Risk (95% CI)[b] [RR of 100 ppb O_3, 1-h max]
New York City, NY[c]	Thurston et al. (1992)	All		
Buffalo, NY[c]	Thurston et al. (1992)	All		
Ontairo, Canada[c]	Burnett et al. (1994)	All		
Toronto, Canada[c]	Thurston et al. (1994)	All		
Montreal, Canada[d]	Delfino et al. (1994a)	All		
Birmingham, AL[e]	Schwartz (1994a)	Pneumonia in elderly	0.73 (± 0.54)	
Birmingham, AL[e]	Schwartz (1994a)	COPD in elderly	0.83 (± 0.33)	
Detroit, MI[e]	Schwartz (1994b)	Pneumonia in elderly	0.82 (± 0.26)	
Detroit, MI[e]	Schwartz (1994b)	COPD in elderly	0.90 (± 0.41)	
Minneapolis, MN[e]	Schwartz (1994c)	Pneumonia in elderly	0.41 (± 0.19)	1.117 (1.03 to 1.39)
Minneapolis, MN[e]	Schwartz (1994c)	COPD in elderly	[f]	[f]

[a]See Appendix A for abbreviations and acronyms.
[b]One-way (□ ± 1.65 SE).
[c]1-h daily maximum ozone data employed in analysis.
[d]8-h daily maximum ozone data employed in analysis.
[e]24-h daily average ozone data employed in analysis. (1 h/24 h avg ratio = 2.5 assumed to compute effects and RR estimates).
[f]Not reported (nonsignificant).

approximately the difference between the maximum and the mean 1-h daily maximum O_3 in these studies (e.g., in Toronto, the 1988 mean = 69 ppb; maximum = 159 ppb). The absolute effect estimates relative to total population have the advantages that the total effect can be readily "partitioned" into subcategories (e.g., by age group or disease subcategory), and it also can be applied easily to other situations (i.e., only the population and O_3 levels are required), but this may not be appropriate if the other population makeup is very different from the study populations (e.g., in age distribution). The relative risk estimates are intuitively interpretable but are not as readily applied elsewhere (i.e., the respiratory disease prevalence rates must be known), and the effect will vary depending on the prevalence, which differs widely between populations and even throughout the year within a single population (as respiratory morbidity is generally higher in winter than summer). For example, this accounts for much of the apparent inconsistency between the Burnett et al. (1994) and Thurston et al. (1994) relative risks, in that the Thurston et al. (1994) Toronto values are for July and August only (when the prevailing number of respiratory admissions per day are generally at an annual minimum), whereas the Burnett et al. (1994) Ontario values are relative to respiratory admissions averages over more months of the year, yielding one-fourth the effect as a relative risk, even though the absolute effect estimate is two-thirds of the Thurston et al. (1994) estimate. In the case of the Schwartz studies of the elderly, the assumption has been made, based on data presented by Schwartz (1994b), that the 1-h daily maximum O_3 is 2.5 times the 24-h average, and the 100-ppb 1-h maximum estimates provided for these studies therefore are derived from a 40-ppb increase in 24-h average O_3. The absolute effect size results from these particular studies suggest that a large portion of the O_3 effects noted in the previous total respiratory admissions studies are contributed by COPD and pneumonia cases in the elderly. Based on results presented by Thurston et al. (1992, 1994), the other major contributor is asthma admissions, which are usually more prevalent in younger age groups. Overall, the results presented in Table 7-23 collectively indicate that ambient O_3 often has a significant effect on hospital admissions for respiratory causes, ranging in these studies from 1 to 3 total respiratory admissions/day/100 ppb $O_3/10^6$ persons, or from a 1.1 to 1.36 relative risk/100 ppb O_3.

Daily Mortality

Past studies of the possible association of O_3 (oxidants) with human mortality summarized in prior O_3 criteria documents (U.S. Environmental Protection Agency, 1978, 1986) were sometimes suggestive of an association, but each study was flawed in some way. These studies are included in Table 7-24, with annotation as to the document in which they were reported. Most of these studies considered daily mortality in Los Angeles, CA, during the 1950s and 1960s. Unlike most historical hospital admissions studies, many of these studies did recognize and attempt to control for seasonality in the data series. Notable exceptions are the California Department of Public Health studies (1955, 1956, 1957), which were further weakened by their qualitative treatment of the air pollution data. The Mills (1957a,b) analyses also employed a questionable exposure assessment method (the Standard Research Institute smog index), which diminishes its usefulness. Massey et al. (1961) reported no significant correlations between community differences in mortality and differences in oxidant levels over time, but the investigators compared two communities with very different populations (e.g., age distributions), a likely confounder in such cross-sectional comparisons. Mills (1960), while reporting mortality-oxidant associations and effects (370 respiratory and cardiovascular deaths/year), did not control for potential temperature

Table 7-24. Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
1.0 peak (undefined)	Oxidant	Relationship between daily concentrations of photochemical oxidants and daily mortality among residents of Los Angeles County aged 65 years and older during the periods August through November 1954 and July through November 1955.	Heat had a significant effect on mortality; no consistent association between mortality and high oxidant concentrations in the absence of high temperature. However, seasonal trends were not addressed, and pollution data treatment was qualitative.	California Department of Public Health (1955[b], 1956[b], 1957[b])
0.38 max 1 h/day	Oxidant	Data extended to include the period from 1956 through the end of 1959.		Tucker (1962)
(Not reported) 0.10 to 0.42 (undefined) for 148 days of 1949	Oxidant O_3	Relationship between daily maximum oxidant concentrations and daily cardiac and respiratory mortality in Los Angeles for the periods 1947 to 1949; August 1953 through December 1954; and January through September 1955.	Positive relationship between daily maximum oxidant concentrations and mean daily death rates on high-smog days vs. low-smog days. Questionable exposure analysis, including use of the SRI smog index.	Mills (1957a[b],b[b])
(Not reported)	Oxidant	Comparison of daily mortality in two Los Angeles County areas similar in temperature, but with different levels of daily maximum and mean oxidant levels (KI); SO_2 and CO concentrations were also measured.	No significant correlations between differences in mortality and differences in pollutant levels. However, the populations differed in socioeconomic and age distribution characteristics.	Massey et al. (1961)[b]
0.02 to 0.37 average of 1-h daily max from all Los Angeles sites	Oxidant	Daily respiratory and cardiac death counts for Los Angeles County, 1956 to 1958, related to daily maximum oxidant concentrations. All days above 96 F daily maximum temperature eliminated from analysis. Each day's average of daily oxidant maxima was related to that day's deviation from monthly mean mortality.	A stratification of the mortality deviations vs. oxidant concentration revealed increasing mortality with increasing oxidant concentration, even in the cooler months. The use of deviations addresses data seasonality. It is estimated that over 300 deaths/year in Los Angeles are associated with oxidants. However, the lack of temperature controls below 96 F is a major weakness.	Mills (1960)

Table 7-24 (cont'd). Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
0.05 to 0.21 monthly avg	Oxidant	Reanalysis of the relationship between KI and daily mortality from cardiac and respiratory diseases in Los Angeles for the years 1956 through 1958.	Used deviations from sine wave fit to reduce seasonality of pollution and mortality, but fit of monthly variations was inadequate. Significant correlations found between pollutants and mortality for cardiorespiratory diseases, but autocorrelation adjustments by authors reportedly reduced these associations to nonsignificance.	Hechter and Goldsmith (1961)[b]
0.003 to 0.128 max 1 h/day	O_3	Relationship between daily mortality and daily 1-h maximum concentrations of O_3 in Rotterdam, The Netherlands, during the months of July and August 1974 and 1975.	Mortality significantly higher during relatively high pollution ($0.05 < O_3 < 0.125$) and heat episodes in 1975. However, no significant mortality difference due to moderate O_3 episodes ($0.05 < O_3 < 0.08$) in 1974, in the absence of high temperature. Such aggregated analyses of serial data makes interpretation difficult.	Biersteker and Evendijk (1976)[c]
0.02 to 0.29 six-site mean of daily 1-h max	O_3	Total, respiratory, and cardiovascular mortality in Los Angeles County, 1970 to 1979, related to O_3, CO, SO_2, NO_2, HC, PM, daily max temperature, and RH. Low-pass filter used to eliminate short-wave associations so that only seasonal associations could be studied.	Frequency domain analysis indicated a significant short-wave O_3-mortality association, but this was not investigated further. The filtered (i.e., long-wave) data analysis indicated O_3 to be a nonsignificant contributor to seasonal variations in mortality.	Shumway et al. (1988)
0.02 to 0.29 six-site mean of daily 1-h max	O_3	Shumway et al. (1988) 1970 to 1979 Los Angeles mortality dataset reanalyzed using a high-pass filter to allow investigation of short-wave (acute) associations with environmental variables, after removing seasonality effects. Environmental variables considered included temperature, RH, extinction coefficient, carbonaceous PM, SO_2, NO_2, CO, and O_3.	Filtered environmental and mortality data analyses demonstrated significant associations between short-term variations in total mortality and pollution, controlling for temperature. Day-of-week effects found not to affect the relationships. Of the pollutants considered, O_3 had the strongest association with total mortality. Similar results found for cardiovascular deaths, but not for respiratory deaths (for which only temperature was significant).	Kinney and Ozkaynak (1991)

Table 7-24 (cont'd). Daily Mortality Associated with Exposure to Photochemical Oxidant Pollution[a]

Concentration(s) (ppm)	Pollutant	Study Description	Results and Comments	Reference
Not reported 1-h daily max	O_3	Total daily deaths in Detroit, MI, 1973 to 1982, analyzed using Poisson methods. Environmental variables considered included TSP, SO_2, temperature, dew point, and O_3.	Significant associations found between mortality and PM, but not O_3. However, O_3 data and results not presented, so it is difficult to evaluate reported conclusion. Seasonality controlled via multiple dummy weather and time variables, and autocorrelation addressed using autoregressive techniques. Possible overspecification of weather controls may be a factor in the nonsignificance of O_3 in this analysis.	Schwartz (1991)
0.000 to 0.064 24-h avg (in TN, no exceedances of 0.12 ppm 1-h max; in MO, five exceedances with max = 0.15 ppm)	O_3	Associations between total daily mortality and air pollution were investigated in St. Louis, MO, and Kingston-Harriman, TN, during September 1985 through August 1986. Environmental variables considered include temperature, RH, PM_{10}, $PM_{2.5}$, sulfate, aerosol acidity, SO_2, NO_2, and O_3.	Statistically significant daily mortality associations were found with PM_{10}, but not with O_3. Autocorrelation removed via season indicators, multiple temperature/climate variables, and AR modeling. The nonsignificant O_3 coefficient may have been contributed to by the more conservative autocorrelation removal measures taken, lower O_3 concentrations, and shorter study period, relative to other recent mortality studies.	Dockery et al. (1992)

[a]See Appendix A for acronyms and abbreviations.
[b]Reviewed in U.S. Environmental Protection Agency (1978).
[c]Reviewed in U.S. Environmental Protection Agency (1986).

influences on mortality below 96 °F daily maximum. Hechter and Goldsmith (1961) reanalyzed the Mills (1960) data using a simple annual sine wave seasonality correction and obtained significant oxidant correlations until an autocorrelation adjustment was applied; this reportedly caused the pollutant-mortality correlations to drop to nonsignificance (results not presented). Biersteker and Evendijk (1976) conducted a t-test of difference analysis of two summers of time series data from Rotterdam for 1974 and 1975. Although significant mortality differences could be seen during 1975 heat-pollution episodes ($0.05 < O_3 < 0.125$ ppm), no significant mortality increase could be seen during the cleaner and cooler summer episodes ($0.05 < O_3 < 0.08$ ppm). Statistical time series methods were needed to address probable confounding by temperature effects. Overall, the various exposure assessment and statistical analyses weaknesses in the studies reported in previous O_3 criteria documents have prevented the drawing of definitive conclusions in those past documents as to whether or not there is a significant association between O_3 and human mortality.

Although relatively few O_3 mortality studies have been conducted and published since the last criteria document (U.S. Environmental Protection Agency, 1986), the statistical methods and pollution data employed in these studies have improved, compared with the older studies discussed above. Shumway et al. (1988) focused on long-wave variations in mortality, finding that O_3 was a nonsignificant contributor to seasonal variations in Los Angeles mortality during 1970 to 1979. As might have been expected, temperature was found to be the principal environmental factor influencing seasonal mortality fluctuations. This paper's exploratory frequency domain analysis did indicate a significant short-wave (i.e., cycles on the order of a few days in period) association between O_3 and mortality, but this result was not pursued in the subsequent regression analyses.

Kinney and Ozkaynak (1991) reanalyzed the 1970 to 1979 Los Angeles County mortality and environmental data set for short-wave pollution-mortality associations using seasonal and day-of-week controls. After prefiltering the environmental and mortality time series using a high-pass filter, significant associations were demonstrated between air pollution and short-wave (acute) variations in total mortality, even after controlling for temperature influences. Day-of-week effects also were accounted for but were found not to affect pollutant-mortality associations. In the regression models considered, the 1-day lag of O_3 concentration gave the strongest pollutant associations with total mortality. This O_3 coefficient was statistically separable from the other significant pollutants in the analysis (CO, NO_2, and PM), although these other three pollutants were too intercorrelated to separate from each other. Expressed as an elasticity, the O_3 regression coefficient (0.03 ± 0.01 [SE] deaths/ppb) over all years indicated that a 1% increase in O_3 concentration was associated with a 0.015% increase in total mortality. This result would imply an O_3 mean effect on the order of 1.5% of total mortality throughout the year (i.e., 830 total deaths/year). Results for individual years varied widely in terms of the O_3 coefficient size and significance, which indicates the need for multiple years of data to discern an effect of such a small size, relative to other mortality causes. Ozone regression results for cardiovascular deaths (average = 87/day) were qualitatively similar to those for total mortality (average = 152/day), but only temperature was significant for respiratory deaths (average = 8/day), probably due to low count number effects for this category (i.e., Poisson models may have been required). Overall, although the Shumway et al. (1988) analysis of these 1970 to 1979 Los Angeles data indicates that disease factors and other pollutants dominate the overall seasonal cycles in mortality in Los Angeles, the Kinney and Ozkaynak (1991) short-wave analysis documents that

O_3 explained a small but statistically significant portion of day-to-day variations in total mortality in that city over a 10-year period.

Schwartz (1991) analyzed total daily human mortality in Detroit, MI, during the 10-year period from 1973 to 1982, primarily investigating the effects of PM using Poisson methods. Although actual results are not presented for O_3, it is stated in the discussion of results that O_3 was "highly insignificant as a predictor of daily mortality." Weather is controlled for extensively in the model specification before the introduction of the air pollution variables. The fact that O_3 is usually correlated over time with meteorology raises the concern that the model may be overspecified, but no diagnostics (e.g., correlations of the coefficients) are presented to allow for an evaluation. Although previous-day temperature was included in the model, the only direct seasonality control attempted was to limit the analysis to nonwinter months. Thus, it is not clear to what extent within-season long-wave confounding also may be influencing the results. If present, such long-wave confounding would be expected to bias the O_3 coefficient downward towards nonsignificance in this case (because O_3 is usually highest, and mortality lowest, in summer) and would result in autocorrelation in the model. No model residual diagnostics are reported (e.g., DW statistics or plots of the model residuals), so the extent of this problem, if present, cannot be evaluated directly. However, autoregressive methods were employed, which should have addressed any autocorrelation problems. Overall, the poor documentation of the mortality-O_3 modeling, especially regarding the lack of model specification details or model coefficient intercorrelations, makes the author's statement regarding O_3 and mortality difficult to evaluate.

Dockery et al. (1992) conducted an analysis of total daily human mortality in St. Louis, MO, and Kingston-Harriman, TN, during the 1-year period from September 1985 through August 1986 aimed primarily at assessing the effects of PM on mortality. One of the strengths of this study is the fact that multiple air pollutants were measured and considered. Thus, as part of the analysis, O_3 and other gaseous pollutants also were considered and found to have nonsignificant associations with mortality in these cities. The statistical analysis addressed autocorrelation in the mortality data through the use of multiple climate indices (i.e., daily mean temperature, hot day, cold day, humid day, hot and humid day, season, and interactive terms) and through the incorporation of autoregressive modeling. This approach is possibly more conservative than that employed by Kinney and Ozkaynak (1991), and the lack of a significant O_3 coefficient in this analysis may be due in part to the statistical modeling approach, which may or may not have affected an O_3 mortality relationship in the data in the process of addressing autocorrelation and so extensively controlling for temperature (which is usually correlated with O_3 over time). Also, the lack of any O_3 associations with total mortality may be due in part to the relatively low O_3 levels found in these particular communities (especially in Kingston-Harriman, where no O_3 exceedances occurred) during the study year (maximum 24-h mean O_3 <0.065 ppm). Overall, this study did not show an association between O_3 and mortality, but this may in part be a product of the particular methodological and exposure characteristics of this study vis-ŕ-vis the identification of O_3 health effects.

7.4.1.4 Summary and Conclusions

Recent epidemiology studies addressing the acute effects of ambient O_3 have yielded significant associations with a wide range of health outcomes, including lung function decrements, aggravation of preexisting respiratory disease, and increases in daily hospital

admissions and mortality. Individual-level camp and exercise studies clearly indicate that lung function can decrease in a concentration-related manner in response to O_3 exposures occurring in ambient air. The combined results of these studies provide useful, quantitative information on the pulmonary effects of ambient O_3 exposures. Results from daily life studies, although more difficult to interpret quantitatively due to exposure assessment uncertainties, are qualitatively consistent with camp and exercise studies. There is limited evidence from several studies suggesting that ambient O_3-induced lung function decrements may persist for up to 24 h. Results from lung function epidemiology studies generally are consistent with those of human chamber studies. An O_3-related worsening of symptoms in selected groups of healthy individuals and detrimental changes in symptoms, lung function, and medication use in asthmatics have been observed qualitatively and, to a lesser extent, quantitatively. The relationship is consistent, temporally plausible, and moderately coherent.

Emergency room visit and hospital admission studies considered in this document collectively indicate that, when the major confounders to such analyses are addressed (e.g., seasonality, day-of-week effects), consistent associations are seen between acute occurrences of respiratory morbidity and O_3 exposure. The evidence is especially strong for hospital admissions, as the association has been seen by numerous researchers at a variety of localities using a wide range of appropriate statistical approaches. Although the absolute size of the effect varied somewhat across localities and statistical approaches, these analyses suggest that, in the summertime (when many other respiratory illness causes have abated), O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all respiratory hospital visits and admissions. Moreover, certain of these analyses also indicate that, on the highest O_3 days, this pollutant's estimated contribution can increase to the point where it is associated with nearly half of all respiratory hospital admissions. Moreover, significant associations also are seen between O_3 and hospital visits and admissions at exposures below 0.12 ppm 1-h daily maximum O_3.

As was also the case for the O_3-hospital admissions time series studies, many of the older O_3-mortality studies had methodological or statistical weaknesses that prevented clear conclusions. However, since the release of the previous criteria documents, one of the two most useful new studies (Kinney and Ozkaynak, 1991) indicated statistically significant effects by O_3 on short-term (acute) human mortality. The one relevant new study that did not show any O_3 association (Dockery et al., 1992) employed much more extensive climate and autocorrelation control methods and was conducted over a much shorter time period than the other study. Also, the study that showed an O_3-mortality association considered an urban area experiencing 1-h maximum O_3 concentrations above 0.15 ppm, whereas the other study areas (eastern Tennessee and St. Louis, MO) did not. Thus, although the analysis of daily series of human mortality and air pollution has yielded small but statistically significant associations with O_3 in one study, the sensitivity of this association to statistical modeling methods and to O_3 concentration level needs further investigation.

7.4.2 Chronic Effects of Ozone Exposure

7.4.2.1 Introduction

At the time of the publication of the previous EPA air quality criteria document (U.S. Environmental Protection Agency, 1986), little useful data were available on the chronic effects of O_3 exposure. Table 11-10 of that document summarized the limited number of studies available at that time and concluded "…it is unlikely that any of these studies can be

used to develop quantitative exposure-response relationships for ambient oxidant exposures. Further study of well-defined populations over long periods of time is required before any relationship between photochemical oxidants and the progression of chronic diseases can be conclusively demonstrated from population studies" (U.S. Environmental Protection Agency, 1986). The document noted that existing studies failed to demonstrate any consistent relationship between chronic oxidant exposure and changes in pulmonary function, chronic symptoms, chromosomal abnormalities, or chronic disease mortality.

The largest study that had been performed at the time of the 1986 criteria document was that of Detels et al. at the University of California at Los Angeles (UCLA) (Detels et al., 1979, 1981; Rokaw et al., 1980). This study employed a population-based sample of households in selected communities in the Los Angeles South Coast Air Basin. A standardized interview was administered, and individuals underwent various tests of lung function. Air pollution data were derived from a network of monitoring stations maintained by the South Coast Air Quality Management District of the California Air Resources Board (ARB). The usefulness of the findings of this study was considered to be limited due to a number of factors: (1) variable timing of testing in the several study communities over a 4-year period, (2) paucity of data on self-selection (completion rates between 70 to 79%) and migration in and out of the study communities, (3) inconsistent demonstration of reproducibility of the pulmonary function measurements, (4) mixed ethnicity of the study population, (5) inadequate data on individual exposure and failure to adjust exposure estimates for migration in and out of the study areas, and (6) methods employed for comparisons of health effects.

The 1986 criteria document also summarized the first of the Adventist Health Smog (AHSMOG) studies (Hodgkin et al., 1984) on the occurrence of COPD in relation to chronic air pollution exposures. However, the data from this first publication were felt to be of limited value because only symptom data were reported and the exposure assessment was insufficient.

7.4.2.2 Recent Epidemiological Studies of Effects of Chronic Exposure

By the very nature of the problem of the establishment of a link between chronic exposure to O_3 and the occurrence of chronic health effects, epidemiological studies remain the only approach for obtaining human data. As has been noted in the 1986 document, principal problems for such studies relate to (1) the specification of individual exposures over the relevant periods of life of the study subjects; (2) the coincident effects of other oxidant species (e.g., NO_x, derivative acid species) and other air pollutants (acid aerosols, particulate species); (3) seasonal effects that relate to pollutant and meteorologic factors, which affect specific pulmonary function measurements relevant during the course of longitudinal studies or over studies that utilize multiple cross-sectional samples; and (4) control for effects of factors such as occupational exposures, cigarette smoking, etc. In addition, past epidemiologic studies have not had access to any human histologic specimens in relation to the exposure groups under study nor have specific mechanisms been investigated to explain any of the symptom or functional outcomes observed.

Histologic and Immunologic Effects

Sherwin has presented some provocative preliminary, histologic data and uses it to offer a hypothesis on the importance of pathologic changes in the centriacinar region (CAR) of the lung in relation to chronic pulmonary effects of oxidant air pollution (Sherwin, 1991; Sherwin and Richters, 1991). Only the publication that presents the primary data (Sherwin,

1991) is reviewed here, because there is some redundancy in the two available publications. Sherwin (1991) obtained lungs from 107 subjects, 15 to 25 years of age, who died of a sudden death without evidence of overt disease, lived in Los Angeles County, had no autopsy evidence or history of drug use, and had no lung trauma. Abnormalities of the CAR were evaluated by a pathologist who was "blinded" to basic demographic data. Centriacinar region disease was defined as the extension of a respiratory bronchiolitis into the proximal acinar structures (i.e., chronic inflammatory cells and histiocytes into alveolar ducts, sacs, and alveoli immediately adjacent to a respiratory bronchiole). The odds ratio for severe CAR disease in subjects who lived in metropolitan Los Angeles versus those who lived in other cities in Los Angeles County was 4.0 (95% confidence limit [CL], 1.4 to 11.3; a calculation based on data in Sherwin [1991] Tables 2 and 3).

Unfortunately, no exposure data (or lifetime residence data) were available for the subjects in the Sherwin study, nor were smoking histories, cotinine results, or occupational histories available. The smoking history data is of critical importance because respiratory bronchiolitis has been shown to be an early pathologic change found in the pulmonary airways of young smokers (Niewoehner et al., 1974). Additional problems for this study were the fact that most subjects were of low socioeconomic status, and only 10 of the subjects were female. Furthermore, the study is limited by a lack of quantitative morphometry on the lung specimens and by the lack of a control group from an ambient environment with low oxidant pollution. Therefore, although Sherwin's data are of considerable interest, particularly in relation to the primate O_3 exposure data that show similar effects (see Chapter 6, Section 6.2.4), they currently are not of value in the determination of appropriate human exposure levels for O_3, nor do they even establish the fact that the oxidant environment found in metropolitan Los Angeles, indeed, is responsible for the observed pathologic changes.

Zwick et al. (1991) carried out a study of allergic sensitization and cellular immune responses in children (median age of 11 years) from four schools in two Austrian cities. Two years of meteorologic data and continuously measured levels of SO_2, NO_2, and O_3 were available for both cities. Monitors were within 2 km of the study schools, except for one O_3 monitor that was 13 km from a school in the "high"-O_3 area. "Allergic diseases" (rhinitis, conjunctivitis, and asthma), response to prick test antigens, total IgE concentration, number of subjects with IgE >100 kU/L, and total IgG concentration did not differ between the subjects in the two cities. Adjustment for sex, age, active and passive smoking, and types of cooking and home heating did not alter the results. Children from the high-O_3 environment had small, but statistically significant, decreases in the absolute and relative numbers of OKT4+ (helper/inducer) T cells and OKNK+ (natural killer) cells and increases in OKT8+ (suppressor) T cells. Adjustment for active and passive smoking and recent respiratory illness did not alter the results. The frequency of subjects with a measurable PD_2 to histamine also was increased in the high-O_3 area. No relationship between the T-cell findings and PD_2 or any of the other immunologic markers are provided.

The Zwick et al. (1991) results are limited by lack of any exposure data and by lack of detail for the O_3 and other ambient air pollution data. Except for data on the average percentage time above specific levels of O_3, there are no useful data that can be applied to the observations reported. Moreover, the differences observed in the various T-cell subsets were relatively small and of questionable biological significance. There are no analyses that relate the T-cell findings to the clinical and functional data (see Table 7-25) that are reported. Finally, although the communities were said to be similar on all meteorologic and other

ambient pollution data, inspection of the author's Table 1 (Zwick et al., 1991) indicates that the mean (averaging time not given) NO_2 levels in the low-O_3 community were fourfold greater than those in the high-O_3 community (42 $\mu g/m^3$ versus 11 $\mu g/m^3$). No data on acid species or particles are provided, although both study cities were free of heavy industry and heavy traffic.

Calderon-Garciduenâs et al. (1992) have studied chronic exposure to the ambient air of southwestern metropolitan Mexico City in relation to histologic abnormalities of the nasal mucosa. The exposed group consisted of subjects who spent at least 8 h/day while working at a naval hospital in southwestern metropolitan Mexico City. Ninety-two percent of the group lived in the same area as the hospital, and all had lived in southwestern metropolitan Mexico City for >2 mo (n = 47). Controls consisted of (1) subjects who lived in Veracruz and who had not left this area over a period of at least 5 years before the onset of the study (n = 12) and (2) new arrivals (<30-day residence in southwestern metropolitan Mexico City) at the naval hospital who came from low-O_3, "non-polluted" ports (n = 17). Nasal biopsies were obtained for all subjects in May through June, 1990, as were histories on residence, smoking, occupation, allergies, etc. All three groups were matched for age, sex, and occupation. There were no differences in familial allergy history or personal smoking (specific data not given in paper). There was a progressive increase in both nasal symptoms and nasal histologic abnormalities in relation to presumed O_3 exposure (Veracruz < new arrivals < long-term residents of southwestern metropolitan Mexico City). The principal histologic change was basal cell hyperplasia, with squamous cell metaplasia and mucosal atrophy occurring less frequently. Only 11% of those with >60-day residence in southwestern metropolitan Mexico City showed normal mucosa.

Unfortunately, no ambient air data were presented for SO_2 or particles, which are said to be low relative to other parts of the city, or other pollutants that could be present. In addition, because the monthly average maximal O_3 concentrations are (and have been since late 1986) well above the current U.S. 1-h standard of 120 ppb, the Calderon-Garciduenâs et al. (1992) data are of limited value to understanding low ambient O_3 exposures. (This conclusion probably applies even if one considers the different concentrations represented by a given parts-per-billion value at different altitudes.) Subjects in southwestern metropolitan Mexico City are subjected to O_3 levels of between 100 and 400 ppb for several hours per day in the winter and spring. Despite the lack of data on other air pollutants and specific exposure data for individual subjects, this study does provide useful evidence to suggest upper respiratory damage as a consequence of prolonged exposure to ambient air mixtures.

Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease

The Adventist Health Smog Study. Since the publication of the 1986 criteria document (U.S. Environmental Protection Agency, 1986), a number of studies have been published that attempt to define chronic respiratory system health effects in relationship to ambient O_3 concentrations (see Table 7-26). Among these, the series of publications from the AHSMOG study (Hodgkin et al., 1984; Euler et al., 1987, 1988; Abbey et al., 1991a,b) will be discussed first and as a set.

Table 7-25. Pathologic and Immunologic Changes Associated with Chronic Ozone Exposure[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	g/m				
Not provided		Not provided, Los Angeles County, not further specified	Autopsy study of lungs from sudden death victims 15 to 25 years old whose residence was Los Angeles County; examination of lungs for inflammatory changes in CAR of lungs.	Most severe CAR disease in residents of metropolitan Los Angeles County versus other county areas; data limited by lack of smoking history, personal exposure and occupational data; interesting hypothesis, but role of O_3 unknown.	Sherwin and Richters (1991)
0.095 to 0.188, time metric not given	186 to 368	O	Study of allergic sensitization and cellular immune responses in children (median age, 11 years) in two Austrian cities, 1989.	Small increases in OKT4+ (helper/inducer) and OKT8+ (suppressor) T-cells and small decrease in natural killer cells in "high" ozone community; increase in number of subjects with measurable PD_{20} histamine in "high" ozone area; no relationship between T-cell findings and any clinical immunologic measure, lung function, or PD_{20}; meaning of results unclear.	Zwick et al. (1991)
0.150 to 0.275 monthly average	Approx. 294 to 539	O_3	Study of nasal histology in persons living in southwestern Mexico City and Veracruz; subjects matched on age, sex, occupation; similar allergy and smoking histories.	Increased occurrence of nasal dysplasia in southwestern Mexico City residents, especially those with more than 5 years residence; no data on other air pollutants; data not directly relatable to U.S. conditions because Mexico City residents are exposed to O_3 levels between 0.1 and 0.4 ppm for several hours each day, all year long, with relatively few days below 0.1 ppm.	Calderon-Garciduenâs et al. (1992)

[a]See Appendix A for acronyms and abbreviations.

Table 7-26. Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	μg/m				
0.033 median average annual hourly value	65	O_3	Study of relationship of air pollution to levels of FVC, FEV_1, and PEFR based on 1976 to 1980 supplement to NHANES and data from EPA SAROAD monitoring system; subjects 6 to 24 years of age; exposure values based on hourly O_3 values for previous 365 days; data for TSP, NO_2, and SO_2; and data for important demographic, smoking, and health covariates.	Nonlinear relationship between annual average O_3 and function measurements with threshold at approximately 0.040 ppm; findings limited by inability to control for multiple pollutant effects, relatively crude assignment of exposure; data consistent with effect on forced flow at O_3 levels at or below 0.12 ppm.	Schwartz (1989)
0.034 to 0.050 90th percentile annual mean 1-h daily max	67 to 98	O_3	1983 to 1984 cross-sectional study of 2nd- to 6th-grade students in Ontario and Manitoba, Canada; data on SO_2, NO_2, nitrates, and sulfates; respiratory health, demographic, smoking, and home cooking fuel data; and spirometry.	Ontario town had more O_3 days >0.080 ppm; small decrements (2%) in FVC and FEV_1 were found in the Ontario town compared to the Manitoba town; any O_3 possible effects were completely confounded with SO_4 effects.	Stern et al. (1989)
0.024 to 0.031 annual mean 1-h daily max	47 to 61	O_3	1985 to 1986 cross-sectional study of 7- to 11-year-old children from (n = 3,945) five rural towns in Ontario and five towns in Saskatchewan, Canada; data on SO_2, sulfates, NO_3, NO_2, and PM_{10}; respiratory health multiple covariates; spirometry including flow at mid-lung volumes.	Ontario towns had higher levels of O_3 and SO_4 in summer months and for 90th and 99th-percentiles of distributions; 90th percentile mean 1-h maxima were 80 ppb vs. 47 ppb for O_3 and 11.5 $\mu g/m^3$ vs. 3.1 $\mu g/m^3$ for SO_4; magnitude of FEV_1 and FVC effects was similar to Stern et al. (1989); no effect for mid-volume flows, except for subjects with asthma; coincidence of increased O_3 and SO_4 precludes definite statements concerning O_3 effects.	Stern et al. (1994)
0.008 to 0.118 average hourly concen-trations, 1974 to 1979	16 to 231	O_3	Study of chronic respiratory symptoms in adults with use of 1979 National Health Interview Survey data and 1974 to 1979 EPA SAROAD data; data on respiratory health, demography, and smoking; and data for TSP.	Data for only 29% of those eligible could be used; average hourly O_3 concentration over period 1973 to 1979 associated with report of sinusitis and hay fever after control for covariates and TSP; no association with asthma or emphysema; large amount of data reduction, lack of adequate exposure assignment, lack of occupational exposure histories, and lack of adequate data on other pollutants make results very difficult to interpret.	Portney and Mullahy (1990)

Table 7-26 (cont'd). Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	g/m				
0.015 to 0.052 average HMV	29 to 102	O	Cross-sectional study of children ages 6 to 15 years in a community in Austrian alps divided into three zones based on SO_2, NO_2, and O_3; respiratory health, demographic, and spirometry data.	Only difference in respiratory history was increased adjusted prevalence of asthma in zone with highest O_3 (6.4%; 0.052 ppm HMV) vs. the zones with lower O_3 concentrations (4.8%; 0.015 ppm HMV; 2.7%, 0.026 ppm HMV); no meaningful differences in spirometry indices; data limited by use of single monitoring site for 1,200 km^2 area; effects of SO_2 and NO_2 on asthma prevalence not well studied.	Schmitzberger et al. (1993)
0.10 to 0.20 3-mo mean daily peak hourly values for Lancaster and Glendora, respectively	196 to 392	Oxidants	5-year follow-up of Lancaster and Glendora, CA, cohorts; from UCLA population study of CORD restricted to nonsmoking, non-Hispanic whites, 7 to 59 years old.	No difference in respiratory symptoms over follow-up for either community; across all age groups, slope of Phase III of N_2 washout deteriorated more rapidly in Glendora; in subjects 14 years of age, more rapid decrease in spirometric indices in Glendora; interpretation hampered by large losses to follow-up, inability to disentangle multiple pollutant effects.	Detels et al. (1987)
0.04 to 0.07 mean peak daily peak hourly values 1972 to 1981; Long Beach and Lancester, respectively	78 to 137	Oxidants	5- to 6-year follow-up of Lancaster and Long Beach, CA, cohorts from UCLA CORD study; Long Beach with higher NO_2, SO_4, and TSP than Lancaster.	All reported excess functional decline for Long Beach likely due to bias in decline estimates between locations; data not useful with regard to possible O_3 effects.	Detels et al. (1991)
Not reported		Oxidant	Prevalence of respiratory symptoms in nonsmoking Seventh Day Adventists residing for at least 11 years in high- (South Coast) and low- (San Francisco, San Diego) photochemical air pollution areas of California; ARB regional air basin monitoring data for oxidants, NO_2, SO_2, CO, TSP, and SO_4 from 1973 to 1976.	Slightly increased prevalence of respiratory symptoms in high pollution area; after adjusting for covariables, 15% greater risk for COPD due to air pollution (not specific to oxidants); past smokers had greater risk than never-smokers; when past smokers were excluded, risk factors were similar. In addition, insufficient exposure assessment and confounding by environmental conditions limit the quantitative use of this study.	Hodgkin et al. (1984)

Table 7-26 (cont'd). Effects of Chronic Ozone Exposure on Pulmonary Function, Respiratory Symptoms, and Chronic Respiratory Disease[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	$\mu g/m^3$				
Not reported		Oxidant	Cross-sectional analysis of above populations; uses hours above various "threshold" values for oxidants, TSP, SO_2 based upon California, EPA, and World Health Organization max levels; period covered, 1966 to 1976; data available for important covariates (sex, occupation, environmental tobacco smoke, race, age, education, past smoking).	OX (10) most significantly associated with COPD after adjustment for covariates; number of hours above higher thresholds less significant; when TSP, SO_2, and OX (10) entered in same regression, TSP (200) only pollutant associated with COPD; high correlation between OX (10), TSP (200), and SO_2 (hours more than 4 pphm). Improved exposure assessment over previous paper; however, no clear statement possible about effects of oxidants due to colinearity with TSP and SO_2.	Euler et al. (1988)
Not reported		O_3	Same as Abbey et al. (1991a) but analysis applied to COPD severity and a "multi-pollutant" analysis performed; also evaluated effect of using data for different time periods of ambient air monitoring.	Cumulative incidence of COPD symptoms when each pollutant entered separately, similar to above study; joint effects of OZ (10) and TSP (200) and mean concentrations of each pollutant evaluated only for cumulative asthma incidence; TSP (200) entered logistic regression in preference to OZ (10) but mean O_3 concentration entered in preference to mean TSP; change in asthma severity associated with mean O_3 concentration (1977 to 1987) and with exceedance frequency for OZ (10), OZ (12), and TSP (200) considered separately; findings for asthma severity similar to cumulative incidence when TSP and O_3 evaluated together; in no analysis did TSP and O_3 both remain jointly significant, nor were there any interactions; data unable to unequivocally disentangle effects of individual pollutants.	Abbey et al. (1993)

[a]See Appendix A for acronyms and abbreviations.

The basic population for these studies represents California-resident, Seventh-Day Adventists aged 25 years of age who had lived 11 years or longer (as of August 1976) in either a high-oxidant-polluted area (South Coast Air Basin [Los Angeles and vicinity] and a portion of the nearby Southeast Desert Air Basin) or a low-pollution area (San Francisco or San Diego). This sample was supplemented by an additional group of subjects who met the 11-year residence requirement but who came from low-exposure rural areas in California. The total, baseline sample (March 1977) comprised 8,572 individuals, of whom 7,267 enrolled. From this group, 109 current smokers and 492 subjects who had lived outside of the designated areas for a portion of the previous 11 years were excluded. Detailed respiratory illness and occupational histories were obtained. In these studies, "COPD" refers to "definite chronic bronchitis", "definite emphysema", and "definite asthma" as defined by the study questionnaire. Measures of pulmonary function are not included.

Air monitoring data were obtained from the California ARB monitoring system. Ninety-nine percent of the subjects (excluding the rural supplement) lived at a distance from the nearest ARB monitoring site that was considered to provide relatively reliable concentration estimates for the outdoor, ambient environment at their residence. Concentrations at the monitors were interpolated to the centroid of each residential zip code from the three nearest monitoring sites with the use of a $1/R^2$ interpolation. Subsequent development of exposure indices took account of the improvements in ARB data after 1973. Data were available for total oxidants, O_3, TSP, SO_2, NO_2, CO, and SO_4 (excluding 1973 to 1975).

The initial report from this study was summarized in the 1986 criteria document (U.S. Environmental Protection Agency, 1986). Based upon a multiple logistic regression that adjusted for smoking, occupation, race, sex, age, and education, it was estimated that residence in the South Coast Air Basin conferred a 15% increase in risk for prevalent COPD. No estimates of exposure were provided, and the data were considered to be of limited utility.

In their 1988 publication, Euler et al. provided exposure estimates based on the cumulative number of hours, over 11 years prior to the baseline, that individuals lived in environments at various oxidant thresholds, beginning at 10 pphm [OX (10)] (196 $\mu g/m^3$) and the total dosage to which they would be exposed. The estimates in this report did not correct for time spent indoors. When the OX (10) was the only pollutant considered, each 750 h/year increment in exposure was associated with a 20% increase in risk for COPD in a multiple logistic regression analysis that adjusted for effects of occupation, passive exposure to tobacco smoke, personal smoking, sex, age, race, and education (baseline data only). Moreover, the data were compatible with a threshold effect at 10 pphm. However, when hours above a TSP concentration of 200 $\mu g/m^3$ [TSP (200)] and SO_2 concentration of 4 pphm were included in the logistic regression model, only TSP (200) was associated with the occurrence of COPD. No significant interactions were found between the various pollutant thresholds. The authors noted that their failure to control for time spent indoors may have led to an underestimation of the oxidant effect. Moreover, the fact that 74% of the variance of OX (10) was explained by the other pollutants certainly reduced the power of this study to detect an independent effect of oxidants on the occurrence of COPD. The authors also noted the limitations imposed by the cross-sectional nature of the data that were used in this analysis. Thus, on the basis of this study, no clear statement could be made about the chronic respiratory system effects of oxidant exposure.

A major improvement in the methods for assessment of exposure was presented in Abbey et al (1991a). Previous exposure estimates were refined by the computation of "excess concentrations" (concentration minus cutoff, summed over all relevant time periods and corrected for missing data). Exposures also were corrected for time spent at work and time away from residence, with estimates provided for the environments where work occurred and for geographic areas away from residence. The quality of the interpolations (in terms of distance of monitor from residence zip codes) also was evaluated and incorporated into the estimates. Adjustments were made for the time spent indoors by individuals. New indices were developed that were based on O_3, rather than on total oxidants. The investigators demonstrated correlation coefficients of 0.98 between monthly mean total oxidants and O_3 at concentrations 12 pphm. (It should be noted that a more appropriate comparison would have been between the mean and the differences of the two measurements.)

The above estimates were applied to data that included 6 years of follow-up of the study population (Abbey et al., 1991b). This analysis focused on incident occurrence of obstructive airways disease (AOD—same definition as for COPD above). Incident symptoms of AOD were significantly associated with hours above several TSP thresholds, but not with hours above any O_3 threshold. There was a suggestion of an association between hours above 10 pphm O_3 [OZ (10)] and the 6-year cumulative incidence of asthma [RR for 500 h/year above OZ (10) = 1.40 (95% CL = 0.90 to 2.34)] and definite bronchitis (RR = 1.20 [95% CL = 0.97 to 1.53]). Approximately 43% of the study population experienced at least 500 h in excess of the OZ (10) criterion. Cumulative incidence estimates were adjusted with the use of Cox proportional hazard models for the same variables noted in the original publication of Hodgkin et al. (1984), as well as the presence of possible symptoms in 1977 and childhood respiratory illness history. None of the analyses included both O_3 and TSP thresholds. No data were provided on the details of the subjects available for the prospective analysis and their representativeness versus the entire base population. Therefore, assuming no bias due to selective loss to follow-up, these data are consistent with a small O_3 effect and are limited by the same considerations of colinearity and subsequent reduction of power noted above.

Another analysis by Abbey et al. (1993) evaluated changes in respiratory symptom severity with the TSP and O_3 thresholds noted above. In this analysis, logistic regression, rather than Cox proportional hazard modeling, was used to assess cumulative incidence of components of the COPD/AOD complex; and multiple, linear regression was used to evaluate changes in symptom severity. When O_3 was considered by itself, there was a trend toward an increased risk of asthma for a 1,000-h average annual increment in the OZ (10) criterion (RR = 2.07, 95% CL = 0.98 to 4.89). In this analysis, there was a suggestion that recent ambient O_3 concentrations were more related to cumulative incidence than past concentrations. Change in asthma severity score was associated significantly with the 1977 to 1987 average annual exceedance frequency for O_3 thresholds of 10 and 12 pphm. No significant effects were found for COPD or bronchitis alone. In contrast to the above study of cumulative incidence, the investigators carried out an analysis in which TSP (200) and OZ (10) were allowed to compete for entry into a model to evaluate asthma cumulative incidence and changes in severity. In the cumulative incidence model that employed exceedance frequencies (number of hours above threshold), TSP (200) entered before OZ (10); when average annual mean concentrations were used, O_3 entered before TSP. From this, the authors concluded that both TSP and O_3 were relevant to asthma cumulative incidence. In no case did both pollutants remain significant

simultaneously in the same regression. No interactions were observed between TSP and O_3 for either metric. A similar result was observed for change in asthma severity. As in previous analyses, there was a high correlation between TSP (200) and OZ (10) exceedance frequencies (0.72) and their respective average annual mean concentrations (0.74).

The AHSMOG study represents the most extensive effort to date to provide realistic exposure estimates within the constraints of a large, population-based study. Moreover, the exposure estimates for photochemical oxidants have been tied to current O_3 levels and have taken into account many of the sources of inaccuracy and imprecision in the assignment of exposures to individuals (short of detailed personal monitoring). As such, they do represent a considerable improvement over all other studies to date. Nonetheless, it is not possible from these data to determine if there is an effect of O_3 on the outcomes that were studied. This largely is due to the difficulty of partitioning effects between O_3 and particles.

Other Studies. Subsequent to the publication of the 1986 criteria document, two additional publications have emerged from the UCLA study (Detels et al., 1987, 1991). The data presented are derived from the same population bases that were used in previous publications; and, therefore, they are subject to the same limitations that were cited in the introduction to this section.

In 1987, Detels et al. reported a 5-year follow-up study of white, non-Hispanic subjects from the Lancaster and Glendora study areas. The 12- and 3-mo mean peak hourly total oxidant values from 1972 to 1982 for Lancaster and Glendora were 7 and 10 pphm and 11 and 20 pphm, respectively. Only 47 and 58% of subjects, respectively, were retested with both the questionnaire and measures of lung function. Effects of air pollution on the days of testing were evaluated by comparing lung function test results in a subgroup of individuals who were tested three times at 3- to 4-mo intervals. No effect was observed, but the power to find differences was low. Over the follow-up period, there were no changes in reported respiratory symptoms for either community. In adults (≥19 years of age) who never smoked, all spirometric and nitrogen-washout results showed more rapid deterioration in Glendora. Differences were significant only for mid-expiratory flows and for slope of Phase III from the nitrogen-washout curve. The effects were greater in females, in whom changes in FEV_1 also were significant. In subjects less than 19 years of age, only changes in slope of Phase III were significant, although FVC in Glendora females was lower than that observed in Lancaster.

The results of this study remain limited by the lack of adequate exposure data and the failure to control for the possible effects of other ambient pollutant differences between the communities. Problems with loss to follow-up represent a significant issue, especially for the pulmonary function measurements, given that approximately 50% of the original subjects were not available for repeated testing. Baseline comparability is also of concern because subjects who were retested in Lancaster had a better slope of Phase III than those not retested. Because this measure most consistently differed between the two study communities, the possibility of selection bias is very real. Overall, these results do not strengthen the usefulness of this study for the attribution of an effect of oxidant exposure on respiratory health.

The 1991 report from the UCLA group compared Lancaster with Long Beach, the latter area with relatively high levels of SO_2, sulfates, NO_x, and hydrocarbons, as well as increased total oxidant levels (mean 1-h daily peak values, 1972 to 1982, 30 pphm versus 110 pphm, respectively) (Detels et al., 1991). As above, the analysis was restricted to non-Hispanic whites who never smoked cigarettes and with 5 years of follow-up. Only 47% of the Lancaster cohort and 44% of the Long Beach cohort had pulmonary function retested on two

occasions. Over the age range of 25 through 59 years, changes in slope of Phase III of the nitrogen-washout curve and most spirometric indices were significantly worse in Long Beach, compared to Lancaster. In subjects under 25 years of age, there were significant differences in slope of Phase III, especially in subjects 7 to 10 years of age.

All of the limitations identified for the 1989 report apply to this report as well. Moreover, comparison between the two communities of the interlaboratory differences (mobile laboratory versus UCLA reference laboratory; 3% sample) indicated that average annual decrements in FEV_1 were exaggerated by 13 mL/year (standard error $\pm$7 mL/year) in Long Beach versus 2 mL/year ($\pm$7 mL/year) in Lancaster. Application of this difference to the data in their Table 6A would suggest that the "significant" difference in FEV_1 for both males and females may be largely, if not entirely, due to bias. Thus, all of the functional differences reported in this study are suspect on this basis alone. This, of course, ignores any additional biases that may have been due to the large losses to follow-up in both communities.

A number of additional studies have addressed data relevant to the chronic effects of O_3 on respiratory health (see Table 7-26).

Schwartz (1989) evaluated the effect of air pollution on children and young adults ages 6 to 24 years with the use of data derived from NHANES II (Second National Health and Nutrition Examination Survey, February 1976 to 1980). All individuals in each census tract were assigned average pollutant values derived from monitors located within 10 miles of the centroid of the census tract. Average hourly values for the 365 days preceding spirometry were used, and an annual average was created for O_3 (EPA Storage and Retrieval of Aerometric Data [SAROAD] database). For O_3 (chemiluminescence and ultraviolet [UV] spectroscopy), six of the seven hourly readings between 11:00 a.m. and 5:00 p.m. were required to include a day's data. Only 1,005 of the 3,922 (25.6%) of the subjects lived close enough to a monitor to have O_3 exposures assigned to them. Data for TSP, NO_2, and SO_2 were assigned to 47.1, 13.6, and 21.2% of the subjects, respectively. Analyses were restricted to consideration of single pollutants, because the author reported that there was insufficient overlap between the locations where data were available for all or any combination of pollutants. Data for a variety of relevant personal and demographic covariates were available. Statistical analyses appropriate to the correlation structure of the data (induced by the sampling design of NHANES) were utilized. There was a nonlinear relationship between the annual hourly average O_3 concentration and FVC, FEV_1, and PEFR. A threshold of effect around 0.04 ppm was observed, above which there appeared to be a linear decline in FVC (only data shown graphically). The effect persisted after control for sex, race, age, family income, educational level, chronic respiratory symptoms, and smoking history. Results were little affected by region or use of a 2-year averaging time. Ozone levels above the threshold were significantly associated with an FVC $<$70%, a result not seen for TSP but observed for NO_2.

The major limitation of the Schwartz (1989) analysis is the inability to distinguish between the effects of O_3, TSP, and NO_2 and the choice of only a single metric for O_3 (hourly average). Support for the former concern can be seen in the similarity of the effects of NO_2 and O_3 in the logistic regression analyses, which suggests that the results could reflect the joint effect of a number of species in a complex oxidant environment. The operating assumption that near-term (1- to 2-year) exposure reasonably reflects a "lifetime" of exposure is highly suspect in the mobile U.S. population. In fact, restriction of the analysis to subjects who still resided in the state in which they were born led to slight reductions of O_3 effect, especially for FEV_1 and PEFR. Despite these limitations, the data do suggest that, for children and young

adults, if there is a chronic O_3 effect (or, more accurately for these data, a subchronic effect) on lung function, it could occur at levels at or below 120 ppb. However, the particular pattern of exposure (peaks, season, etc.) that may be relevant cannot be discerned from these data.

In 1989, Stern et al. reported a cross-sectional study, conducted in 1983 and 1984, of the relationship between respiratory health effects of second- through sixth-grade children in two Canadian communities (one in southern Ontario and one in southern Manitoba). The Ontario region was characterized by low levels of gaseous pollutants (SO_2 and NO_2) and moderately elevated levels of particulate sulfate, FPs, and O_3. Frequent episodes of elevated sulfate and O_3 concentrations occurred in the summer and early fall. The Manitoba community was not subject to the same pattern of air pollutants. Gases and O_3 (measured by chemiluminescence) were sampled continuously, and TSP, sulfates, and total nitrates were sampled every sixth day. Fixed monitoring stations were established at the center of each community, and monitoring was carried out from October 1983 to April 1984. Ozone measurements in Ontario were derived from sites between 35 to 45 km from the study area. Average annual maximum O_3 concentrations were similar in the two communities (0.136 and 0.130 ppm in Ontario and Manitoba, respectively), but the frequency of elevated O_3 events (>0.080 ppm, Canadian standard for 1-h maximum) was more frequent in Ontario (30 days) than in Manitoba (3 days) in 1983. Ninety-two percent of the subjects (n = 1,317) provided data from detailed questionnaires, but only 70% (1,010) provided spirometric data (tested in fall and winter months). There were no meaningful differences in the prevalence of all of the respiratory health outcomes studied after adjustment for parental smoking, gas cooking, sex, length of residence, parental education, and past respiratory illness history. Ontario children had a 2% lower FVC (adjusted for age, sex, height, and parental smoking) and a 1.7% lower FEV_1; both differences were statistically significant. The differences were somewhat greater when children with underlying respiratory illness or symptoms were excluded from the analyses. These data are very difficult to interpret in relation to O_3 due to the marked colinearity between the O_3 and sulfate levels in Ontario. Moreover, the differences observed in lung function are very small (an average of 50 mL and 40 mL for FVC and FEV_1, respectively) and of questionable importance without further follow-up data on the subjects. Such follow-up data would need to attempt to identify whether the small decrements observed are "across the board" with respect to the overall population or the result of decrements in a susceptible subset of the population, particularly a set of children at the lower end of the pulmonary function distribution. In these children, small decrements might be associated with adverse respiratory effects as a result of their already lowered (absolute or relative) levels of lung function.

Stern et al. (1994) extended the 1983 and 1984 (Stern et al., 1989) study to 10 rural Canadian communities. Five towns in southwestern Ontario and five towns in Saskatchewan were selected and studied between September 1985 and March 1986. Children 7 to 11 years of age were studied (n = 3,945) with techniques similar to the previous study. In 1986, SO_2, NO_2, and O_3 were monitored continuously through a 10-site network (one site in each town). Particles were sampled every 3 days for 24 h in Saskatchewan and every 6 days in Ontario. Annual mean 1-h maximum O_3 concentrations were slightly higher in Ontario, but the 90th and 99th percentile values were much greater (90th: 80 ppb versus 47 ppb; 99th: 115 ppb versus 57 ppb). This was particularly true for the months of June to August. The levels of PM_1 and nitrate did not vary between the areas and were well within the Canadian Ambient Air Quality

Objectives. Annual mean SO_4 levels were three to four times greater in Ontario communities (6.6 $\mu g/m^3$ versus 1.9 $\mu g/m^3$).

The adjusted (age, sex, parental education, gas cooking, and parental smoking) prevalence of respiratory symptoms did not differ among the 10 communities. Adjusted (height, weight, plus the above adjustment factors) FVC and FEV_1 averaged 1.7 and 1.3% less, respectively, in the five Ontario towns. No differences were observed for PEFR, $FEF_{25\ 75\%}$, or $\dot{V}_{max5\ \%}$. The results did not change when the analysis was restricted to life-long residents or to children without respiratory symptoms. Although not statistically significant, Ontario children with doctor-diagnosed current asthma had $FEF_{25\ 75\%}$ and $\dot{V}_{max5\ \%}$ levels that were 6.6 and 6.5%, respectively, lower than similar children in Saskatchewan. Overall, the prevalence of asthma was 4% for the entire sample.

These results are consistent, in terms of the magnitude of the FEV_1 and FVC effects, with those of the previous Stern study (Stern et al., 1989). In addition, these data provide suggestive evidence of enhanced effects for children with current asthma. The two major limitations of the study are recognized by the authors: (1) the effects observed cannot be attributed to O_3 or to SO_4 (or acid) aerosols and could be due to either part of the pollutant mixture or attributed to the combination of the component, and (2) the differences in the mean values reported do not take into account the variability in the pulmonary function distribution and the variability of responses across the distribution (see above).

Portney and Mullahy (1990) used the 1979 U.S. National Health Interview Survey and SAROAD data to explore the relationship between O_3 and TSP and chronic respiratory disease. Average hourly O_3 concentrations from 1974 to 1979 were used; data from 1974 to 1979 and data from 1979 alone were evaluated. Individuals were matched to the nearest centroid of the census tract in which they lived in 1979. Individuals were excluded if they lived >20 miles from the nearest monitor. Only 29.3% of the 4,500 adults surveyed who participated in the smoking and respiratory disease supplemental interview and for whom residential data were available could be included. Seven different model specifications (probit analysis) evaluated cumulative (5-year) and 1-year effects of O_3 on various respiratory diseases. Hourly average O_3 concentrations, but not TSP concentrations, over 1974 to 1979 were significantly associated with the report of sinusitis and hay fever after control for smoking, sex, income, race, education, temperature, and stability of residence. In contrast, neither O_3 nor TSP were associated with reported asthma and emphysema. An enormous amount of data reduction, the lack of individual exposure data, lack of specification of the age and sex distribution of the study population, lack of data on occupational exposures, the use of a single O_3 metric, and the restricted formulation of the particulate data all severely limited the usefulness of these data.

Kilburn et al. (1992) studied the effect of "air pollution" on expiratory flows and vital capacity in Mexican-American children in Los Angeles. In 1984, 556 second- and fifth-grade students were studied, and 251 of these were studied again in 1987. The analytical strategy, the losses to follow-up, and the lack of reasonable exposure data make the data from this study virtually uninterpretable.

A study by Castillejos et al. (1992) evaluated the effects of acute exposures to ambient O_3 concentrations on pulmonary function and respiratory symptoms. One-hundred and forty-eight 9-year-old children in the southwest part of Mexico City were studied between January and June 1988. Weekly spirometric measurements were made over 10 weeks. Ambient air data were obtained from the monitoring system maintained by the Mexican

government and included hourly values for temperature, RH, and O_3 concentration. Ozone concentration exceeded 120 ppb on 74% of days and "frequently" exceeded 240 ppb. No data are presented for SO_2 or particles. All subjects had to live within 5 km of a monitoring station. The study demonstrated that levels of FEV_1 and $FEF_{25\ 75\%}$ were associated with mean hourly O_3 levels in the preceding 24, 48, and 168 h. The authors interpreted their data as consistent with a subchronic effect of O_3 on measures derived from spirometry that may be due to an "inflammatory process". However, this interpretation seems at odds with the statement in the paper that the initial FEV_1 measurements for the group did not differ from those observed in a comparable age group in the Harvard Six Cities Study who were not exposed to O_3 concentrations as high as those reported in this study. If the overall level of pulmonary function of this group does not differ from those children who live in ambient environments with far lower O_3 concentrations, the data would suggest that the subchronic effects observed are not translated into persistent abnormalities, at least as can be observed with spirometry.

Austrian investigators (Schmitzberger et al., 1992) described a cross-sectional study of the effects of O_3 on the respiratory health of 1,156 children, ages 6 to 15 years. Pulmonary function in two different areas with differing "annual" O_3 concentrations (actual metric on which "annual" based not given) were compared (52 ppb versus 26 ppb). No differences were observed for FVC. All flow measures (FEV_1, $FEF_{5\ \%}$, and $FEF_{75\%}$) were significantly lower in the children in the "high"-O_3 area. These data are of limited value for a variety of reasons, the most important being lack of individual exposure data, lack of data on other pollutants, the uninterpretable specification of "annual" O_3 concentration, lack of data on chronic respiratory illness (especially asthma), and the lack of data on smoking for the teenage members of the subject group.

Schmitzberger et al. (1993), following up on their preliminary data (Schmitzberger et al., 1992), studied additional subjects in the Austrian Tyrolian Alps. Three zones were identified based upon ambient air conditions: (1) Zone 1 was characterized by annual mean SO_2 (UV fluorescence) of 20 $\mu g/m^3$, monthly mean NO_2 (Palmes tubes) of 17 ppb, and annual mean O_3 (chemiluminescence) of 15 ppb (maximum half-hour mean = 102 ppb); (2) Zone 2 was characterized by values of 14 $\mu g/m^3$, 13 ppb, and 26 ppb (112 ppb), respectively; and (3) Zone 3 was characterized by 12 $\mu g/m^3$, 8 ppb, and 52 ppb (146 ppb), respectively. Children ages 6 to 15 years who lived in the study areas for $\geq$3 years were enrolled. Respiratory health questionnaire data and forced expiratory flows were obtained. Full data were available from 81% of the enrolled subjects. Adjusted (age, sex, environmental tobacco smoke, socioeconomic status, and home heating) levels of FVC and forced flows did not follow the gradient in O_3 concentrations. Although Zone 3 differed significantly from Zone 2 on several measures, there were no meaningful differences with Zone 1. Adjusted asthma prevalence was highest in Zone 3 (6.4% versus 4.8 and 2.7% for Zones 1 and 2, respectively). There were no differences for other respiratory symptoms. Although the authors conclude that "residence in the area of elevated O_3 increases the risk…of low small airway-related lung function," careful inspection of the data does not support this conclusion. This conclusion is based on the supposed increased frequency of FEV_1 of less than 70% in Zone 3 relative to the other zones, although the specific data are not provided. Moreover, the mean levels for all functional measurements are lowest in Zone 1, the zone with the lowest O_3 concentrations and the highest SO_2 and NO_2 concentrations. This report is handicapped by the lack of any information that could be used to access individual exposures. Moreover, only a single monitoring station was employed that was placed at the center of Zone 1, which itself was at

the center of the study area (1,200 km^2). No information is provided as to how the concentrations of the various pollutants were estimated for Zones 2 and 3. Therefore, these data virtually are of no quantitative value.

Other Chronic Disease Morbidity and Mortality

Only AHSMOG study has provided any data on possible O_3-related health effects other than those related to the respiratory system or malignant disease of the respiratory system (Abbey et al., 1991b; Mills et al., 1991) (see Table 7-27). The population studied and the assignment of exposures has been presented previously (Hodgkin et al., 1984; Euler et al., 1988; Abbey et al., 1991a).

In their initial study based on 6 years of follow-up, Mills et al. (1991) found that for 500 h in excess of the OZ (10) threshold, there was a relative risk of 2.24 for respiratory cancer incidence after adjustment for a number of factors listed previously. When the TSP (200) and OZ (10) thresholds were allowed to compete for entry into a Cox proportional hazards model for respiratory cancer incidence, the O_3 threshold entered in preference to TSP. Ozone exposure was not associated with excess respiratory cancer mortality or incidence of nonrespiratory cancer over the 6-year follow-up period.

A second chronic disease study from the AHSMOG population extended the above observations to include myocardial infarction and all-cause mortality (Abbey et al., 1993). Incident chronic respiratory disease also was included in this analysis. Ambient levels of O_3 were not associated with incidence of myocardial infarction at any of the threshold indices that were tested. Neither the mean concentration of O_3 nor any of the thresholds were associated with incidence of chronic respiratory diseases, as previously defined. However, there was a trend toward an association between 6-year cumulative incidence of asthma and 500-h exceedance of the OZ (10) threshold (RR = 1.40, 95% CL = 0.99 to 2.34).

7.4.2.3 Conclusions

The body of data that has accumulated since publication of the previous air quality criteria document for O_3 (U.S. Environmental Protection Agency, 1986) provides only suggestive evidence for health effects of chronic O_3 exposure. Most of the studies suffer from one or another of the following limitations: (1) simplistic assignment of exposure in terms of choice of O_3 metrics or adequate adjustment for relevant covariates and (2) lack of ability to isolate effects related to O_3 from those of other pollutants, especially the particulate fraction. The AHSMOG study has made substantive progress in the problem of the assignment of individual exposures (Abbey et al., 1991a). Unfortunately, the results from this study cannot disentangle the effects of chronic O_3 exposure from those due to chronic exposure to the particulate fraction of ambient pollution. The study also lacks sufficient power to evaluate the possibility of interactions between O_3 and particulate pollution in relation to health effects. Thus, the overall data are not conclusive, but current evidence is suggestive of possible health effects from chronic exposure to O_3.

Table 7-27. Effects of Chronic Ozone Exposure on the Incidence of Cardiovascular and Malignant Diseases[a]

Concentrations(s)		Pollutants and Environmental Variables	Study Description	Results and Comments	Reference
ppm	g/m				
Not reported		Hodgkin et al. (1984)	Hodgkin et al. (1984) and Abbey et al. (1991a); analysis based upon exceedance frequencies, 1973 to 1977, and cancer cumulative incidence, 1977 to 1982.	Exceedance of OZ (10) threshold borderline associated with respiratory cancer; no association with mean concentration; multipollutant analysis with TSP (200) and OZ (10), only OZ (10) entered the logistic regression for respiratory malignancy; TSP (200) was significant for females for all malignancy; no association between O_3 and any measure of cancer mortality; overall results suggestive of O_3 effect on respiratory cancer morbidity at level of exposure within range experienced by large percentage of study population.	Mills et al. (1991)
Not reported		See above	See above	No association between any O threshold and all-cause mortality or incidence of myocardial infarction.	Abbey et al. (1991b)

[a]See Appendix A for acronyms and abbreviations.

7.5 Summary and Conclusions

7.5.1 Controlled Human Studies of Ozone Exposure

7.5.1.1 Effects on Pulmonary Function

Controlled human O_3 exposure studies have provided the strongest and most quantifiable exposure-response data on the health effects of O_3. This chapter reviews the results of studies involving subjects exposed to O_3 concentrations ranging from 0.08 to 0.75 ppm O_3 while at rest or during CE or IE of varying intensity for periods of up to 8 h. In many of these studies, small sample size and suboptimal experimental design limit the ability to generalize to the larger population. Of particular concern in considering studies with small sample sizes is the risk of making a beta (Type II) error: the incorrect conclusion that no difference exists between treatments when comparisons are not significantly different. The likelihood of making a Type II error greatly limits the ability to determine the minimum O_3 concentration that results in a significant pulmonary response in the larger population. As a result, the conclusions drawn from many of the studies cited in this chapter may underestimate the presence of responses at low O_3 concentrations in healthy, young adults.

Healthy Subjects

Results from studies of at-rest exposures to O_3 for 2 h in healthy adult subjects have demonstrated decrements in forced expiratory volumes and flows occurring at and above 0.5 ppm O_3 (Folinsbee et al., 1978; Horvath et al., 1979). Airway resistance is not clearly affected during at-rest exposure to these O_3 concentrations.

With moderate IE for 2 h, eliciting a $\dot{V}_E$ of 30 to 50 L/min, decrements in forced expiratory volumes and flows, secondary to decreases in IC, have been observed in healthy adult subjects at and above 0.3 ppm O_3 (Folinsbee et al., 1978; Seal et al., 1993). With IE ($\dot{V}_E$ ≈ 65 L/min), pulmonary symptoms and decrements in forced expiratory volumes and flows are present following 2-h exposures to 0.12 ppm O_3 (McDonnell et al., 1983). Symptoms are present and decrements in forced expiratory volumes and flows occur at 0.16 to 0.24 ppm O_3 following 1 h of continuous heavy exercise ($\dot{V}_E$ ≈ 55 to 90 L/min) (Adams and Schelegle, 1983; Folinsbee et al., 1984; Avol et al., 1984; Gong et al., 1986) and following 2 h of intermittent heavy exercise ($\dot{V}_E$ ≈ 65 to 68 L/min) (McDonnell et al., 1983; Kulle et al., 1985; Linn et al., 1986). With longer exposures of 4- to 8-h duration, responses have been observed at lower O_3 concentrations and lower ventilation rates. In the range of concentrations between 0.08 and 0.16 ppm, a number of studies using moderate IE and durations between 4 and 8 h have shown significant responses under the following conditions: 0.16 ppm for 4 h of IE at $\dot{V}_E$ ≈ 40 L/min (Folinsbee et al., 1994), 0.08 to 0.12 ppm for 6.6 h of IE at $\dot{V}_E$ ≈ 35 to 40 L/min (Folinsbee et al., 1988; Horstman et al., 1990), and 0.12 ppm for 8 h of IE at $\dot{V}_E$ ≈ 40 L/min (Hazucha et al., 1992). Symptom and spirometry responses were increased, with increased duration of exposure, O_3 concentration, and total ventilation. Airway resistance is only modestly affected with moderate or even heavy exercise combined with O_3 exposure to concentrations as high as 0.5 ppm O_3 (Folinsbee et al., 1978; McDonnell et al., 1983; Seal et al., 1993). Increased breathing frequency (f) and decreased V_T, while maintaining $\dot{V}_E$, occur with exposure to 0.20 to 0.24 ppm O_3 when combined with heavy exercise for 1 to 2.5 h (McDonnell et al., 1983; Adams and Schelegle, 1983). Differences in response to a given O_3 concentration among individuals have been shown to be reproducible (Gliner et al., 1983; McDonnell et al., 1985b), indicating some individuals are consistently more responsive to O_3 than others.

Group mean decrements in pulmonary function can be estimated roughly when expressed as a nonlinear function of effective (i.e., exposure) dose of O_3, the simple product of O_3 concentration, mean ventilation, and exposure duration (Silverman et al., 1976; Folinsbee et al., 1978; Adams et al., 1981). The O_3 concentration appears to make a greater impact on the pulmonary function response than does $\dot{V}_E$ or exposure duration (Folinsbee et al., 1978; Adams et al., 1981), and, indeed, Larsen et al. (1991) suggest an exponent of approximately 4/3 for the O_3 concentration. Another way of expressing this relationship is that doubling the O_3 concentration under any given exposure scenario will have a greater impact on spirometry responses than doubling either $\dot{V}_E$ or exposure duration. However, at any given O_3 concentration, the major external determinants of response are $\dot{V}_E$ and exposure duration. Because of the broad range of intersubject variability, and the inability to identify characteristics that influence this variability (other than age), efforts to estimate or model individual responses have so far been fruitless (McDonnell et al., 1993). Nevertheless, prediction of group mean FEV_1 responses using the variables of the O_3 concentration, $\dot{V}_E$, and exposure duration can be successful (Adams et al., 1981; Folinsbee et al., 1978, 1988; Hazucha, 1987; Hazucha et al., 1992; Larsen et al., 1991; McDonnell et al., 1993).

In acute O_3 exposure studies of 3 h or less in duration, the responses observed during and following acute exposure to O_3 at concentrations between 0.12 and 0.50 ppm in normal, healthy human subjects include decreases in TLC, IC, FVC, FEV_1, $FEF_{25\ 75\%}$, and V_T and increases in SR_{aw}, f, and airway responsiveness. Ozone exposure also has been shown to result in the symptoms of cough, PDI, SB, throat irritation, and wheezing. Similar responses are seen with 4- to 8-h exposures in the O_3 concentration range between 0.08 and 0.16 ppm.

When viewed collectively, these physiological and symptom responses may be separated into four general categories, including (1) symptoms, (2) changes in lung volume or spirometry, (3) changes in R_{aw}, and (4) changes in airway responsiveness. These categories are based on the absence of correlation between spirometry responses and change in R_{aw} or airway responsiveness. The attenuation by atropine of R_{aw} but not spirometry responses supports the notion of independent mechanisms. The attenuation by indomethacin or ibuprofen of spirometry responses, but not changes in R_{aw} or airway responsiveness, also supports this categorization. A bronchodilator, albuterol, given to healthy subjects prior to O_3 exposure did not prevent changes in spirometry, symptoms, or airway responsiveness. Symptoms ratings represent reflex responses (e.g., cough) or a perceptual evaluation of consciously appreciated afferent information (e.g., chest tightness, PDI), and it is therefore somewhat difficult to separate these responses from the more objective physiological responses. However, cough and pain on deep inspiration are related temporally to spirometry and breathing pattern responses (i.e., volume-related changes). In repeated exposure studies, changes in spirometry and breathing pattern become attenuated with the same time course as the changes in symptom responses.

Recent multihour O_3 exposure studies indicate that spirometry and symptom responses to concentrations as low as 0.08 ppm occur in healthy subjects with exposures lasting 6 to 8 h. Prolonged exposures (8 h) at lower O_3 concentrations (0.12 ppm) also indicate that there is a plateau of response to O_3 (Hazucha et al., 1992). Although suggested in previous studies (Gliner et al., 1983), such a plateau is difficult to verify with the typical duration of less than 2 h and the large responses seen with higher concentrations. The level of the response plateau (i.e., the spirometry decrement at which the response no longer changes) must be dependent on the dose rate of exposure (i.e., the product of the O_3 concentration and

$\dot{V}_E$) because the magnitude of response at a higher dose rate may greatly exceed the response plateau seen at a lower dose rate. Prolonged exposure studies also suggest that O_3-induced spirometry responses depend on the immediate exposure history. With relatively low dose rates (e.g., Hazucha et al., 1992), responses to exposure that occurred 2 to 4 h previously may influence the current response. The cumulative effect of exposures has not been studied at higher dose rates, but greater persistence of effects may be expected based on the longer recovery period at higher doses rates.

Recovery from O_3 exposure has not been systematically investigated in a large group of subjects, but available information indicates that an initial phase of recovery proceeds relatively rapidly, and some 40 to 65% of the acute response appears to be recovered within about 2 h (Folinsbee and Hazucha, 1989). However, there is some indication that the spirometric responses, at least to higher O_3 concentrations, are not fully recovered within 24 h (Folinsbee and Horvath, 1986; Folinsbee et al., 1994). Collectively, these observations suggest that there is a rapid recovery of O_3-induced spirometry and symptom responses, which may occur during resting exposure to O_3 (Folinsbee et al., 1977) or as O_3 concentration is reduced during exposure (Hazucha et al., 1992), and a slower phase, which, in some cases, may take at least 24 h to complete. Repeated exposure studies at higher concentrations typically show that the response to O_3 is enhanced on the second of several days of exposure. This enhanced response suggests a residual effect of the previous exposure, about 22 h earlier, even though the preexposure spirometry may be the same as on the previous day. The absence of the enhanced response with repeated exposure at lower O_3 concentrations may be the result of a more complete recovery or less damage to pulmonary tissues.

Studies of repeated daily exposure to O_3 have shown that O_3-induced changes in spirometry, symptoms, R_{aw}, airway responsiveness, and airway inflammation are attenuated with repetitive exposure. At higher dose rates, symptom and spirometry responses may be enhanced on the second exposure. Attenuation of response within 3 to 5 days is a consistent finding in repeated exposure studies, regardless of O_3 exposure dose rate, although attenuation of response occurs after fewer exposures at the lower dose rates. The attenuation of response appears to occur more rapidly in less responsive individuals (Horvath et al., 1981) or in responsive subjects exposed to lower O_3 dose rates (Folinsbee et al., 1978, 1994). Loss of attenuation is relatively rapid, with O_3 responsiveness being partially restored within 4 to 7 days (Kulle et al., 1982; Linn et al., 1982b), and normal responsiveness restored within 1 to 2 weeks after a series of 4 or 5 daily O_3 exposures. The attenuation of airway responsiveness may occur somewhat more slowly than that of symptom and spirometry responses. Airway inflammation also appears to attenuate, but less completely than the spirometry responses and with a more gradual recovery (Devlin et al., 1995; Folinsbee et al., 1995). Some markers of inflammation (e.g., LDH and elastase) have not demonstrated attenuation.

The mechanisms leading to the observed pulmonary responses induced by O_3 are beginning to be better understood. The available descriptive data suggest several possible mechanisms, some leading to alterations in lung volumes, symptoms, and exercise breathing patterns, and others leading to increases in central and peripheral R_{aw}. These mechanisms appear to involve (1) O_3 reactions with the airway lining fluid and epithelial cell membranes; (2) local tissue responses, including injury and inflammation; and (3) stimulation of neural afferents (bronchial C fibers) and the resulting reflex responses and symptoms. Much remains to be understood in order to determine how each event in this cascade contributes to the pulmonary responses induced by acute O_3 inhalation in human subjects.

Subjects with Preexisting Disease

Of the subpopulations studied, those with preexisting impediments in pulmonary function and exercise capacity are of primary concern in evaluating the health effects of O_3 because even a small change in function is likely to have more impact on a person with reduced reserve. Inherent in these studies are several limitations that, at present, hamper the ability to make definitive conclusions regarding the relative O_3 responsiveness of the various groups of subjects studied. Furthermore, it is ultimately necessary to determine whether their responses are representative of the larger population with preexisting disease. These limitations include subject selection (in controlled studies, typically only people with milder disease are selected or volunteer for study), standardized methods for the characterization of some responses, and limited range of exposure doses utilized to examine some endpoints.

These limitations are evident in studies on subjects with COPD, chronic bronchitis, and ischemic heart disease. For patients with COPD performing light to moderate IE, no decrements in pulmonary function were observed after 1- and 2-h exposures to 0.30 ppm O_3 (Linn et al., 1982a, 1983a; Solic et al., 1982; Kehrl et al., 1985) and only small decreases in forced expiratory volume were observed for 3-h exposures of chronic bronchitics to 0.41 ppm O_3 (Kulle et al., 1984). Small decreases in arterial blood oxygen saturation also have been observed in some of these studies, but the interpretation of these results and their clinical significance is uncertain.

Similar limitations also apply to the early studies examining O_3 effects in adult and adolescent asthmatics. Decrements in pulmonary function were not observed for adult asthmatics exposed for 2 h at rest (Silverman, 1979) or with intermittent light exercise (Linn et al., 1978) to O_3 concentrations of 0.25 ppm and less. Similarly, no significant changes in pulmonary function or symptoms were found in adolescent asthmatics exposed for 1 h at rest to 0.12 ppm O_3 (Koenig et al., 1985) and in adolescent asthmatics and nonasthmatics exposed to 0.12 and 0.18 ppm O_3 with intermittent moderate exercise up to 1 h (Koenig et al., 1987, 1988), although a small decrease in forced expiratory flow at 50% of FVC was observed in asthmatics after exposure to 0.12 ppm O_3. More recent observations by Kreit et al. (1989), Eschenbacher et al. (1989), and Linn et al. (1994) suggest that mild to moderate asthmatics are at least as sensitive to the acute effects of O_3 inhalation as healthy subjects when the asthmatics are exposed to O_3 under conditions that elicit a significant response in healthy subjects. Kreit et al. (1989) and Eschenbacher et al. (1989) exposed adult asthmatic and nonasthmatic subjects to 0.4 ppm O_3 with intermittent moderate exercise for 2 h and observed a greater response in R_{aw}, FEV_1, and $FEF_{25\ 75\%}$ in the asthmatic subjects, although changes in FVC and symptoms were similar in both groups. Ozone exposure also resulted in a marked increase in airway responsiveness to methacholine in both the asthmatic and nonasthmatic subjects. These responses take on greater importance when it is considered that the observed O_3-induced pulmonary effects were superimposed on preexisting impairment of pulmonary function and airway responsiveness. In addition, the observations of Koenig et al. (1990) and Molfino et al. (1991) suggest the possibility that acute exposure to O_3 at doses that do not produce measurable pulmonary function decrements may increase the responsiveness of asthmatics to inhaled SO_2 or antigens.

7.5.1.2 Symptom Responses to Ozone

Following exposure to O_3 many subjects report respiratory symptoms, the most common of which are cough, shortness of breath, and PDI. There is a broad range of severity in rating symptom responses among subjects in these studies. A number of 2-h O_3 exposure studies that have examined the exposure dose-pulmonary function and symptom response relationships have included a semiquantitative analysis of symptom responses (Avol et al., 1983; Kulle et al., 1985; McDonnell et al., 1983; Seal et al., 1993). In each of these studies, as O_3 concentration increased, the pulmonary function response became more negative (a decrease in FEV_1), and the level of the respiratory symptoms (cough, shortness of breath, PDI) increased. The mean decrement in FEV_1 in each of these studies was highly correlated ($r > 0.98$ in all cases) with the mean change in symptom rating or symptom score (the determination of symptoms varied between studies). This high correlation results primarily because each of these variables is highly correlated with O_3 concentration. Correlation of individual changes in symptoms and changes in pulmonary function seldom exceed $r = 0.6$ (Horstman et al., 1990). Contributing to this low individual correlation is the fact that symptoms scores have lower test-retest reliability than tests of lung function. For example, McDonnell et al. (1985b) report test-retest coefficients of about 0.9 for spirometry responses but only about 0.8 for symptoms. Thus individual symptom responses are not good predictors of individual pulmonary function responses. However, group mean symptom responses still provide a good marker of the average FEV_1 response to O_3 exposure. In two of the very heavy exercise studies (McDonnell et al., 1983; Avol et al., 1983) symptoms of cough or total respiratory symptom scores were increased significantly at 0.12 and 0.16 ppm O_3, respectively. In the heavy exercise study (Seal et al., 1993), cough symptoms increased significantly at 0.18 ppm O_3. Other studies that support this relationship of symptoms and pulmonary function have been conducted with various exposure durations and exercise intensities (Gong et al., 1986; Horstman et al., 1990) ranging from 1 h of severe exercise to 6.6 h of moderate exercise at O_3 concentrations from 0.08 to 0.20 ppm.

In comparing the spirometry and symptom responses of older adults and young adults exposed to O_3 under the same conditions, Drechsler-Parks et al. (1989) found significantly lower spirometry responses (19% versus 6% FEV_1) in the older adults. In addition, the incidence of respiratory symptom responses for the three symptoms most commonly reported with O_3 exposure were almost twice as high in the young adults, whereas the incidence of symptoms unrelated to O_3 exposure (e.g., eye irritation, muscle soreness) typically was greater in the older adults. The comparable or greater incidence of nonrespiratory symptoms in the older adults clearly indicates that they felt less respiratory discomfort in conjunction with their smaller spirometry responses. Asthmatics, when compared with nonasthmatics, tend to have greater changes in R_{aw} and expiratory flow with O_3 exposure (Kreit et al., 1989; Horstman et al., 1995) but similar changes in lung volume (i.e., FVC). Asthmatics also have similar symptom responses for cough, PDI, and shortness of breath, although, in one study (Horstman et al., 1995), asthmatics reported a higher incidence of wheezing.

In repeated exposure studies (Folinsbee et al., 1994; Linn et al., 1982b), the changes in symptoms track the changes in spirometry. With repeated exposure to high O_3 concentrations, the change in FEV_1 is typically greatest on the second exposure day. Correspondingly, symptoms are increased on the second exposure day and diminish to near baseline levels by the fourth or fifth exposure when the spirometry responses become negligible. With repeated exposure to lower O_3 concentrations, the largest spirometry

response is seen on the first day and attenuates by the third or fourth day. Symptom responses also are largest on the first day and are attenuated with the same time course. In a single 2-h study in which symptom and spirometry responses were measured during exposure and recovery, the mean changes in symptoms and spirometry responses followed similar time courses (McDonnell et al., 1987).

Intervention studies examining the effects of various drug treatments on the responses to O_3 also report parallel changes in symptoms and spirometry. Although atropine blocked the increase in R_{aw} in response to O_3 exposure, it did not alter the spirometry or symptom responses (Beckett et al., 1985). Similarly, albuterol and salbutamol, which had no effect on O_3-induced changes in spirometry, also had no effect of symptom responses (McKenzie et al., 1987; Gong et al., 1988). The anti-inflammatory medications indomethacin and ibuprofen, which partially inhibit the spirometry responses to O_3 exposure, also cause a reduction in respiratory symptoms (Schelegle et al., 1987; Hazucha et al., 1994).

The individual correlations between symptoms and spirometry responses are relatively low (<0.6) and are of little predictive value. However, the group mean responses have similar exposure-response characteristics and follow a similar time course of response to exposure and recovery. Symptom and spirometry responses also follow a similar time course of attenuation to repeated exposure and they are affected similarly by a number of medication interventions.

7.5.1.3 Effects on Exercise Performance

Endurance exercise performance and $\dot{V}O_{2max}$ may be limited by acute exposure to O_3 (Adams and Schelegle, 1983; Schelegle and Adams, 1986; Gong et al., 1986; Foxcroft and Adams, 1986; Folinsbee et al., 1977; Linder et al., 1988). Gong et al. (1986) and Schelegle and Adams (1986) found that significant reductions in maximal endurance exercise performance may occur in well-conditioned athletes while they perform CE ($\dot{V}_E > 80$ L/min) for 1 h at O_3 concentrations 0.18 ppm. Data from Linder et al. (1988) suggest that small decrements in maximal exercise performance may occur at O_3 concentrations less than 0.18 ppm. The mechanisms that lead to these responses and the minimum O_3 concentration at which these effects occur have not yet been defined clearly. Reports from studies of exposure to O_3 during high-intensity exercise indicate that breathing discomfort associated with maximal ventilation may be an important factor in limiting exercise performance. However, these studies do not exclude the possibility that some as yet undefined physiological mechanism may limit exercise performance.

7.5.1.4 Effects on Airway Responsiveness

Ozone exposure causes an increase in nonspecific airway responsiveness as indicated by a reduction in the concentration of methacholine or histamine required to produce a given reduction in FEV_1 or increase in SR_{aw}. Increased airway responsiveness is an important consequence of exposure to O_3 because its presence means that the airways are predisposed to narrowing on inhalation of a variety of stimuli (e.g., specific allergens, SO_2, cold air). Markedly increased airway responsiveness is a classical feature of asthma and also may be present with other respiratory diseases (e.g., chronic bronchitis, acute viral infections) and even in a sizeable percentage of the healthy asymptomatic population. Many studies have demonstrated O_3-induced increases in nonspecific airway responsiveness in healthy subjects after a 1- to 2-h exposure with exercise to concentrations in the range of 0.20 to 0.60 ppm

(Golden et al., 1978; Holtzman et al., 1979; König et al., 1980; Dimeo et al., 1981; Gong et al., 1986; Folinsbee and Hazucha, 1989) and after 6.6 h of exposure to concentrations in the range of 0.08 to 0.12 ppm (Folinsbee et al., 1988; Horstman et al., 1990). Ozone-induced increases in airway responsiveness tend to resolve within 24 h after exposure but may persist in selected individuals for longer periods (Golden et al., 1978).

Ozone exposure of asthmatic subjects, who characteristically have increased airway responsiveness at baseline, can cause further increases in responsiveness (Kreit et al., 1989). The difference in baseline airway responsiveness between healthy and mild asthmatic subjects may be as much as 100-fold, whereas the changes in airway responsiveness induced by O_3 are typically two- to fourfold. Similar relative changes in airway responsiveness are seen in asthmatics exposed to O_3 despite their markedly different baseline airway responsiveness. One study (Molfino et al., 1991) has been published suggesting an increase in specific (i.e., allergen-induced) airway reactivity. This response was observed after a 1-h resting exposure of atopic asthmatics to 0.12 ppm O_3. One of the important aspects of this observation of increased airway responsiveness after O_3 exposure is that this represents a plausible link between ambient O_3 exposure and increased hospital admissions for asthma. However, experimental design flaws preclude the use of this study in the determination of a lowest-observed-effect level.

Changes in airway responsiveness after O_3 exposure appear to be resolved more slowly than changes in FEV_1 or respiratory symptoms. Furthermore, in studies of repeated exposure to O_3, changes in airway responsiveness tend to be somewhat less susceptible to attenuation with consecutive exposures than changes in FEV_1 (Dimeo et al., 1981; Kulle et al., 1982; Folinsbee et al., 1994). The question of whether chronic O_3 exposure can induce a persistent increase (or decrease) in airways responsiveness has not been studied adequately.

Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms. This conclusion is based on studies in healthy subjects; however, asthmatics who have widely different baseline airway responsiveness exhibit FEV_1 changes after O_3 exposure that are similar to those seen in healthy subjects (Kreit et al., 1989).

The mechanism of O_3-induced increases in airway responsiveness is only partially understood, but it appears to be associated with a number of cellular and biochemical changes in airway tissue. Airway inflammation may be temporally associated with the presence of increased airway responsiveness (Holtzman et al., 1983; O'Byrne et al., 1984; Seltzer et al., 1986), but many animal models of induced neutrophilia report a conflicting role of these cells in eliciting nonspecific bronchial hyperresponsiveness. Several animal species, for example, have shown an increased airway responsiveness induced by O_3 exposure in the absence of an influx of PMNs into the airway mucosa (Evans et al., 1988; Okazawa et al., 1989; Li et al., 1992). In one human study (Ying et al., 1990), preexposure treatment with the anti-inflammatory drug indomethacin blocked the effect of O_3 on FEV_1 and FVC but not on airway responsiveness; however, cyclooxygenase inhibitors have not been effective at blocking the O_3-induced influx of PMNs into BAL fluid (Hazucha et al., 1996; Kleeberger and Hudak, 1992). Therefore, O_3-induced airway responsiveness may not be due to the presence of PMNs in the airway or to the release of arachidonic acid metabolites. Rather, it seems likely that the mechanism for this response is multifactorial, possibly involving the presence of cytokines, prostanoids, or neuropeptides; activation of macrophages, eosinophils, or mast cells; and

epithelial damage that increases direct access of mediators to the smooth muscle or receptors in the airways that are responsible for reflex bronchoconstriction.

7.5.1.5 Inflammation and Host Defense Effects

A number of studies clearly show that a single acute exposure (1 to 4 h) of humans to moderate concentrations of O_3 (0.2 to 0.6 ppm) while exercising at moderate to heavy levels results in a number of cellular and biochemical changes in the lung, as assessed by measurement of BAL constituents (Seltzer et al., 1986; Kehrl et al., 1987; Koren et al., 1989a,b, 1991; Schelegle et al., 1991; McGee et al., 1990; Aris et al., 1993a; Devlin et al., 1995). These exposures result in an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial cells lining the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins. This response can be detected as early as 1 h after exposure (Koren et al., 1991; Schelegle et al., 1991) and persists for at least 18 h (Koren et al., 1989a; Aris et al., 1993a). The response profile of these mediators is not defined adequately, although it is clear that the time course of response varies for different mediators and cells (Schelegle et al., 1991, Koren et al., 1989a, 1991).

A single study (Devlin et al., 1991) provides evidence that many of these changes also occur in humans exposed to 0.08 and 0.10 ppm O_3 with moderate exercise for 6.6 h. Decrements in the ability of AMs to phagocytose microorganisms also were reported in this study.

Ozone also causes inflammatory changes in the nose, as indicated by increased levels of PMNs and albumin, a marker for increased epithelial cell permeability. Increases in tryptase levels immediately after O_3 exposure suggested the release of mast cell products.

There appears to be no strong correlation between any of the measured cellular and biochemical changes and changes in lung function measurements, suggesting that different mechanisms may be responsible for these processes. Alternatively, the absence of a correlation may reflect either the temporal misalignment of these measurements, the fact that changes detected in the lavage fluid do not quantitatively reflect events occurring in tissues where functional or symptomatic events originate, or that BAL fluid may not be collected from the same lung region primarily implicated in pulmonary function responses. The idea of different mechanisms is supported by a study in which ibuprofen, a cyclooxygenase inhibitor, blunted the O_3-induced decrements in lung function without altering the O_3-induced increase in PMNs or epithelial cell permeability, although ibuprofen did change the concentration of a number of mediators, some of which may be related to changes in function (Hazucha et al., 1994).

In vitro studies suggest that epithelial cells are the primary target of O_3 in the lung and that O_3 induces them to produce many of the mediators found in the BAL fluid of humans exposed to O_3. Although O_3 does not induce AMs to produce these compounds in large quantities, it does directly impair the ability of AMs to phagocytose and kill microorganisms.

7.5.1.6 Factors Modifying Responsiveness to Ozone

Many variables that at least have potential for influencing response to O_3 remain inadequately addressed in the available clinical data. Factors such as smoking status, age, gender, race or ethnic group, season, and mode of breathing during exposure have been evaluated inadequately for their potential influence on responses to O_3 exposure.

Information derived from O_3 exposure of smokers is limited. Some degree of attenuation appears to occur in active smokers and may be reversed following smoking cessation (Emmons and Foster, 1991), but available results should be interpreted with caution. The possibility of age-related differences in response to O_3 has been explored to some extent since the publication of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986). Young adults historically have provided the subject population for air pollutant exposure studies. Pulmonary function responsiveness appears to decrease with age, although symptom rates remain similar to those of young adults (Drechsler-Parks et al., 1987b, 1989, 1990; Bedi et al., 1988; Reisenauer et al., 1988; McDonnell et al., 1993). The limited information available on the responses of children and adolescents to O_3 (McDonnell et al., 1985a; Avol et al., 1985a, 1987; Koenig et al., 1987, 1988) does not indicate that children and adolescents are either more or less responsive than young adults. Of the studies that have investigated gender differences in responsiveness to O_3, some (Lauritzen and Adams, 1985; Horvath et al., 1986; Adams et al., 1987; Drechsler-Parks et al., 1987a,b; Messineo and Adams, 1990) have suggested that women are more responsive to O_3 than men. However, the absence of consistent findings with respect to gender differences indicates that it cannot be concluded that men and women respond differently to O_3. Comparison of responses across gender, racial, ethnic, and age groups is complicated by the determination of equivalent exposures. For example, women and children have smaller lungs than adult men. Thus, with a given exposure concentration, duration, and ventilation, humans with smaller lungs will presumably receive a large relative intrapulmonary exposure. Some attempts have been made to normalize responses according to BSA or lung capacity (e.g., FVC). The only study in which this factor has been investigated systematically (Messineo and Adams, 1990) found no influence of lung size on the spirometry responses under identical exposure (O_3 concentration, $\dot{V}_E$, and T) conditions. Three studies (Fox et al., 1993; Seal et al., 1995; Weinmann et al. (1995) have compared pulmonary function responses of women during different phases of the menstrual cycle, but the results are conflicting. The responses of black and white young adults to various concentrations of O_3 have been compared in one study (Seal et al., 1993). The data suggested that black males experienced significant decrements in pulmonary function at a lower concentration of O_3 than white males, but that there were no differences among the responses of white males and black and white females. Thus, the question of ethnic or racial differences in responsiveness to O_3 is answered inadequately, and the available results should be interpreted with caution. No new studies are available on the effects of heat stress (i.e., increased temperature or RH) on O_3 responses. One study (Linn et al., 1988) suggests that sensitivity to O_3 may be related to seasonal variations in ambient O_3 concentrations; this finding needs to be confirmed. Two studies (Hynes et al., 1988; Adams et al., 1989) have reported that differences in the inhalation route (e.g., oral versus nasal or oronasal) appear to be of negligible importance in the responses of exercising adults to O_3 exposure. Studies of O_3 uptake in the upper airway (Gerrity et al., 1988) confirm the negligible differences between oral and nasal inhalation (also see Chapter 8). None of these potential influences on O_3 responsiveness (age, gender, race, hormonal fluctuations, smoking, seasonal variations in responsiveness, and ambient environmental factors) has been investigated thoroughly. However, the observation that healthy older adults appear to be less responsive to O_3 exposure than young adults has been confirmed to the point that it can be considered in risk assessment. Nevertheless, this does not address fully the question of age differences because children and adolescents remain inadequately studied.

7.5.1.7 Extrapulmonary Effects of Ozone

It still is believed that O_3 reacts immediately on contact with respiratory systems fluids and tissues and is not absorbed or transported to extrapulmonary sites to any significant degree. A number of laboratory animal studies reported in the previous chapter (Chapter 6) and early studies on human subjects reported in this chapter suggest that reaction products formed by the interaction of O_3 with respiratory system fluids or tissues may produce effects measured outside the respiratory tract—either in the blood, as changes in circulating blood lymphocytes, erythrocytes, or serum, or as changes in the structure or function of other organs, such as the parathyroid, the heart, the liver, and the central nervous system.
No extrapulmonary effects have been reported to date in other organ systems of O_3-exposed human subjects, except for limited data indicating that acute (1- to 2-h) exposures with exercise at concentrations 0.35 ppm O_3 caused transient changes in blood cells and plasma. The interpretation of all these effects in regards to potential human health effects at ambient levels of exposure ($<$0.35 ppm O_3) is not clear. However, the demonstration in this chapter of an array of inflammatory mediators and immune modulators released at the airway surface in response to O_3 provides a possible mechanism for effects to occur outside of the lung. Additional studies are needed, therefore, in order to determine if there are any significant extrapulmonary effects of O_3 exposure and at what levels of exposure they might occur.

7.5.1.8 Effects of Ozone Mixed with Other Pollutants

No significant enhancement of respiratory effects (i.e., more than additive) has been consistently demonstrated for mixtures of O_3 with SO_2, NO_2, H_2SO_4, HNO_3, or particulate aerosols, or with multiple combinations of these pollutants. There is general agreement among studies of simultaneous exposure of healthy adults and asthmatic adolescents to mixtures of O_3 and NO_2, SO_2, H_2SO_4, or HNO_3 that pulmonary function responses are not significantly different from those following exposure to O_3 alone when compared to studies conducted at the same O_3 concentration. Exposure to high PAN concentrations (i.e., 0.3 ppm) combined with O_3 has been reported to induce greater pulmonary function responses than exposure to O_3 alone (Horvath et al., 1986), but when the PAN concentration is reduced to the ambient range, any additional effect of PAN in the mixture appears to be negligible (Drechsler-Parks et al., 1989).

In addition to simultaneous exposures to pollutant mixtures, studies of the responses to O_3 exposure either preceded or followed by another pollutant have been performed. To the extent that these exposure sequences mimic real ambient conditions, the results could be useful in the risk assessment process. Koenig et al. (1990) demonstrated that exposure of allergic (and probably asthmatic) adolescents to O_3 and then to SO_2 resulted in significant pulmonary function decrements not seen with an O_3-O_3 sequence or FA-SO_2 sequence. These results also can be interpreted in light of the fact that O_3 increases nonspecific bronchial responsiveness and that the increased SO_2 responses may simply reflect this increased responsiveness. Such responses would be unlikely in nonatopic healthy adolescents. Other studies (Aris et al., 1991; Hazucha et al., 1994; Linn et al., 1994; Utell et al., 1994) have assessed the responses to O_3 after previous exposure to another pollutant. Aris et al. (1991) found that preexposure to water or HNO_3 fog appeared to attenuate responses to O_3, whereas Hazucha et al. (1994) observed an increased airway responsiveness after O_3 exposure preceded by NO_2 exposure relative to O_3 exposure alone. Two studies of combined or sequential exposure to H_2SO_4 aerosol and O_3 suggest a possibly enhanced response to O_3 in asthmatics when the exposure is

combined with or preceded by exposure to H_2SO_4 aerosol (Linn et al., 1994; Utell et al., 1994). These findings are intriguing, but must be replicated before they can be useful for quantitative health assessment. Much is unknown about responses to air pollutant mixtures. Only a limited number of pollutant combinations and exposure protocols have been investigated, and subject groups are small and may not be representative of the general population. Few studies have included more than two pollutants, and most combinations have been evaluated in single studies. Furthermore, only rarely are endpoints other than pulmonary function and plethysmography measured.

7.5.2 Field and Epidemiology Studies of Ozone Exposure

Individual-level camp and exercise studies provide useful, quantitative information on the exposure-response relationships linking human lung function declines with O_3 exposure occurring in ambient air. Their utility derives largely from the reliability with which individual exposures can be estimated using outdoor measurements in studies of these kind. Although it usually has not been possible to isolate O_3 exposures from other copollutants (e.g., acid aerosols) and environmental factors (e.g., temperature) in the design of such studies, the available body of evidence now strongly supports a dominant role of O_3 in the observed lung function decrements.

The most extensive epidemiologic database on pulmonary function responses to ambient O_3 comes from camp studies. Six recent key studies from three separate research groups provide a combined database on individual exposure-response relationships for 616 children ranging in age from 7 to 17 years, each with at least six sequential measurements of FEV_1 and previous-hour O_3 exposures while attending summer camps (Avol et al., 1990; Higgins et al., 1990; Raizenne et al., 1987, 1989; Spektor et al., 1988a, 1991). When analyzed together using consistent methods, these data yielded an average relationship between FEV_1 and previous-hour O_3 concentration of 0.50 mL/ppb. The highest 1-h O_3 levels measured in five of the six studies ranged from 100 to 160 ppb, with one study reporting concentrations as high as 245 ppb. Minimum O_3 values ranged from 10 to 60 ppb. Although the regression results noted above were based on 1-h O_3 levels, exposure in camp studies usually extended for multiple hours. Because of the high level of correlation between single- and multiple-hour averages in the studies, these results may therefore, represent, to some extent, the influence of multihour exposures. In addition to the camp study results, two key studies involving lung function measurements before and after well-defined exercise events in adults have yielded exposure-response slopes of 0.40 and 1.35 mL/ppb (Spektor et al., 1988b; Selwyn et al., 1985). Ozone concentrations during exercise events of approximately 0.5 h duration ranged from 4 to 135 ppb in these studies. Consistent with chamber studies, there is no clear evidence from individual-level studies for a response threshold for the average population effects of O_3 on pulmonary function decline. However, as with chamber studies, there is evidence that responsivity varies across individuals. Thus, pulmonary function decline as a function of ambient O_3 exposure for an individual may be either greater than or less than the mean responses noted above.

Recent results of daily-life studies also support a consistent relationship between ambient O_3/oxidant exposure and acute respiratory morbidity in the population. Respiratory symptoms (or exacerbation of asthma) and decrements in PEFR occur with increasing ambient O_3, especially in asthmatic children (Lebowitz et al., 1991; Krzyzanowski et al., 1992; Thurston et al., 1995). Concurrent temperature, particles, H^+, aeroallergens, and asthma

severity or medication status also may contribute as independent or modifying factors. The aggregate results show greater responses in asthmatic individuals than in nonasthmatics (Lebowitz et al., 1991; Krzyzanowski et al., 1992), indicating that asthmatics constitute a sensitive group in epidemiologic studies of oxidant air pollution.

Recent aggregate population time series studies of O_3-related health effects provide relevant evidence of acute responses, even below a 1-h maximum of 0.12 ppm O_3. Emergency room visits, hospital admissions, and mortality all have been examined as possible outcomes of exposure to O_3. In the case of ER visits, the evidence is limited (e.g., Bates et al., 1990; Cody et al., 1992; White et al., 1994; Weisel et al., 1995), but results generally are consistent with an effect of O_3 on morbidity. Mortality studies vis-ŕ-vis O_3 also are rather limited, but are more mixed in their results. One of two new, well-designed studies indicate a significant association between O_3 and total mortality in Los Angeles, CA, even after controlling for the potentially confounding effects of temperature and PM (Kinney and Ozkaynak, 1991). Los Angeles experienced peak 1-h maximum O_3 concentrations above 0.2 ppm during this study period. However, at lower concentrations, over a shorter time span, and with different statistical methods, a second study (Dockery et al., 1992) did not detect a significant O_3 association with mortality. The strongest and most consistent evidence of O_3 effects, both above and below 0.12 ppm O_3, then, is provided by the multiple studies that have been conducted over the last decade on summertime daily hospital admission for respiratory causes in various locales in eastern North America (Bates and Sizto, 1983, 1987, 1989; Thurston et al., 1992, 1994; Lipfert and Hammerstrom, 1992; Burnett et al., 1994). These studies consistently have shown that O_3 air pollution is associated with an increased incidence of admissions, accounting for roughly one to three excess respiratory hospital admissions per 100 ppb O_3 per million persons. This association has been shown to remain even after statistically controlling for the possible confounding effects of temperature and copollutants (e.g., H^+, SO_4, and PM_1), as well as when considering only days having 1-h maximum O_3 concentrations below 0.12 ppm. Furthermore, these results imply that O_3 air pollution can account for a substantial portion of summertime hospital admissions for respiratory causes on the most polluted days. Overall, the aggregate population time series studies considered in this chapter provide strong evidence that ambient exposures to O_3 can cause significant exacerbations of preexisting respiratory disease in the general public at concentrations below 0.12 ppm O_3.

Studies of chronic health effects that may relate to long-term exposure to ambient pollutants still have not provided enough data to determine if there are respiratory or other health effects that result directly from chronic O_3 exposure. However, the aggregate evidence to date suggests that chronic O_3 exposure, along with other environmental factors, could be responsible for health effects in exposed populations.

The most useful set of data has been provided by the AHSMOG studies (Hodgkin et al., 1984; Euler et al., 1987, 1988; Abbey et al., 1991a,b, 1993). These studies have provided the most refined measures of chronic exposure to date (including adjustment for quality of the monitoring data as determined by distance of monitoring sites from subject residences, topography, time spent indoors, and time spent at work). The most consistent effects that can be attributable, in part, to O_3 relate to an increase in 10-year cumulative incidence of asthma (RR = 2.07 for each 1,000 h above 10 pphm) and an increase in asthma severity. Unfortunately, for the entire set of studies, the colinearity between O_3 and TSP reduces the confidence that effects can be attributed to O_3 alone, O_3 in combination with the particulate fraction of ambient pollution, or the combination of the two. Some support for an

effect on persons with asthma also can be derived from a recent Canadian study (Stern et al., 1994) that demonstrated nonstatistically significant 6.6 and 6.5% reductions in $FEF_{25\ 75\%}$ and $\dot{V}_{max5\ \%}$ for people living in Ontario relative to those in Saskatchewan. Again, however, the effects of O_3 are impossible to disentangle from the other contributors such as the acid summer haze that characterizes the United States east of the Mississippi River.

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8

Extrapolation of Animal Toxicological Data to Humans

8.1 Introduction

A full evaluation of the health effects of ozone (O_3) requires an integrated interpretation of human clinical, epidemiological, and animal toxicological studies. Each of these three research approaches has inherent strengths and limitations. Animal toxicological data are valuable because they provide concentration- and duration-response information on a fuller array of effects and exposures than can be studied in humans. However, historically, use of animal toxicological data has been limited because of difficulties in quantitative extrapolation to humans. Recent advances in the state-of-the-art of extrapolation have reduced several uncertainties, which will be discussed in this chapter.

Qualitative animal-to-human extrapolation generally is accepted because O_3 causes similar types of effects in several animal species, from mouse to nonhuman primate (Chapter 6). Also, when similar endpoints (e.g., inflammation and pulmonary function) have been examined in O_3-exposed animals and humans, similar effects are observed. However, quantitative extrapolation (i.e., if a certain exposure causes a specific effect in animals, what exposure is likely to cause that same effect in humans?) is the goal but is controversial. Such an extrapolation requires an integration of dosimetry and species sensitivity. *Dosimetry* is defined as the dose delivered to a site in the respiratory tract (RT). As can be seen in Section 8.2, substantial information is available on dosimetry in several species, including humans. Dosimetric studies that are referenced in the earlier O_3 criteria document (U.S. Environmental Protection Agency, 1986) are summarized only briefly here; newer research is the focus. Species sensitivity, discussed in Section 8.3, refers to the sensitivity of a specific species to the delivered dose. For example, even if the same dose of O_3 were delivered to a specific respiratory tract site in rats and humans, differences in species sensitivity to that dose are likely because of variations in defense mechanisms and perhaps other factors. Section 8.3 also provides a more holistic approach to extrapolation by quantitatively comparing exposure-response data obtained in animals and humans. Sections 8.4 and 8.5 are intended to draw the forgoing information together, reaching conclusions about the potential for acute and chronic human health effects based on animal studies. Lastly, Section 8.6 presents the summary and major conclusions from the chapter.

Although this chapter focuses on animal-to-human extrapolation, dosimetric studies also can be used to elucidate interpretations of the human studies described in Chapter 7. For

example, knowledge of dosimetry in humans as related to age and exercise can enhance understanding of human susceptibility factors.

8.2 Ozone Dosimetry

8.2.1 Introduction

Dosimetry refers to measuring or estimating the quantity or rate of a chemical absorbed by target sites within the RT. The compound most directly responsible for toxic effects may be the inhaled gas O_3 or its chemical reaction products. Complete identification of the actual toxic agents and their integration into dosimetry are complex issues that have not been resolved. Thus, most dosimetry investigations are concerned with the dose of the primary inhaled chemical. In this context, a further confounding aspect can be the units of dose (e.g., mass retained per breath, mass retained per breath per body weight, mass retained per breath per respiratory tract surface area). That is, when comparing dose between species, what is the relevant measure of dose? This question has not been answered; units are often dictated by the type of experiment or by a choice made by the investigators.

Experimental and theoretical (dosimetry modeling) studies are used to obtain information on dose. Experiments have been carried out to obtain direct measurements of absorbed O_3 in the RT, the upper RT (URT; region proximal to the tracheal entrance), and the lower RT (LRT; region distal to tracheal entrance); however, experimentally obtaining dosimetry data is extremely difficult in smaller regions or locations, such as specific airways or the centriacinar region (CAR; junction of conducting airways and gas exchange region), where lesions caused by O_3 occur (see Chapter 6, Section 6.2.4). Nevertheless, experimentation is important for determining dose, making dose comparisons between subpopulations and between different species, assessing hypotheses and concepts, and validating mathematical models that can be used to predict dose at specific respiratory tract sites and under more general conditions.

Theoretical studies are based on the use of mathematical models developed for the purposes of simulating the uptake and distribution of absorbed gases in the tissues and fluids of the RT. Because the factors affecting the transport and absorption of gases are applicable to all mammals, a model that uses appropriate species or disease-specific anatomical and ventilatory parameters can be used to describe absorption in the species and in different-sized, aged, or diseased members of the same species. Importantly, models also may be used to make interspecies and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not possible or feasible experimentally, and to better understand the processes involved in toxicity.

8.2.2 Summary of 1986 Review of Experimental and Theoretical Dosimetry

A summary of the more relevant experimental and theoretical dosimetry studies contained in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) is presented. The reader is referred to the earlier document for completeness.

Experiments on the nasopharyngeal removal of O_3 in laboratory animals suggested that the fraction of O_3 uptake depends inversely on flow rate (Yokoyoma and Frank, 1972), uptake was greater for nose than mouth breathing (Yokoyoma and Frank, 1972), and tracheal and chamber concentrations were related linearly (Yokoyoma and Frank, 1972; Miller et al., 1979). Only one investigation measured uptake by the LRT, finding 80 to 87% uptake by the LRT of dogs (Yokoyoma and Frank, 1972). At the time, there were no reported results for

human URT or LRT uptake. With the exception of two relatively crude studies by Clamann and Bancroft (1959) and Hallett (1965), there were no data on O_3 uptake in humans at the time of the earlier criteria document (U.S. Environmental Protection Agency, 1986).

Several mathematical dosimetry models were developed to simulate the processes involved in O_3 uptake and to predict O_3 uptake by various regions and sites within the RT. The model of Aharonson et al. (1974) was used to analyze nasopharyngeal uptake data. Applied to O_3 data, the model indicated that the average mass transfer coefficient of this region and the mass retained increased with increasing air flow, but the percent uptake decreased.

Models were developed to simulate LRT uptake (Miller et al., 1978, 1985). The models were very similar in their treatment of O_3 in the airways and airspaces and in their use of morphometric data to define the dimensions of the air compartments and liquid lining. Both the 1978 and the 1985 models of Miller and co-workers took into account reactions of O_3 with constituents of the liquid lining. However, these models differed in their treatment of chemical reactions in the liquid lining, and the later model included transport and chemical reactions within tissue and blood, whereas the first model did not (an instantaneous reaction at the liquid-tissue interface was assumed, so the O_3 concentration was defined as zero). In both models, tissue dose was defined as the O_3 flux to the liquid-tissue interface. Both models predicted O_3 tissue dose to be relatively low in the trachea, to increase to a maximum in or near the CAR, and then to decrease distally; this was characteristic for both the animal and the human simulations (Miller et al., 1978, 1985).

Prior to 1986, there were no experimental results that were useful in judging the validity of the modeling efforts. However, a comparison of the results of Miller and co-workers with morphological data (showing the CAR to be most affected by O_3; see Chapter 6, Section 6.2.4) indicated qualitative agreement between the site of predicted maximum tissue dose and the site of observed maximum morphological damage in the pulmonary region.

8.2.3 Experimental Ozone Dosimetry Data

8.2.3.1 Introduction

Models of O_3 uptake in the RT have reached a scale of sophistication that provide some highly specific predictions regarding the location and magnitude of O_3 dose. However, before these models can be exploited to their fullest degree in extrapolating dose within and between species, validation of the models with experimental data is essential. This section will review the experimental database on which the modeling of O_3 dosimetry is both based and validated. This will help facilitate discussion of the models themselves in subsequent sections. Table 8-1 provides a summary of all post-1986 experimental O_3 dosimetry studies.

8.2.3.2 In Vivo Ozone Dosimetry Studies

The model predictions of Overton et al. (1987), based on the original model of Miller et al. (1985), provided specific predictions about the regional and total uptake efficiencies of O_3 in laboratory rats. It was therefore necessary to test these predictions with actual data. The first data on total RT uptake of O_3 in rats were obtained by Wiester et al. (1987). Ozone uptake was measured in 30 awake, unanesthetized Sprague-Dawley (S-D) rats receiving a nose-only exposure. Each rat was situated within a plethysmograph that continuously monitored the animal's breathing pattern. Air with O_3 flowed by rats' noses at 1,200 mL/min for 1 h at a concentration of 0.3, 0.6, or 1.0 ppm O_3. Determination of RT

Table 8-1. Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vivo, nose only	Rat (S-D)	Total RT	V_T = 2.8 mL f = 150 bpm $\dot{V}_E$ = 400 mL/min	Uptake measured in 30 rats exposed to 0.3, 0.6, or 1.0 ppm O_3 for 1-h. Uptake measured using mass balance. Total RT uptake efficiency measured at 40%. Uptake efficiency was independent of O_3 concentration.	Wiester et al. (1987)
In vivo	Rat (F344) Rat (S-D) Rat (Long-Evans) Guinea pig (Hartley)	Total RT	For rats: V_T = 2.6 mL f = 120 bpm $\dot{V}_E$ = 330 mL/min For guinea pigs: V_T = 2.4 mL f = 77 bpm $\dot{V}_E$ = 188 mL/min	All uptake measurements at 0.3 ppm O_3. In addition F344 rats were measured at 0.6 ppm. Uptake measurements made with system of Wiester et al. (1987). Total RT uptake efficiency averaged 47% and was strain and species independent. Uptake efficiency again was shown to be independent of O_3 concentration in F344 rats.	Wiester et al. (1988)
In vivo	Rat (F344)	Total RT, head, larynx/trachea, lung	V_T = 2.05 mL f = 150 bpm $\dot{V}_E$ = 290 mL/min	Regional uptake measured by assaying for recoverable ^{18}O from respiratory tract tissue after animal inhaled ^{18}O-enriched 1 ppm O_3 for 2 h. Fifty-four percent of inspired O_3 was taken up by total RT. Of the O_3 taken up, 49.6% taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs.	Hatch et al. (1989)
In vivo	Human	Total RT, URT, LRT	V_T = 800 mL f = 12 and 24 bpm $\dot{V}_I$ = 350 and 634 mL/s oral, nasal, and oronasal breathing	Uptake efficiencies of URT, LRT, and total RT measured by sampling inspired and expired air from catheter inserted through nose to posterior oropharynx. Uptake efficiencies computed from peak plateau concentrations on inspiration and expiration. Inspiratory URT uptake efficiencies averaged 40%; inspiratory plus expiratory LRT uptake efficiencies averaged 92%. Small but significant decreases in URT and LRT uptake efficiencies with increasing f. No effect of concentration on uptake. Uptake efficiency of mouth relatively greater than nose by about 10%.	Gerrity et al. (1988)
In vivo	Human	Total RT, URT, LRT	V_T = 1,239-1,650 mL f = 25-35 bpm $\dot{V}_E$ = 41 L/min	Twenty healthy male subjects exposed to 0.4 ppm O_3 while exercising at $\dot{V}_E$ of 41 L/min for 1 h. Uptake efficiencies of URT, LRT, and total RT measured at beginning and at end of exposure by method of Gerrity et al. (1988). Subjects mouth-breathed only. Uptake efficiencies computed as mass fractions. URT inspiratory efficiency was 40% and did not change during exposure. LRT efficiency dropped from 68% to 62% during exposure. LRT decrease correlated with drop in V_T. Cumulative dose of O_3 to LRT was predictive of V_T drop.	Gerrity and McDonnell (1989); Gerrity et al. (1989); Gerrity et al. (1994)
In vitro	Pig Sheep	Trachea	$\dot{V}_A$ = 50-200 mL/s	Unidirectional O_3 uptake efficiencies of trachea decreased with increasing flow from 0.5 to 0.15 for the sheep and 0.12 for the pig. Mass transfer coefficients generally were independent of flow.	Ben-Jebria et al. (1991)

Table 8-1 (cont'd). Experimental Studies on Ozone Dosimetry[a]

Type of Study	Species (Strain)	Uptake	Breathing Patterns	Results	Reference
In vitro	N/A	N/A	N/A	Ozonolysis studies on various unsaturated fatty acids, rat erythrocyte ghost membranes and rat BAL. Dominant processes are the production of aldehydes and peroxides due to reactions between O_3 and olefins.	Pryor et al. (1991)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Uptake efficiencies by measuring recovery of O_3 boluses delivered at 20 mL increments into lung to depth of 200 mL. At deepest depth, only 6% of O_3 could be recovered. Ozone uptake by conducting airways larger than predicted by Miller et al. (1985).	Hu et al. (1992b)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 150, 250, 500, 750, 1,000 mL/s	Same technique as Hu et al. (1992b), but investigating flow effects. Increasing flow caused marked shift of delivered O_3 toward the periphery of the conducting airways (i.e., the greater the inspiratory flow, the greater the amount of O_3 delivered to the lung periphery), where it is available for absorption. Mass transfer coefficients in upper airways independent of flow, but in conducting airways they increase proportional to flow. Lung liquid lining mass transfer coefficient computed to be 1.4 cm/s in the URT, falling to 0.17 cm/s in the respiratory airways. Reaction rate constant between O_3 and the lung liquid lining was computed as 7.3×10^6/s in the URT, falling to 8.2×10^5/s in the distal conducting airways.	Hu et al. (1994)
In vivo	Human	20-200 mL depth into total RT	V_T = 500 mL $\dot{V}_I$ = 250 mL/s	Comparison of O_3 bolus uptake between oral and nasal routes. Nose was found to be 30% more efficient at removing O_3 from the air stream than the mouth.	Kabel et al. (1994)
In vitro	Rat (S-D)	Lung	V = 2.71 mL f = 50-103 bpm FRC = 4 and 8 mL	Perfused and nonperfused rat lungs ventilated with 1 ppm O_3. Uptake efficiency of lungs dropped from 95% at 50 bpm to about 50% at 103 bpm. No change in uptake efficiency when lungs inflated from FRC = 4 to 8 mL.	Postlethwait et al. (1994)
In vivo	Human	Total RT, URT, trachea, mainstem bronchi	V_T = 810 mL f = 12 bpm $\dot{V}_I$ = 320 mL/s	O_3 uptake efficiencies in conducting airway structures determined by sampling air from anatomical sites ranging from the vocal cords to bronchus intermedius in 10 subjects undergoing transnasal bronchoscopy while being exposed to 0.4 ppm O_3. Total RT uptake efficiency was 91%. Uptake efficiencies of mouth-vocal cords: 17.6%; vocal cords-upper trachea: 12.8%; upper trachea-main bifurcation carina: 11.5%; main bifurcation carina-bronchus intermedius: 0%.	Gerrity et al. (1995)
In vivo	Human	Total RT	V = 593-642 mL f = 16 bpm $\dot{V}_E$ = 9.2-9.8 L/min Nasal and oral breathing	Total RT uptake efficiency measured using the same technique (Wiester et al., 1987; Wiester et al., 1988) applied to rats. During nasal breathing, total RT uptake efficiency was 73%. During oral breathing, total RT uptake efficiency was 76% and significantly higher than with nasal breathing.	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

O_3 uptake was determined by mass balance. The uptake was the difference in mass in the upstream air and in the air downstream of the rat. Total RT O_3 uptake efficiency of approximately 40% was measured and was independent of O_3 concentration. During data acquisition, the animals had an average tidal volume (V_T) of about 2.8 mL, an average breathing frequency (f) of about 150 breaths per minute (bpm), and an average minute ventilation ($\dot{V}_E$) of about 400 mL/min. This study was followed by another (Wiester et al., 1988) in which total RT uptake was measured in three strains of rats and in the guinea pig. Specifically, Fischer 344 (F344), S-D, and Long-Evans rats and Hartley guinea pigs were exposed for 1 h to 0.3 ppm O_3; F344 rats also received a 0.6-ppm exposure. All rats in the Wiester et al. (1988) study had f's between 112 and 132 bpm, V_Ts between 2.4 and 2.8 mL, and $\dot{V}_E$s between 299 and 364 mL/min. The guinea pigs had a V_T of 2.4 mL (not different from rats), an f of 77 bpm, and a $\dot{V}_E$ of 188 mL/min. Uptake was measured as in the previous experiment (Wiester et al., 1987). Total RT uptake of O_3 was species-independent and averaged 47%; this was higher than in the previous study because a different calculation method for fractional uptake was used. As in the first experiment, exposure concentration did not affect uptake. Wiester et al. (1987) corrected all flows used in uptake calculations for body temperature and relative humidity. In their later work, however, they found that this correction was not warranted, resulting in a slightly higher computed O_3 uptake efficiency (Wiester et al., 1988).

These data are in disagreement with the model predictions of Overton et al. (1987), who made predictions of O_3 uptake in two different rat anatomical lung models (Kliment, 1973; Yeh et al., 1979). Simulations were conducted in both anatomical models, varying f and V_T at fixed $\dot{V}_E$; functional residual capacity (FRC) also was varied as a fraction of total lung capacity (TLC). The Kliment (1973) anatomical lung model gave consistently high predictions for uptake when compared with actual data. The predictions using the Yeh et al. (1979) model came closer. Total RT uptakes (not including the head) for the Yeh et al. (1979) model were predicted to range between 46 and 60% at $f = 154$ bpm and $V_T = 1.25$ mL and between 70 and 80% for $f = 81$ bpm and $V_T = 2.4$ mL. However, when the fact that these predictions do not include the head of the animal is considered, it is evident that the model predictions overestimate the total RT uptake in rats. The question is whether the measurements are accurate or whether there is a problem with the model formulation. The data of Postlethwait et al. (1994), presented below, suggested that the data of Wiester et al. (1987, 1988) may be reasonable. The Postlethwait et al. (1994) data in the excised rat lung suggested a clear inverse dependence of lung uptake on f. At a V_T of 2.5 mL, the O_3 uptake efficiency of the excised lung fell from nearly unity at $f = 50$ bpm to almost 50% at $f = 100$ bpm. For extrapolation purposes, a key question here is what should be considered the normal resting f of a rat. Although Wiester et al. (1987, 1988) allowed their rats to acclimate to the plethysmograph by monitoring f and began uptake measurements only after f had plateaued at a minimum, it is still uncertain whether f's of 120 to 150 bpm are reasonable. In a summary of studies of the pulmonary function of rats in response to O_3, Tepper et al. (1993) found typical f's of 100 bpm. Although the models appear to overestimate the O_3 uptake efficiency of the rat RT, the discrepancy is not large, and the near agreement indicates that the O_3 dosimetry models have predictive capability.

In addition to data on the total RT O_3 uptake efficiency, in vivo data on regional O_3 dosimetry in animal models have begun to emerge. Hatch and Aissa (1987) and Aissa and Hatch (1988) first described a method to measure O_3 uptake in animals by exposing them nose-

only, while in a plethysmograph, to O_3 enriched with ^{18}O, a stable isotope of oxygen. After exposure, bronchoalveolar lavage (BAL) fluid and respiratory tract tissue were assayed for excess ^{18}O using isotope ratio mass spectrometry. One problem with this technique is that all of the absorbed ^{18}O cannot be accounted for, thus possibly leading to an underestimation of dose. The technique used by Hatch and Aissa (1987) involves the detection of excess ^{18}O in $^{18}O_3$ reaction products after tissue pyrolysis. Thus, $^{18}O_3$ that is degraded to $H_2{}^{18}O$ or $^{18}O_2$ is lost and cannot be detected in dry tissue. Eight male F344 rats (four previously exposed chronically to O_3 for 1 year to an urban pattern generally consisting of a 0.06-ppm baseline with 1-h daily spikes rising to 0.25 ppm) were exposed for 2 h to 1 ppm of ^{18}O-enriched O_3 (Hatch et al., 1989). During exposure, the rats breathed at 150 bpm and had a V_T of 2.05 mL and a $\dot{V}_E$ of 290 mL/min. After exposure, the lung, trachea, and head were analyzed separately for ^{18}O. Overall, the animals took up 54.3% of inspired O_3. Although this value of O_3 uptake efficiency is higher than that found by Wiester et al. (1987, 1988), considering the fact that the coefficient of variation for O_3 uptake efficiency measurements is around 20% in all studies, the result of Hatch and Aissa (1987) is consistent with the data of Wiester et al. (1987, 1988). Of the O_3 taken up by the animals, 49.6% was taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs. By assuming equal uptake efficiencies by compartments on inspiration and expiration, inspiratory uptake of O_3 by these regions was computed. It was determined that the rat nasopharynx (NP) had an inspiratory efficiency of 17.4%, and that the larynx/trachea removed 2.7% of the remaining O_3.

This technique recently has been extended to humans. Hatch et al. (1994) showed that when human subjects were exposed to 0.4 ppm $^{18}O_3$ while exercising intermittently at $\dot{V}_E$ = 60 L/min for 2 h, the amount of recovered ^{18}O in lavagable cells indicated that the human cells incorporated 4 to 5 times the O_3 dose (i.e., concentration of $^{18}O_3$) that was incorported by the BAL cells from rats exposed to 0.4 ppm O_3 for 2 h at rest. Consequently, to compare absorbed $^{18}O_3$ doses between rats and humans using BAL requires the assumption that the amount of lavagable cell membrane available to react with $^{18}O_3$ is comparable between the two species. The difference between rats and humans could be accounted for by the fact that the humans were exercising, whereas the rats were not. However, as was noted above, not all absorbed $^{18}O_3$ can be accounted for.

8.2.3.3 In Vitro Ozone Dosimetry Studies

The use of whole, intact animals to study O_3 uptake is needed to ascertain the actual amounts of O_3 absorbed. However, it is also important to understand some of the more fundamental processes governing O_3 uptake, such as the biochemistry of O_3/liquid and O_3/tissue interactions to determine chemical reaction rates essential to O_3 dosimetry models. Furthermore, the use of intact animals does not allow more precise determinations of the role of physiological parameters on O_3 uptake. For this reason, there have been some limited attempts at utilizing animal tissue explants and whole lungs to study O_3 uptake.

Ben-Jebria et al. (1991) studied O_3 uptake by the trachea of sheep and pigs to investigate mass transfer coefficients. Ozone boluses of 1 ppm were passed through excised tracheae. Tracheae were obtained from a slaughter house 0.5 to 2 h after slaughter, and, although they were kept coated with physiologic saline, they were not maintained at body temperature, possibly resulting in underestimation of in vivo uptake. The lengths and diameters of the pig tracheae were not too different from human tracheal dimensions. The flow dependence of mass uptake and the mass transfer coefficient (K) were determined for

flows between 50 and 200 mL/s. Uptake efficiencies in the pig decreased with increasing flow from about 0.5 to 0.12 and in the sheep decreased from about 0.5 to 0.15. Mass transfer coefficients generally were independent of flow (K = 0.5 cm/s in pigs and 0.35 cm/s in sheep), indicating the lack of dependence of uptake on gas-phase diffusion processes. This contrasts with the conclusion of Aharonson et al. (1974) for the NP of dogs where the investigators observed that the slight inverse dependence of uptake on flow observed by Yokoyama and Frank (1972) leads to the conclusion that the mass transfer coefficient for the NP of the dog should increase with flow, suggesting a role of the boundary layer in limiting diffusion of O_3 to the wall of the NP. The different geometries of a trachea and an NP may account for the differing observations.

A significant feature of the Ben-Jebria et al. (1991) study was the use of a rapidly responding O_3 analyzer. In order to conduct their O_3 uptake studies, Ben-Jebria and Ultman (1989) and Ben-Jebria et al. (1990) developed a rapidly responding O_3 analyzer. The analyzer relies on the reaction of O_3 with alkenes such as ethylene, propylene, cyclohexane, etc. Ten alkenes were tested. Ninety percent step-response times of 130 to 540 ms were achieved with varying degrees of linear response with O_3 concentration. The authors concluded that the best alkene was 2-methyl-2-butene, with optimum 10 to 90% responses of 110 ms and minimum detectable limits of O_3 of 18 ppb. Interference with CO_2, however, was found, requiring measurements of CO_2 to correct the analyzer response.

Postlethwait et al. (1994) used an isolated rat lung preparation to investigate the effects of vascular perfusion, inspired dose, temperature, and distal lung surface area on O_3 absorption by the LRT. Vascular perfusion had little or no effect on uptake efficiency of O_3. When the lung was exposed to 1 ppm O_3 and ventilated with a V_T of 2.5 mL, a FRC of 4 mL, and an f of 50 bpm, uptake efficiency was 95%. As f increased with fixed V_T, uptake efficiency began to drop, reaching nearly 50% at an f of 100 bpm. When the lung temperature was dropped from 37 to 25 °C, uptake efficiency dropped from 95 to 85% at 50 bpm. This drop was exploited to investigate other factors (such as flow, volume, and lung surface area) governing uptake because it moved respiratory tract uptake further below 100%. The observation of a dependence of uptake on temperature indicates that uptake efficiency is chemical-reaction dependent, thus possibly coupling uptake to reaction product formation. Another interesting result from this study was the lack of dependence of uptake on FRC. When FRC was doubled from 4 to 8 mL at 25 °C, fractional O_3 uptake was unchanged. This latter result suggests that O_3 uptake is virtually complete by the time O_3 reaches the alveolar spaces of the lung. Otherwise it would have been expected that the uptake efficiency would have risen with increased FRC.

To further investigate the reactions of O_3 with the lung, Pryor et al. (1991) performed ozonolysis studies of various unsaturated fatty acids (UFAs), rat erythrocyte ghost membranes, and rat BAL. These studies demonstrated significant production of hydrogen peroxide and aldehydes and that production of hydrogen peroxide was due primarily to reactions between O_3 and olefins. The authors concluded that the reaction of O_3 with UFAs in the lung fluid lining and cell membranes produce hydrogen peroxide and aldehydes that may be important mediators in the toxicity of O_3. The quantitative results of these studies led Pryor (1992) to hypothesize about the degree to which O_3 reacts with the liquid lining of the lung and with lung tissue. A simple model calculation was performed using the Einstein-Smoluchowski equation to estimate the half-life of O_3 in bilayers and cell membranes. Pryor (1992) concluded that a substantial fraction of O_3 reacts in the bilayer, and that only in regions of the

lung where the lung lining fluid layer is less than 0.1 μm thick will O_3 penetrate to tissue, and only then will O_3 react in cell membranes before penetrating further. The overall conclusion is that the toxic effects of O_3 may be mediated not just by O_3 directly but by reactive intermediates such as aldehydes and hydrogen peroxide. This raises the question as to the relevant dose of O_3: is it the total dose, the dose to the liquid lining, the tissue dose, or the dose of reactive intermediates delivered to tissue?

8.2.3.4 Human Ozone Dosimetry Studies

Significant progress has been made in the area of human O_3 dosimetry since the previous criteria document (U.S. Environmental Protection Agency, 1986). Studies have been conducted defining total and regional respiratory tract uptake, the dependence of uptake on physiological parameters, and the role of uptake in modulating response.

Gerrity et al. (1988) reported on measurements of O_3 uptake by the extrathoracic airways (airways proximal to the posterior oropharynx) and intrathoracic airways (airways distal to the posterior oropharynx) in 18 healthy, young male volunteers. Ozone uptake was measured by placing a small polyethylene catheter through the nose and positioning the distal tip in the posterior oropharynx. Breath-by-breath samples of O_3 were collected, and the peak plateau concentrations were compared with chamber concentrations. The effects on uptake of O_3 concentration (0.1, 0.2, 0.4 ppm), f (12 and 24 bpm at fixed V_T), and mode of breathing (oral, nasal, and oronasal) were tested. The O_3 analyzer had a moderately rapid response with a 90% response time of 700 ms. Inspiratory V_T ranged between 754 and 848 mL; mean inspiratory flow at 12 bpm was 350 mL/s; at 24 bpm it was 634 mL/s. The authors measured extrathoracic uptake efficiency of O_3 on inspiration at approximately 40% and intrathoracic uptake efficiency (inspiration plus expiration) at approximately 92%. They essentially found no effect of O_3 concentration on uptake (intrathoracic uptake was significantly higher at 0.4 ppm, but the difference was very small). They did find that both intrathoracic and extrathoracic uptake decreased with increasing f (at fixed V_T), falling by about 7% for extrathoracic uptake and by about 3% for intrathoracic uptake when f increased from 12 to 24 bpm. The finite response time of the analyzer may have affected the results at the 24 bpm f by overestimating extrathoracic uptake and underestimating intrathoracic uptake. However, because uptake was defined relative to plateaus of concentration, the response time of the analyzer was adequate to reach a plateau in the 1.2-s inspiratory time at 24 bpm. It is important to note here that, when utilizing the data from this study to compare with other studies and models, the uptake efficiencies measured are comparable to steady-state unidirectional measurements of uptake. Another feature is that Gerrity et al. (1988) consistently measured a small, non-zero plateau of O_3 on expiration. This plateau is not consistent with the suggestion from models of O_3 uptake and the data of Postlethwait et al. (1994) (nor with the later work of Gerrity et al. [1995] that is presented below) that no O_3 should be washed out from lung volumes beyond the conducting airways. This observation of Gerrity et al. (1988) may have been an artifact of the manner in which O_3 was measured by sampling from the posterior oropharynx. There may have been entrainment of O_3 in the pharyngeal airspaces that was washed out after expiration of dead-space air. Regardless, the concentration of O_3 exhaled from the alveolar phase of washout was very low.

One of the most startling results from the work of Gerrity et al. (1988) was the finding that there was only a small, but statistically significant, difference between uptake by the nose and by the mouth. The mouth had approximately 10% greater uptake efficiency than

the nose. The combined oronasal passage had an uptake efficiency greater than the nose by another 8%. This suggests that persons who breathe nasally are at no less risk than persons who breathe oronasally. Adams et al. (1989) investigated this possibility by comparing functional responses in subjects acutely exposed to O_3 while breathing either orally or oronasally. Healthy subjects were exposed on five separate days to 0.4 ppm O_3. In the first four exposures, subjects were exposed by face mask (with or without nose clip) for 30 min at an exercise level of 75 L/min or for 75 min at exercise level of 30 L/min. The fifth exposure was for 30 min at 75 L/min, with exposure through a mouthpiece. There were no differences in pulmonary function response (forced expiratory volume in 1 s [FEV_1], forced vital capacity [FVC], or forced expiratory flow) with face-mask exposure among all experimental groups (i.e., no nose clip, $\dot{V}_E$, or time effect). Pulmonary function response was, however, greater with a mouthpiece. Adams et al. (1989) speculated that the greater response with the mouthpiece was due to O_3 scrubbing by the face mask or by facial hair. It also may have been due to different oral configurations imposed by a mouthpiece. Hynes et al. (1988) also investigated whether functional responses were affected by the mode of breathing. Healthy subjects were exposed to 0.4 ppm O_3 for 30 min in an exposure chamber. On two different occasions, each subject breathed either through the nose or the mouth exclusively. There was no difference in pulmonary function response between these two routes of exposure. Taken together, the studies of Adams et al. (1989) and Hynes et al. (1988) are consistent with the observations of Gerrity et al. (1988) on the equal efficiency of all routes of breathing for extrathoracic O_3 scrubbing.

This study was followed by another study (Gerrity and McDonnell, 1989; Gerrity et al., 1989, 1994) in which the relationship between O_3 uptake and functional response was investigated. Healthy subjects were exposed to 0.4 ppm O_3 for 1 h while exercising continuously at 40 L/min. Ozone uptake was measured at the beginning and at the end of exposure while the subjects were still exercising, using the technique of Gerrity et al. (1988). In contrast to the work of Gerrity et al. (1988), uptake was computed in this study by integrating concentration times flow instead of using peak plateau measurements. Also, in this work, the 90% response time of the analyzer was 1.2 s (compared with 0.7 s in the previous work). The authors found that about 40% of the inspired O_3 was taken up by the URT (i.e., the same as the extrathoracic airways described in Gerrity et al., 1988) during inspiration, and that this did not change during exposure. Total RT uptake efficiency was approximately 80%, and it did not change during exposure. However, LRT (i.e., the intrathoracic airways described in Gerrity et al., 1988) uptake efficiency fell during exposure from 68 to 62% and was correlated with the O_3-induced fall in V_T (V_T fell from 1,650 to 1,239 mL; f increased from 25.2 to 34.8 bpm; inspiratory flow fell from 1,506 to 1,357 mL/s; $\dot{V}_E$ increased slightly from 40.8 to 40.9 L/min), suggesting that the V_T reduction may have a protective effect on dose delivered to the periphery of the lung. It is not likely that the finite analyzer response time affected uptake measurements. Evidence of this is the lack of dependence of URT uptake on changes in V_T or f. The low values for uptake in the LRT may have been due to an artifact from the relatively slow response time of the analyzer, which was approximately equal to inspiratory and expiratory times. As a check on their results, the authors compared their data with the previous work of Gerrity et al. (1988) by computing uptake by the original technique using peak plateau concentrations. When that was done, the URT uptake efficiencies were 17 and 22% at the beginning and end of exercise, respectively, and the LRT efficiencies were 96 and 92% at the beginning and end of exercise, respectively. The URT change computed this

way was not significant, but the LRT efficiency drop was. When viewed at in this manner, the data from this experiment are consistent with those from the previous experiment.

Because there is a need to compare human O_3 uptake data with rat O_3 uptake data, it is essential that there be confidence in the reliability of the different approaches. To help establish the comparability of techniques, Wiester et al. (1996) measured total RT uptake in humans using a similar, although obviously scaled-up, system to that used for rats (Wiester et al., 1987, 1988). Healthy subjects breathed 0.3 ppm O_3 while seated in an exposure chamber; their faces were placed in a sealed face mask. The face mask was attached to a large tube through which chamber air was circulated with a pump at a rate of 40 L/min. Upstream and downstream O_3 concentrations were measured continuously, as was ventilation with an induction plethysmograph. Subjects breathed at rest, either through nose or mouth (average f = 16 bpm, V_T = 598 to 642 mL, $\dot{V}_E$ = 9.2 to 9.8 L/min). While nose breathing, 73% of inspired O_3 was taken up by the total RT, and, while mouth breathing, 76% of inspired O_3 was taken up, which was significantly higher than that found with nose breathing. This difference is probably not, however, biologically significant. Significant negative correlations between f and uptake in both mouth- and nose-breathers were found, similar correlations were found with $\dot{V}_E$, but no correlations were found between uptake and V_T or any other measure of breathing pattern or pulmonary function.

The observations in Wiester et al. (1996) of a slight increase of total RT uptake efficiency with oral breathing and the inverse correlation of total RT uptake efficiency with f are consistent with those of Gerrity et al. (1988). Furthermore, the data on total RT uptake are consistent overall with that of Gerrity et al. (1988, 1994). The data from Gerrity et al. (1988) reporting total RT uptake efficiencies of about 95% were based on minimum plateau measurements, thus reflecting uptake during steady-state flow conditions, as opposed to the cyclical conditions of actual breathing. The data of Gerrity et al. (1994), on the other hand (in which total RT efficiencies of 80% were reported), were obtained by integrating the product of concentration and flow, thus more accurately reflecting the actual mass uptake of O_3 during cyclical breathing when Gerrity et al. (1994) computed uptake using the methodology from Gerrity et al. (1988), they found that the total RT uptake measurements were comparable. Thus total RT mass uptake efficiencies at rest of 80% are not unreasonable.

Hu (1991), Hu et al. (1992b, 1994), and Ultman et al. (1993) took a different approach to measuring respiratory tract uptake of O_3. They exploited the development of a rapid responding O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) to measure the recovery of small boluses of O_3 delivered to different volumetric depths of the RT. Ozone uptake was measured in a set of four experiments. In the baseline experiments, absorption of O_3 boluses was measured in healthy male subjects at rest. The O_3 boluses were 10 mL in size, with a peak concentration of 3 ppm. The O_3 analyzer characteristics were sample flow of 400 mL/min, 2-methyl-2-butene as reactive alkene, 10 to 90% step-response time of 110 ms, and lower detection limit (18 ppb). In the baseline experiments, the V_T was 500 mL, and the inspiratory and expiratory flow rates were 250 mL/s. In a complete set of measurements, bolus recovery was examined for penetrations of 10 to 200 mL, in 10 mL increments. In the second set of experiments, the effects of flow were measured by measuring bolus recovery as a function of penetration depth for flows of 150, 250, 500, 750, and 1,000 mL/s at a fixed V_T of 500 mL. In a third set of experiments, bolus recovery was measured as a function of penetration depth at a flow of 250 mL/s and a V_T of 500 mL; the bolus delivered to a rubber mouthpiece or to a nasal cannula was compared, thereby examining

potential uptake differences between the two pathways. In the fourth set of experiments, the effects of O_3 concentration on uptake were determined by delivering boluses with peak concentrations of 0.5, 1.0, 2.0 and 4.0 ppm. The latter experiments were conducted because acute studies in isolated dog airways showed that absorption efficiency was inversely related to inhaled concentration between 0.1 and 20 ppm (Vaughan et al., 1969; Yokoyama and Frank, 1972). However, later experiments in guinea pigs, rabbits (Miller et al., 1979), and humans (Gerrity et al., 1988) showed a lack of concentration dependence, implying a linear relationship between concentration and dose. The dependence or lack of dependence of uptake efficiency on O_3 concentration provides information on the order of reactions of O_3 with lung fluid lining and tissue. Under steady-state conditions, concentration independence of uptake efficiency suggests that first-order processes play a role. However, because O_3 absorption is coupled to both interfacial transfer (gas-phase to solute O_3) and subsequent reaction, at face value, the conclusion cannot be reached that saturated absorption rates are solely due to saturation of the reaction components.

In all four experiments, Hu and colleagues computed the first three moments of the inspired and expired bolus distributions with respect to volume. The zeroth moment of a bolus is the O_3 mass contained in the bolus. Thus the zeroth moments of the inspired and expired boluses were used to compute O_3 uptake efficiency (η; or absorbed fraction), breakthrough volume (the mean volume of the exhaled bolus), and bolus dispersion. The first moment of a bolus is its mean volumetric position. The first moment on inspiration gives the penetration volume (V_p), and the first moment on expiration gives the breakthrough volume (V_B). In the absence of any O_3 uptake, a longitudinally mixed bolus should have $V_B = V_p$. The second moment of a bolus is its variance. The difference in variance between the expired and inspired bolus (σ^2) is a measure of gas mixing, or dispersion, in the lung.

In the baseline experiments, the breathing pattern was a resting pattern with a V_T of 500 mL and an average inspiratory flow of 250 mL/s. These experiments were performed on nine male subjects and showed that almost all O_3 was absorbed beyond a penetration depth of 180 mL. Only about 6% of inhaled O_3 was recovered at the 180 mL penetration depth, and, beyond that depth, it was very difficult to obtain an accurate measurement of recovery. The investigators also found that V_B was greater than V_p at penetration depths less than 100 mL, after which V_B leveled out at a constant value. Dispersion was insensitive to penetration depth. An important finding of the baseline experiment was that at quiet resting ventilation, about 50% of the O_3 mass in a bolus inhaled through the upper airways is taken up by the upper airways. To compare these data with results of Gerrity et al. (1988), it is necessary to assume that inspiratory and expiratory uptake efficiencies are equal. Then the unidirectional uptake efficiency of the upper airways to a depth comparable to that at which Gerrity et al. (1988) positioned their sampling catheter is about 30%, which is approximately 25% less than the 40% results of Gerrity et al. (1988). This difference might be due to the presence of a mouthpiece in the experiments of Hu and colleagues, which could reduce the uptake efficiency of the oral pathway. The functional response data of Adams et al. (1989) suggest that this might be the case.

The flow experiments showed that there was a general right shifting of the η vs. V_p curves with increasing flow (i.e., increasing flow causes a deeper penetration of O_3 into the lung with lower fractional uptake by the conducting airways). Eventually, all of the O_3 is still absorbed. Breakthrough volume showed a similar pattern at all flows (i.e., greater than penetration volume at small V_p but flattening out at larger V_p). As flow increased, the level of

the plateau increased. Dispersion, although constant as a function of V_p at all flows, increased linearly with increasing flow.

The studies of Hu and colleagues investigating the role of exposure route in modulating O_3 uptake efficiencies reported that the nose absorbed approximately 30% more than the mouth. This result is at variance with the findings of Gerrity et al. (1988), which indicate that there was only a slightly higher uptake by the oral pathway when compared with the nasal pathway. Gerrity et al. (1988) studied uptake by the two pathways without the use of a mouthpiece or any other delivery system. Subjects were free to breathe naturally. It is possible that the use of mouthpieces and nasal canulas in the studies of Hu and colleagues caused artifacts, resulting in their findings for nasal and oral uptake efficiencies. The study of Adams et al. (1989) supports this, showing enhanced pulmonary function response to O_3 during a mouthpiece exposure compared to face-mask/oral exposure. Finally, the concentration-dependence studies showed that uptake efficiency was not affected by the concentration of inspired O_3 between 0.3 to 4 ppm, implying that O_3 uptake is governed by linear processes.

One of the very unique features of the approach to measuring uptake efficiency taken by Hu and colleagues is that the O_3 bolus recovery data can be used to derive local mass transfer coefficients for the conducting airways. Regional mass transfer coefficients derived experimentally in this way can then be used as input into mathematical model simulations, thereby potentially leading to more accurate models of O_3 dose to the RT.

Hu and colleagues define the parameter Ka (per second) as one that is suitable to characterize local O_3 absorption. It is the product of the overall K (centimeters per second), which reflects the combined contribution of diffusion and chemical reaction to uptake, and the local surface/volume ratio (a; per centimeter). From the □ □ V_p curves, these investigators derived values for Ka. Thus, the experimentally derived mass transfer coefficients depended on assumptions about airway anatomy and morphology. As a result of the various experiments, these investigators found a number of important properties of Ka:

- The proximal subcompartment of the nose has a Ka that is 70% larger than the Ka for the proximal mouth compartment (see, however, the comment made above regarding the nose/mouth differences).
- Ka's in the upper airway compartment were between 1.20 and 2.24/s and relatively insensitive to flow, indicating that diffusion resistance of O_3 through the gas boundary layer is much less than through the mucus film. These data also are consistent with the pig and sheep tracheae experiments of Ben-Jebria et al. (1991).
- In the proximal and distal conducting airway subcompartments, $(Ka)^{-1}$ was linearly related to $(flow)^{-1}$, suggesting that the gas-phase absorption rate constant is directly proportional to flow. In lower airways, therefore, diffusion resistance of the gas boundary layer is important. Hu and colleagues concluded that the gas boundary layer contributes 80 to 90% of the overall diffusion resistance in the central airway compartment.
- The mass transfer coefficient in the lung liquid lining is estimated to fall from 1.4 cm/s in the URT to 0.17 cm/s in the respiratory airways.
- The chemical reaction rate between O_3 and the lung liquid lining was estimated to be 7.3×10^6/s, 2.3×10^6/s, and 8.2×10^5/s in the upper airways, proximal conducting airways, and distal conducting airways, respectively.

- The overall mass uptake coefficients determined in the work of Hu (1991) are significantly higher than those used in the model of Miller et al. (1985). If the mass transfer coefficients in the model are adjusted upward, the total uptake efficiency would be higher than measurements have shown, requiring a downward adjustment of pulmonary region mass transfer coefficients.

Gerrity et al. (1995) took a somewhat more conventional approach in an attempt to acquire regional information on O_3 absorption in the human RT. Healthy subjects underwent transnasal bronchoscopy while in an exposure chamber in which 0.4 ppm O_3 was present. Subjects were asked to breathe at 12 bpm. Inspired and expired air was sampled through a Teflon catheter that had been passed through the biopsy channel of the bronchoscope and positioned approximately in the center of the airway lumen. The air was drawn into a rapid response O_3 analyzer (Ben-Jebria and Ultman, 1989; Ben-Jebria et al., 1990) with a 90% response time of 250 mL/s while using ethylene as the reactive alkene. Average V_T was 810 mL, and average inspiratory flow was 320 mL/s. Air was sampled for five breaths from above the vocal cords, at the entrance to the trachea, above the main bifurcation carina, and midway through the bronchus intermedius. Flow was measured simultaneously by a pneumotach attached to a simple cylindrical mouthpiece. Before and after each measurement, a set of samples from the mouth was collected for reference. Uptake was defined as the fraction of O_3 mass lost across any anatomical segment; mass was determined by integrating the product of O_3 concentration and flow. By way of comparison with the other human O_3 uptake studies, Gerrity et al. (1994) found that total RT uptake of O_3 measured in this fashion was 91%. This is higher than the resting data of Wiester et al. (1996); however, the average V_T in this study of 810 mL, compared with the 600 mL VT reported by Wiester et al. (1996) may account for this difference. When Gerrity et al. (1995) computed the unidirectional uptake efficiencies between the mouth and the various sampling sites, they found that 17.6, 27.0, 35.5 and 32.5% of the O_3 passing into the mouth is taken up by structures up to the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. They also computed the unidirectional uptake efficiencies across individual airway segments: 17.6% between the mouth and just above the vocal cords, 12.8% from above the vocal cords to the upper trachea, 11.5% from the upper trachea to the main bifurcation carina, and essentially zero between the carina and bronchus intermedius. The uptake between the mouth and just above the vocal cords is considerably lower than that measured earlier by Gerrity et al. (1988), even considering the fact that, in the earlier study, peak plateaus were used. As has been noted earlier, it is possible that the mouthpiece played a role in reducing the uptake efficiency of the mouth. The uptake efficiency of O_3 across the trachea is in line with the data from sheep and porcine tracheae at the higher flow rates (Ben-Jebria et al., 1991). The present data are also consistent with the bolus uptake data of Hu (1991) and colleagues, which also were acquired with a mouthpiece. When the O_3 bolus data are used to compute unidirectional uptake efficiencies (assuming that the segmental efficiencies are the same on inspiration and expiration), the Hu et al. (1992b) data yield uptake efficiencies of 21, 36, 44, and 46% between the mouth and the vocal cords, the upper trachea, the main bifurcation carina, and the bronchus intermedius, respectively. The O_3 bolus data are, therefore, in good accord with the data of Gerrity et al. (1995). The measured uptake efficiencies across airway segments clearly appear to be higher than those predicted by the model of Miller et al. (1985). If higher uptake coefficients in the conducting airways were used in the model of Miller et al. (1985), the model would overestimate total RT O_3 uptake.

To adjust for this, pulmonary uptake coefficients would have to be reduced. Unfortunately, the data of Hu and associates cannot provide information beyond the conducting airways.

Gerrity et al. (1995) also measured O_3 washout volumes (i.e., the expired volumes required to cause a specified drop in O_3 concentration). This type of data provides insight into the location of major sites of O_3 uptake. At the mouth, the 90% washout volume was 142 mL, and, at the upper trachea, the 90% washout volume was 62 mL. By the time the entire anatomical dead space of the lungs was washed out, the O_3 concentration had fallen to zero (Gerrity et al., 1995). It is unclear whether the absence of recovered O_3 after washout of the conducting airways was due to O_3 not penetrating beyond the conducting airways or to all of the O_3 that penetrated beyond the conducting airways being absorbed. The latter possibility is more likely based on the observations of Hu (1991).

In assessing the work of Gerrity et al. (1995), it is significant to note that these investigators measured expired plateaus of O_3 concentration that were zero. This contrasts with the earlier work of Gerrity et al. (1988, 1994) in which a non-zero expiratory plateau was observed. The non-zero expiratory plateau may have been due to a number of factors that are unclear. Because ethylene was the reacting alkene in all cases, it is unlikely that interference with other gases such as carbon dioxide (CO_2) was responsible. Another possibility is that O_3 in the early expiratory phase became entrained in the posterior oropharynx and persisted for the duration of expiration.

8.2.3.5 Intercomparison of Ozone Dosimetry Studies

The previous sections emphasized the methods and results of individual experimental studies on O_3 dosimetry. This section will focus on comparisons of the in vivo studies with each other and will draw on these comparisons to arrive at conclusions regarding the utility of these data for extrapolation purposes. The discussion will be divided into three sections, focusing on total RT uptake efficiency, unidirectional URT uptake efficiency, and LRT uptake efficiency.

There are two categories that distinguish various data sets among each other. The first category is the mouth/nose category listed in the Tables 8-2 to 8-4. Studies indicated by "M" or "N" were performed with unencumbered breathing by either oral (M) or nasal (N) breathing. *Unencumbered* indicates the absence of a mouthpiece or canula. Data listed as "M/N" are pooled from data encompassing oral and nasal breathing. Data shown as "Mouthpiece" or "Nasal canula" are acquired using these devices to deliver the O_3 to the animal or human subject, or to measure flow.

The second category is the method used to compute O_3 uptake efficiency. There are essentially two methods. One method, referred to as the steady-state method, relies on measuring the loss of O_3 from a steady air flow moving across an anatomical structure. An example is the data of Yokoyama and Frank (1972) in which a constant flow of ozonated air through the URT of a dog was maintained by a tracheal canula attached to a pump. Uptake efficiency was measured by changes in equilibrium O_3 concentration. Another example is the study of Gerrity et al. (1988), which used the steady-state method by measuring the peak inspiratory and minimum expiratory O_3 concentrations through a catheter in the posterior pharynx. These measurements were compared to the ambient chamber concentration to obtain uptake efficiencies of the URT and LRT. The second method is referred to as the non-steady state method. This method uses the integration of the product of flow and O_3 concentration to compute O_3 masses that, in turn, are used to compute

Table 8-2. Total Respiratory Tract Uptake Data[a]

Species	Mouth/Nose	Method	V_T(mL)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_t	Reference
Human	M	Steady	832	509	18	0.97	Gerrity et al. (1988)[c]
Human	N	Steady	754	456	18	0.96	Gerrity et al. (1988)
Human	M/N	Steady	832	350	12	0.97	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.96	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.88	Gerrity et al. (1994)[c]
Human	M	Non-steady	1,239	1,360	35	0.87	Gerrity et al. (1994)
Human	M	Steady	1,650	1,350	25	0.97	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.95	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	825	330	12	0.91	Gerrity et al. (1995)
Human	M	Non-steady	631	539	16	0.76	Wiester et al. (1996)
Human	N	Non-steady	642	514	16	0.73	Wiester et al. (1996)
Human	Mouthpiece	Non-steady	500	250	15	0.86	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.93	Hu et al. (1992b)
Rat (F344)	N	Non-steady	2.8	12.2	118	0.44	Wiester et al. (1988)
Rat (S-D)	N	Non-steady	2.4	9.6	123	0.46	Wiester et al. (1988)
Rat (Long-Evans)	N	Non-steady	2.7	12.3	132	0.48	Wiester et al. (1988)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.54	Hatch et al. (1989)
Guinea pig	N	Non-steady	2.4	7.5	77	0.53	Wiester et al. (1988)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from reported flows and volumes.
[c]Total RT uptake reported by Gerrity et al. (1988) and Gerrity et al. (1994) did not include the contribution from URT uptake efficiency during expiration. The data include an expiratory URT contribution, assuming it equals inspiratory URT uptake efficiency.

uptake. The studies of Wiester et al. (1988) in rodents, and Wiester et al. (1996) in humans are examples of this technique, as is the study of Gerrity et al. (1994).

Total Respiratory Tract Uptake Efficiency

Table 8-2 provides a summary of in vivo data in all animal species of respiratory tract O_3 uptake efficiency (F_t). The data reported for the studies of Gerrity et al. (1988, 1994) have been adjusted from the published values to account for the fact that the F_t cited in those papers did not include uptake in the URT on expiration. To make the adjustment, the URT uptake efficiency on expiration was assumed to equal the inspiratory uptake efficiency. The F_t data listed for the study of Hu et al. (1992b) were derived from their bolus recovery data by integrating the data over the desired V_T. Because Hu et al. (1992b) could not recover boluses from a depth greater than 220 mL, it was assumed that any bolus delivered

Table 8-3. Unidirectional Upper Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{urt}	Reference
Human	M	Steady	509	18	0.40	Gerrity et al. (1988)
Human	N	Steady	456	18	0.43	Gerrity et al. (1988)
Human	M/N	Steady	350	12	0.41	Gerrity et al. (1988)
Human	M/N	Steady	634	24	0.38	Gerrity et al. (1988)
Human	M	Non-steady	1,360	25	0.37	Gerrity et al. (1994)
Human	M	Non-steady	1,360	35	0.41	Gerrity et al. (1994)
Human	M	Steady	1,360	25	0.16	Gerrity et al. (1994)
Human	M	Steady	1,360	35	0.22	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	337	12	0.18	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	250	15	0.30	Ultman et al. (1993)
Human	Mouthpiece	Non-steady	250	15	0.47	Ultman et al. (1993)
Dog (beagle)	Nasal canula	Steady	83.3	N/A[c]	0.72	Yokoyama and Frank (1972)
Dog (beagle)	Nasal canula	Steady	667	N/A	0.37	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	83.3	N/A	0.34	Yokoyama and Frank (1972)
Dog (beagle)	Mouthpiece	Steady	667	N/A	0.12	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	11.3	113	0.17	Hatch et al. (1989)
Guinea pig	N	Steady	2.7	N/A	0.62	Miller et al. (1979)
Rabbit	N	Steady	16.7	N/A	0.41	Miller et al. (1979)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

to a depth greater than 220 mL was absorbed completely. The derivation of F_t from the bolus data was done for V_Ts of 500 and 1,000 mL.

To assess the consistency of the data, it is useful to examine it as a function of flow. Figure 8-1 shows F_t as a function of inspiratory flow for all human studies. The F_t from the bolus recovery data of Hu et al. (1994) are shown for V_Ts of 500, 1,000, and 1,500 mL. An overview of the data suggests that, with respect to F_t, there is good agreement among the various experimental methods for humans. The data clearly show that F_t decreases with increasing flow and increases with increasing V_T, both of which are qualitatively consistent with model predictions.

One observation is quite prominent: the rat data of Wiester et al. (1988) and Hatch et al. (1989) (not shown in Figure 8-1) are considerably lower than the human data. Even if it is assumed that the rats were breathing up to three times resting ventilation

Table 8-4. Lower Respiratory Tract Uptake Efficiency Data[a]

Species	Mouth/Nose	Method	V_T (mL/s)	Inspiratory Flow (mL/s)	f (bpm)[b]	F_{lrt}	Reference
Human	M/N	Steady	832	350	12	0.93	Gerrity et al. (1988)
Human	M/N	Steady	778	634	24	0.89	Gerrity et al. (1988)
Human	M	Non-steady	1,650	1,360	25	0.68	Gerrity et al. (1994)
Human	M	Non-steady	1,239	1,360	35	0.62	Gerrity et al. (1994)
Human	M	Steady	1,650	1,360	25	0.96	Gerrity et al. (1994)
Human	M	Steady	1,239	1,360	35	0.92	Gerrity et al. (1994)
Human	Mouthpiece	Non-steady	844	337	12	0.95	Gerrity et al. (1995)
Human	Mouthpiece	Non-steady	500	250	15	0.78	Hu et al. (1992b)
Human	Mouthpiece	Non-steady	1,000	250	7.5	0.89	Hu et al. (1992b)
Dog (beagle)	N/A[c]	Non-steady	168	112	20	0.87	Yokoyama and Frank (1972)
Dog (beagle)	N/A	Non-steady	168	168	30	0.83	Yokoyama and Frank (1972)
Rat (F344)	N	Non-steady	2.6	11.3	113	0.33	Hatch et al. (1989)

[a]See Appendix A for abbreviations and acronyms. M = mouth exposure by natural breathing; N = nasal exposure by natural breathing; M/N = pooled data from mouth and nasal exposure; Mouthpiece = exposure by mouthpiece; Nasal canula = exposure by nasal canula; Steady = uptake computed during constant unidirectional flow; Non-steady = uptake computed by integration during cyclic breathing; F_t = total RT uptake.
[b]f is either measured or is computed from flows and V_T.
[c]N/A = not applicable.

(equivalent to an inspiratory flow in humans of approximately 1,000 mL/s), the rat data would still be significantly lower than what was measured in humans. The consistency of the human data of Wiester et al. (1996) (in which the same methodology was used to measure F_t in humans as was used for rats) with the other human data strongly suggests that the low F_t in rats is not a function of the methodology employed. Overall, the evidence reasonably points to the conclusion that F_t in a rat is smaller than in a human.

Unidirectional Upper Respiratory Tract Uptake

Table 8-3 summarizes the data on the unidirectional O_3 uptake efficiency of the URT (F_{urt}). In general, F_{urt} describes the uptake efficiency of anatomical structures proximal to the larynx. A possible exception to this are the data of Hu et al. (1992b). In that study, and other bolus studies, it was assumed that the URT is the volume of the RT 50 mL distal to the lips. This may or may not include the larynx.

The wide variety of URT uptake data in different species and under different flow conditions allows some intra- and interspecies comparisons. To make comparisons among different species, however, requires assumptions about the scaling of breathing patterns among species. Gerrity (1989) examined nasopharyngeal uptake data available in different species at that time by examining the data as a function of the ratio of predicted resting flow to measured flow (scaled flow). The predicted flows were obtained using the allometric

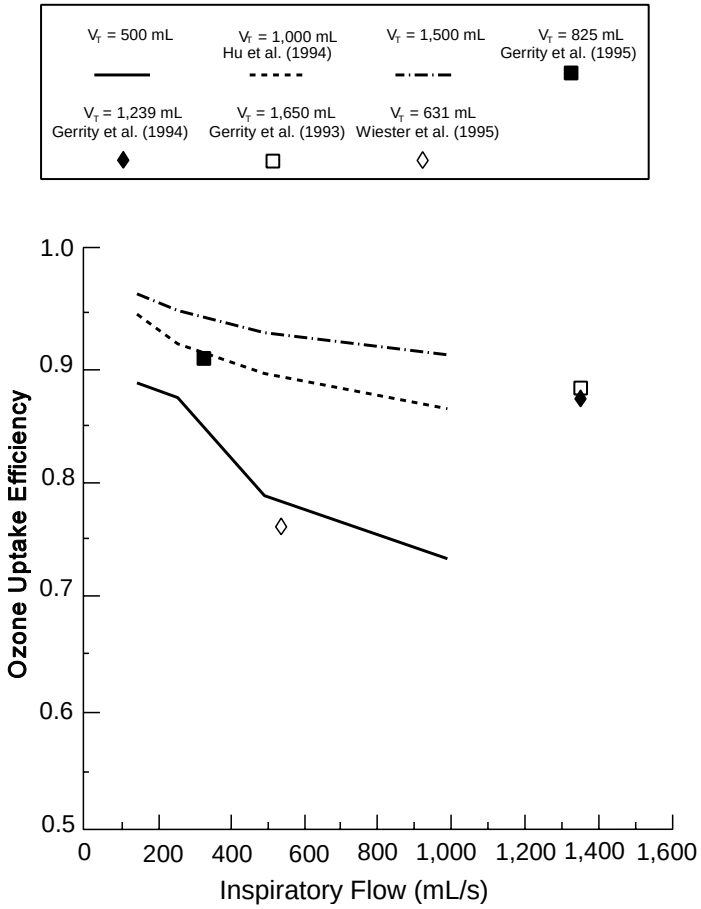


Figure 8-1. Total respiratory tract uptake as a function of inspiratory flow in humans.

equations of Guyton (1947). Ozone uptake efficiency of the URT was then described as a single function of scaled flow and species weight. When newer data become available, this approach can be extended to URT data acquired by both mouth and nasal breathing.

Figure 8-2 shows $1 - F_{urt}$ for nasal breathing plotted as a function of the ratio between predicted and measured flow. As with the previous work of Gerrity (1989), all of the data, except the beagle dog data, are roughly consistent with each other. The body weight dependence of the Gerrity (1989) analysis is illustrated by the two dashed lines representing the predicted range of flow dependencies between rats (lower line) and humans (upper line). All species, except the beagle dogs, fall within these boundaries.

Figure 8-3 shows the data for $1 - F_{urt}$ by the oral route plotted in the same manner. All of the data are for humans except for the two beagle dog data points. Several observations are worth noting. First, the data for the beagle dog appear to be consistent with the human data. The analysis of Gerrity (1989) predicts that the human data generally would lie above the dog data, although not to a large degree. This suggests that the oral passage of the dog may have O_3 scrubbing properties similar to the human oral passage. Second, within the human data, use of a mouthpiece appears to reduce the uptake efficiency of the oral passageway. The closed diamonds in Figure 8-3 are the data from the study of Gerrity et al.

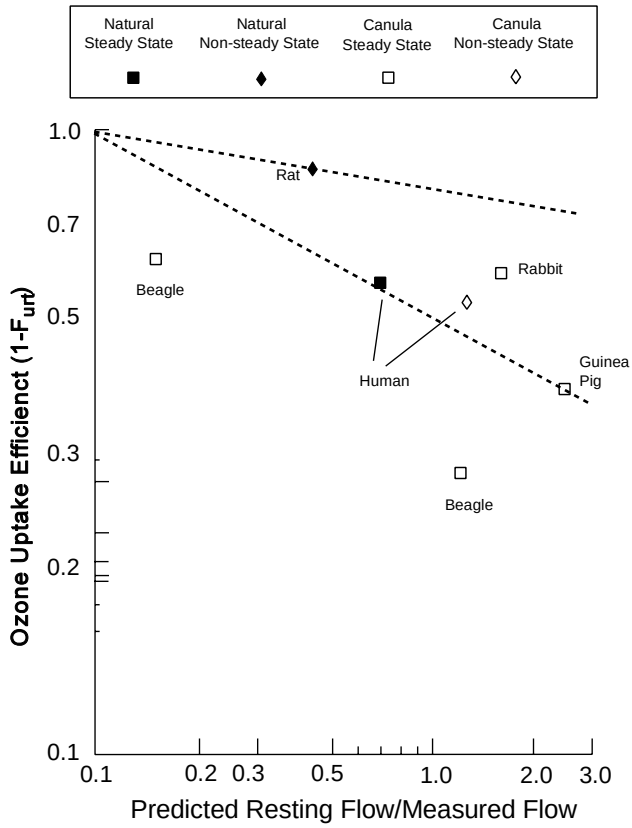


Figure 8-2. Unidirectional uptake efficiency in the upper respiratory tract by the nasal pathway. The ratio of predicted resting flow to measured flow is a way to scale flow to allow for interspecies comparisons. The beagle dog data are from Yokoyama and Frank (1972), the rat data are from Hatch et al. (1989), the rabbit and guinea pig data are from Miller et al. (1979), and the human data are from Gerrity et al. (1988) (closed square) and Ultman et al. (1993) (open diamond). Lines representing predictions for uptake efficiency are from Gerrity (1989).

(1994), which involved unencumbered breathing. These data generally are lower than the data of Hu et al. (1994) and of Gerrity et al. (1995). The fact that the Hu et al. (1994) and Gerrity et al. (1995) data are consistent with each other supports this speculation. This observation may account for the result of Ultman et al. (1993) that the uptake efficiency of the URT is greater by the nasal pathway than by the oral pathway, which is counter to the observations of Gerrity et al. (1988).

Lower Respiratory Tract Uptake

Table 8-4 summarizes the data on the uptake efficiency of the LRT tract (F_{lrt}). In this discussion, F_{lrt} is the uptake efficiency of the LRT relative to the concentration of

O_3 entering the LRT. The human data of Gerrity et al. (1988, 1994, 1995) and the rat data of Hatch et al. (1989) include the larynx in the LRT. The beagle dog data of Yokoyama and

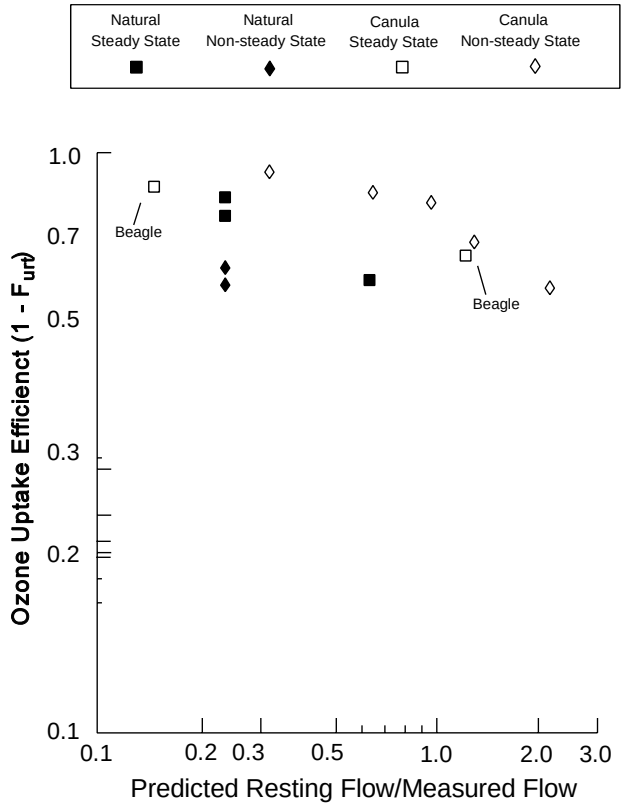


Figure 8-3. Unidirectional uptake efficiency in the upper respiratory tract by the oral pathway. The ratio of predicted resting flow to measured flow scales the flow to allow for interspecies comparison. The closed squares and diamonds at a scaled flow of 0.25 are from Gerrity et al. (1994), the closed square at a scaled flow of 0.65 is from Gerrity et al. (1988), the open diamond at a scaled flow of 0.97 is from Gerrity et al. (1995), the remaining open diamonds are from Hu et al. (1994).

Frank (1972) does not include the larynx. The influence of the larynx in the human data of Hu et al. (1992b) is uncertain because of the volumetric definition of the URT (see discussion above).

Human data obtained using non-steady state methods by the oral pathway are plotted in Figure 8-4 as a function of inspiratory flow. As in Figure 8-1, the data of Hu et al. (1994) are plotted for three different V_Ts: 500 mL, 1,000 mL, and 1,500 mL. The first observation is that the single data point of Gerrity et al. (1995) is approximately 20% higher than the data of Hu et al. (1994) at a comparable flow and V_T. Second, the data of Gerrity et al. (1994) are markedly lower than that suggested for comparable flows and V_Ts by the data of Hu et al. (1994). In assessing this discrepancy, it is important to keep in mind that the URT is defined in the work of Hu et al. (1994) as a fixed volume of 50 mL distal to the lips. This is

an inaccurate definition that could influence greatly the estimation of LRT uptake efficiency. The coherence of all of the data on F_t and F_{urt} by the oral pathway

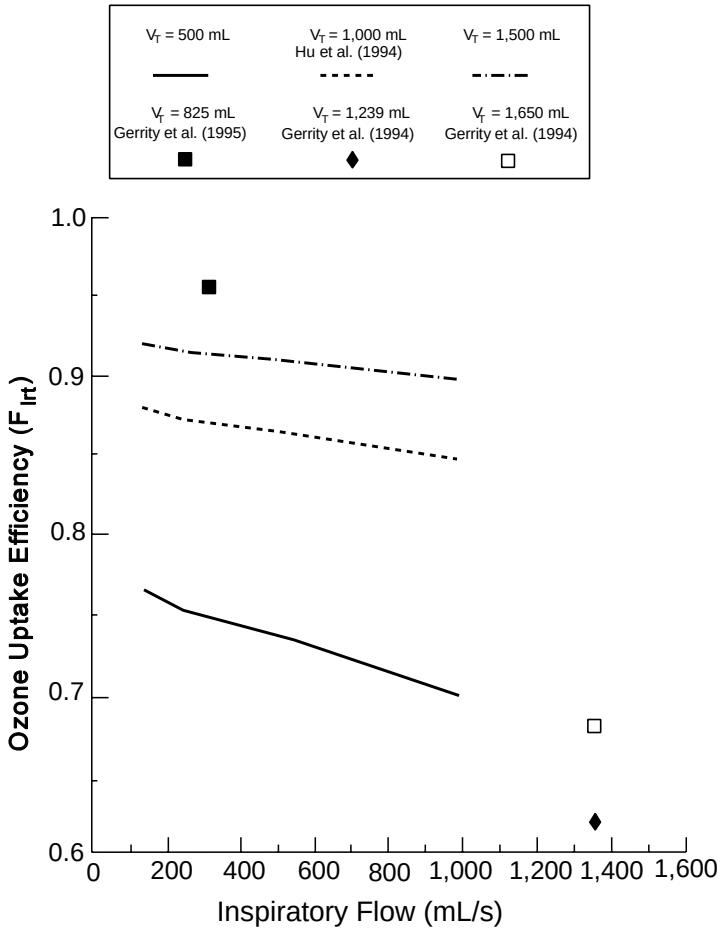


Figure 8-4. Uptake efficiency of the lower respiratory tract as a function of inspiratory flow in humans.

suggests that F_{lrt} also should be consistent among studies. If it is assumed that the data represented by the Gerrity et al. (1994, 1995) studies more accurately reflect human LRT uptake efficiency, then the flow dependence for F_{lrt} would be considerably steeper than suggested by the data of Hu et al. (1994).

Finally, the F_{lrt} data of Yokoyama and Frank (1972) in beagle dogs were acquired with flows that were about 100 and 150% of resting flow rates and V_Ts approximately 150% of those predicted (Guyton, 1947). When flow is scaled in the dogs, the F_{lrt}s of 0.87 and 0.83 at the two flow rates are consistent with the human data of Gerrity et al. (1995) but higher than the Hu et al. (1994) data.

8.2.4 Dosimetry Modeling

8.2.4.1 Background

Table 8-5 presents a summary of theoretical studies of the uptake of O_3 by the RTs (or regions) of humans and laboratory animals that have become available since the 1986 review. Although there are 10 investigations listed, there are only five distinct

Table 8-5. Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation[b]	Results/Predictions	Reference
Guinea pig LRT/Kliment (1973), Schreider and Hutchens (1980); rat LRT/ Kliment (1973), Yeh et al. (1979)	Miller et al. (1985)	Enhanced Miller et al. (1985). Investigates the effect on predictions of anatomical models, FRC, ventilation, and TB liquid lining rate constant. Simulates O_3 uptake in anatomical models of rat lobes.	With respect to different anatomical models for the same and different species: qualitative similarity in the shape of net and tissue dose versus airway number curves, but significant differences in regional fractional uptakes. Maximum tissue dose in vicinity of PAR. PAR dose decreases with increasing time of flight to this region. Maximum tissue dose in the vicinity of the first pulmonary region segment of anatomical models.	Overton et al. (1987)
Human, rat, rabbit, and guinea pig LRT/not specified	Miller et al. (1985) and Overton et al. (1987)	Miller et al. (1985) and Overton et al. (1987). Compares O_3 dose profiles of human, rat, guinea pig, and rabbit. Uses model and experimental data to estimate O_3 dose-response curves for decrements in FEV_1 (humans) and for BAL proteins in rat, guinea pig, and rabbit. Compares LRT uptake predictions to the human experimental data of Gerrity et al. (1988).	Similarity among species in the shape of the airway segment curve: tissue dose increases distally in the TB region, reaches a maximum in the first pulmonary region segment for human, rat, and guinea pig and in the last TB segment of the rabbit, and then decreases distally in the pulmonary region. Predictions of uptake distal to the oropharynx are in agreement with Gerrity et al. (1988).	Miller et al. (1988)
Rat total RT/URT: Schreider and Raabe (1981); TB: Uses rat cast data (Raabe et al., 1976) to define TB region paths; PUL: Yeh et al. (1979) for model of the acinus	TB and pulmonary region mass transfer coefficients based on Overton et al. (1987)	Enhanced Overton et al. (1987). Illustrates dose distribution along the longest and shortest (as defined by time of flight) paths from the trachea to the most distal alveoli.	Threefold difference in PAR doses of the shortest and longest paths from trachea to PAR. Dose distributions along the longest or shortest path were qualitatively similar to Overton et al. (1987), with maximum tissue dose in the first pulmonary region generation.	Overton et al. (1989)
Human LRT (newborn to adult)/TB: based on Yeh and Schum (1980); PUL: based on Hansen and Ampaya (1975)	Miller et al. (1985)	Enhanced Overton et al. (1987). From various sources, develops age-dependent LRT anatomical models. For quiet and maximal exercise breathing, applies the dosimetry model of Miller et al. (1985) to several ages from birth to adult, illustrating the LRT distribution of absorbed O_3.	For quiet breathing, the LRT distribution of dose, the percent uptake, and the PAR dose are not very sensitive to age; but are more sensitive during exercise. Regardless of age and breathing state, the largest O_3 dose occurs in the PAR. No uptake in the URT.	Overton and Graham (1989); Miller and Overton (1989)

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation	Results/Predictions	Reference
Human total RT/URT: Hanna and Scherer (1986); LRT: Weibel (1963)	Time-dependent molecular diffusion and first-order reactions in liquid lining and interstitium; transfer through epithelium modeled as a permeability process—no reactions in this layer. URT and LRT liquid lining rate constants: 50 and 1 times that of Miller et al. (1985), respectively	Model development. Lung dimensions scaled to those of a young male and a young female. Contrasts LRT air-phase concentrations during exercise and rest. Compares male and female air-phase O_3 concentrations and male and female subepithelial concentrations.	URT uptake may be greater in cold than in warmer air. For the ventilatory parameters used, (1) subepithelial O_3 concentrations are a maximum in the terminal bronchioles or the first respiratory bronchioles, and (2) these concentrations are greater in the female than in the male for most of the RT.	Hanna et al. (1989)
Human, distal segment of a lobe/ based on Horsfield et al. (1971)	Similar to Miller et al. (1985)	Monte Carlo simulation; transport processes defined in terms of probabilities based on physical and chemical principles. The effect of lung asymmetries on the distribution of uptake in the pulmonary region.	Tissue dose in the PAR along the shortest path is approximately 50% larger than that along the longest path.	Ultman and Anjilvel (1990)
Human LRT/Weibel (1963)	Investigates two formulations: (1) Miller et al. (1978) and (2) Miller et al. (1985)	Model development; assumes quasi-steady conditions. Air-phase concentration and tissue dose profiles for the two reaction schemes, various ventilatory parameters, and various liquid lining transport and chemical parameters. Compares predictions of first-order reaction scheme to results of Miller et al. (1985).	This model does not conserve mass. Predictions should only be considered qualitatively. Maximum tissue dose in respiratory bronchioles for both chemical reaction schemes.	Grotberg et al. (1990) (Grotberg [1990])
Rat LRT/based on serial reconstruction of a set of intrapulmonary airways and their ventilatory units combined with a single path from the larynx to the reconstructed set based on Yeh et al. (1979)	TB and pulmonary region mass transfer coefficients based Overton et al. (1987)	Model development. Illustrates the influence of ventilatory unit size and proximal anatomic dead space and on the uptake and distribution of inhaled O_3 in ventilatory units. (Illustrates the influence of ventilatory unit volume on the distribution of inhaled O_3 within ventilatory units.)	Ventilatory unit uptake is significantly influenced by both proximal airway dead space and ventilatory unit volume. Flux of O_3 to air-liquid interface in the proximal portions of larger ventilatory units are significantly greater than in smaller units.	Mercer et al. (1991) (Mercer and Crapo [1993])

Table 8-5 (cont'd). Theoretical Ozone Dosimetry Investigations[a]

Species and Region Modeled/Anatomical Model	Liquid Lining and Tissue Transport and Chemical Reactions	Dosimetry Model /Subject of Investigation[b]	Results/Predictions	Reference
Rat ventilatory unit/ Mercer et al. (1991)	Mercer et al. (1991)	Mercer et al. (1991). Along a path distally from a bronchiolar-alveolar duct junction; compares experimentally determined changes in ventilatory unit wall thickness due to an O_3 exposure to dosimetry model predictions of flux to the air-liquid interface.	As a function of distance from the BADJ; experimentally determined relative changes in ventilatory unit wall thickness due to O_3 exposure are very similar to predicted relative fluxes to the air-liquid interface.	Pinkerton et al. (1992); Miller and Conolly (1995)
Human total RT/URT: Fredberg et al. (1980); LRT: Weibel (1963)	Pseudo steady-state diffusion and first order reactions combined with biochemical data of Miller et al. (1985)	Model development. Illustrates LRT distribution of (1) air-phase concentration at various times during the breathing cycle and (2) O_3 flux (dose) to liquid lining and to tissue.	Flux of O_3 to air-liquid interface decreases distally; flux to tissue increases along the conducting airways, reaches a maximum in the terminal bronchioles, then decreases rapidly in the gas exchange region.	Hu et al. (1992a)

[a]See Appendix A for abbreviations and acronyms. Generally, for modeling purposes, PAR is the first pulmonary region, generation, or model segment; PUL = pulmonary region.
[b]Refers to the theoretical or mathematical formulation aspects of gas transport and reactions without the specification of morphometric and physiological parameter values.

dosimetry models (with respect to groups of co-workers and independent model formulation): the models of (1) Overton et al. (1987) and Miller et al. (1985); (2) Hanna et al. (1989); (3) Grotberg et al. (1990), although they considered two reaction schemes; (4) Mercer et al. (1991); and (5) Hu et al. (1992a). In some cases, several references have been grouped into one investigation. This is because the multiple studies came from the same co-workers or laboratory and added to or were complementary to the original or common dosimetry modeling theme.

Major factors affecting the local uptake of reactive gases in the RT were respiratory tract morphology and anatomy; the route of breathing (nose or mouth); the depth and rate of breathing (V_T and f); the physicochemical properties of the gases; the processes of gas transport; and the physicochemical properties of the liquid lining of the RT, respiratory tract tissue, and capillary blood. A detailed discussion of these factors can be found in Overton (1984), Ultman (1988), and Overton and Miller (1988).

Because all of the dosimetry models listed in Table 8-5 were developed to simulate uptake in the LRT or the total RT, these models have some common aspects. These include the formulation of O_3 transport and wall loss in the air compartments of the RT, the use of species-dependent morphometric models or data to define air and liquid lining compartment dimensions, and a description of the transport and loss of O_3 in the liquid lining and tissue.

In all the dosimetry models that have become available since 1986, except for Ultman and Anjilvel (1990) and Grotberg et al. (1990), which are discussed later, O_3 transport and loss processes in air compartments were approximated in terms of a one-dimensional, time-dependent, partial differential equation of continuity. This type of equation accounts for axial convection and dispersion or diffusion and the loss of O_3 by absorption at the gas-liquid interface. The use of this approximation is very common in modeling the transport in the LRT of gases such as oxygen, nitrogen, helium, and CO_2 (e.g., Scherer et al., 1972; Paiva, 1973; Chang and Farhi, 1973; Yu, 1975; Pack et al., 1977) and has been assumed to be applicable to O_3. Ultman and Anjilvel (1990) used a Monte Carlo method to simulate O_3 uptake. Based on the physical and chemical principles of mass transport in the RT, probabilities were assigned to the fate of a molecule in a way so as to account for convection, dispersion, and loss to the liquid lining.

Dosimetry models published since 1986 can be grouped according to how transport and chemical reactions are modeled in respiratory tract fluids and tissues: those based on the formulation of (1) Miller (1977) and Miller et al. (1978), who used an instantaneous reaction scheme, and (2) Miller et al. (1985), who used a quasi-steady, first-order reaction scheme. These two approaches are discussed in the earlier criteria document (U.S. Environmental Protection Agency, 1986). In addition to the use of similar formulations for liquid and tissue transport/reactions, all of the post-1986 studies used essentially the same biochemical data of either Miller et al. (1985) for humans or Overton et al. (1987) for laboratory animals. The implication is that most of the studies are expected to predict qualitatively similar results.

There are minor variations on the second chemical reaction formulation. Hanna et al. (1989) used a time-dependent diffusion-reaction equation, instead of the time-independent (quasi-steady) equation used by Miller et al. (1985). Based on the rate constants used by Hanna et al. (1989) and on discussions in Miller et al. (1985) and in Grotberg et al. (1990), the modeled transport processes in the liquids and tissues can be inferred as essentially quasi-steady, which is equivalent to the second formulation. Another variation uses mass transfer coefficients determined by the second formulation and the biochemical assumptions of Miller

et al. (1985) or Overton et al. (1987). The liquid and tissue transport/reaction formulation for specific investigations is indicated in column 3 of Table 8-5.

In addition to the assumptions and the formulation of equations that describe the transport and loss of O_3 in the RTs of humans or laboratory animals, it is important to evaluate whether simulation results reflect accurate solutions to the mathematical dosimetry model formulation. Of the five distinct model formulations listed in Table 8-5, Overton et al. (1987), Mercer et al. (1991), and Hu et al. (1992a) discuss most or all of the relevant issues of stability, solution convergence, and mass conservation. In addition, using steady unidirectional flow in a straight tube as a test case, they report successfully simulating analytical solutions to their equations of transport and uptake. Neither Hanna et al. (1989), Ultman and Anjilvel (1990), nor Grotberg et al. (1990) address the issue of accuracy. There is no reasonable way to judge whether the solutions of Hanna et al. (1989) or of Ultman and Anjilvel (1990) accurately represent solutions to their dosimetry model assumptions and formulations; however, with the exception of Grotberg et al. (1990), there are no reasons to assume that the solutions of these models are not accurate.

Because the Grotberg et al. (1990) model formulation is different than the others, an explanation is needed. Based on the smallness of relevant parameters, Grotberg et al. (1990) assume quasi-steady conditions for O_3 concentration and air velocity in the air compartments and obtain approximate analytical solutions to the time-independent, three-dimensional equation of continuity for a model airway and apply the results to the morphometric model of Weibel (1963). One advantage of analytical solutions is that they account naturally for parameters (such as dispersion and gas-phase, mass-transfer coefficients) or local processes (e.g., possibility of high uptake at airway entrances) that must be known and estimated for, or incorporated into, the one-dimensional approach. Grotberg et al. (1990) carried out simulations using anatomical and physiological conditions based on Miller et al. (1985) and compared their results. Although qualitatively similar to Miller et al. (1985), Grotberg and co-workers predicted significantly larger pulmonary tissue doses (up to 10-fold).
A comparison of the pulmonary region doses predicted by Grotberg et al. (1990) to those predicted by Miller et al. (1985) indicates that the Grotberg et al. (1990) model does not conserve mass (it predicts that the pulmonary region absorbs over 3.4 times the amount of O_3 inhaled). The overprediction may be an artifact of the quasi-steady approximation, because effects due to differences in the time of flights from the trachea to different LRT locations are not taken into account. In any case, the quantitative predictions reported by Grotberg et al. (1990) are questionable.

Chemical data more recent than that used for the dosimetry models in Table 8-5 show that compounds other than unsaturated fatty acids (the only compound with which O_3 is assumed to react in the models using the second chemical reaction formulation) are as reactive or more reactive with O_3 (Pryor, 1992). Using these data, estimates of O_3 diffusion coefficients in the liquid lining and bilayers, layer thicknesses, and data on the concentrations of biocompounds in these layers, Pryor (1992) estimates that most of any O_3 that penetrates into a cell bilayer reacts within the layer (very little if any penetrates to the cell interior), and O_3 will not penetrate the liquid lining where it is greater than 0.1 μm thick. Several relevant comments are given by Pryor (1992): the calculations are considered a crude first approximation; the possibility that a small fraction of O_3 may penetrate the bilayer and reach the cell interior can not be excluded; and surfactant layers can be very thin, and some cells may not be protected very well or at all.

If the conclusions of Pryor (1992) are essentially correct, they have implications for past and future dosimetry modeling studies because past investigations have underestimated the reactivity of O_3 with biocompounds; with respect to cellular damage, products of O_3 reactions in the liquid lining may be the main toxic compound; increasing the value of rate constants would have no effect on predictions of dosimetry models using the instantaneous reaction scheme because rate constants in this approach are assumed to be infinite, but increasing the concentration of reacting biocompounds would increase uptake; (4) use of unsaturated fatty acid data only (with a first-order reaction scheme) results in an underestimate of the reactivity of O_3 in the liquid lining and an overestimate of the O_3 tissue dose, and a possible underestimation of the toxic dose due to reaction products; and (5) with higher O_3 reaction rates, the first-order chemical-reaction formulation would result in larger predicted uptakes.

8.2.4.2 Dosimetry Model Predictions

Similarity of Model Predictions

A survey of dosimetry modeling results shows that, in some areas of investigation, there is a qualitative similarity in predictions by models of different groups of investigators for different species or subpopulations.

(1) *Distribution of LRT dose (dose profiles or dose versus generation)*. As shown in Figure 8-5, beginning at the trachea, net dose (O_3 flux to air-liquid interface) slowly decreases distally in the tracheobronchial region (TB) and rapidly decreases distally in the pulmonary region. Tissue dose (O_3 flux to liquid-tissue interface) is very low in the trachea, increases to a maximum in the terminal bronchioles or first airway generation in the pulmonary region, and rapidly decreases distally from this location (e.g., Miller et al., 1978, 1985, 1988; Overton et al., 1987, 1989; Overton and Graham, 1989; Grotberg et al., 1990; Hu et al., 1992a).

If O_3 were the only toxic agent and all the tissues of the LRT were equally sensitive to the same dose, the models predict that the greatest morphological damage would occur in the vicinity of the junction of the conducting airways and the pulmonary region and decrease rapidly (distally) from this area, which is consistent with observations in laboratory animals (see Chapter 6, Section 6.2.4). On the other hand, using the best estimates of morphometric and physiologically based biochemical parameters of Miller et al. (1978, 1985) and Overton et al. (1987), the models predict extremely (relatively) low tissue doses in the trachea and large bronchi; this suggests very little or no tissue damage should occur there, which is contrary to observations (see Chapter 6, Section 6.2.4). However, this is moot, if, as suggested by Pryor (1992), the toxic substances are primarily reaction products of O_3 and not O_3 itself. In this case, the O_3 net local dose, not the local O_3 tissue dose, may be a better estimator of local toxic tissue dose, because the rate of production of products would be related to the rate of O_3 uptake.

(2) *Effect of exercise or increased ventilation*. The effect of exercise is to slightly increase the TB dose and to significantly increase the pulmonary region total dose (mass of O_3) and the CAR dose (mass per unit surface area) (e.g., Miller et al., 1979, 1985; Overton et al., 1987, 1989; Overton and Graham, 1989; Hanna et al., 1989; Grotberg et al., 1990).

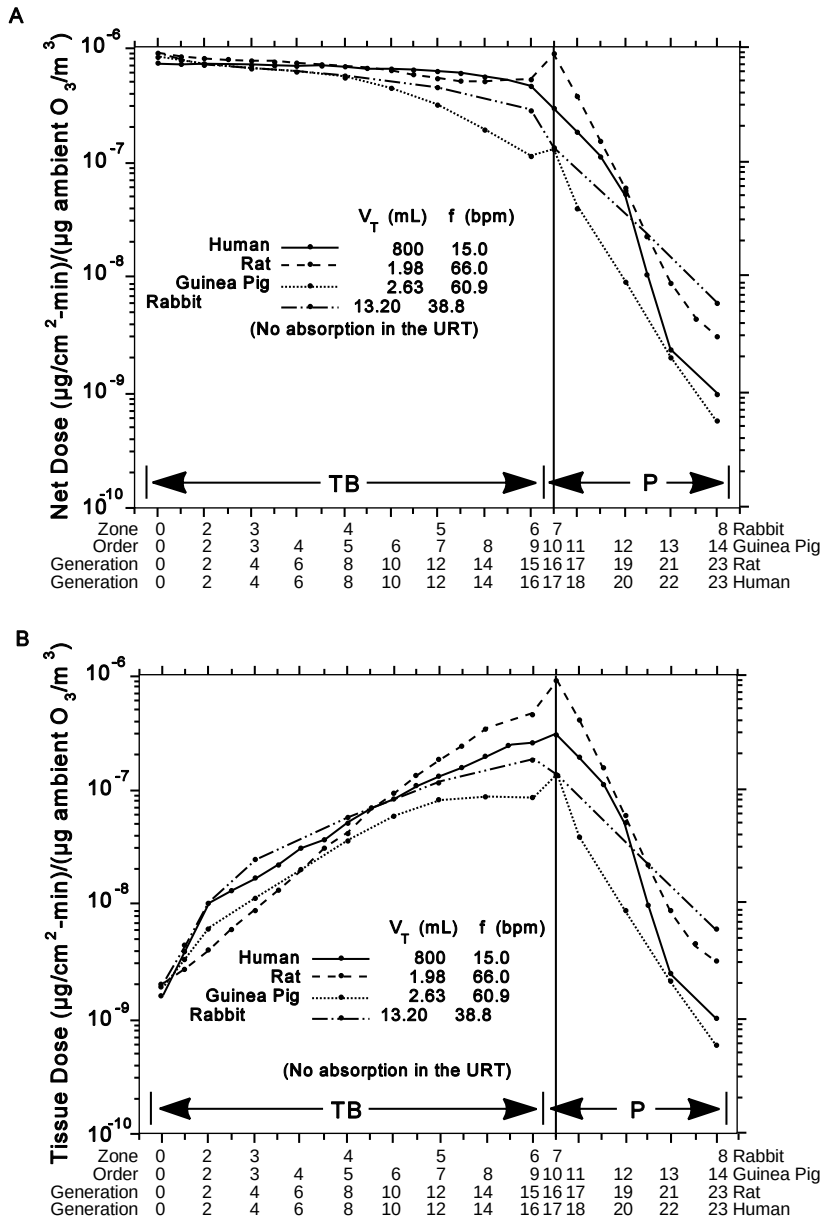


Figure 8-5. Net dose (A) and tissue dose (B) versus sequential segments along anatomical model airway paths for human, rat, guinea pig, and rabbit. In general, each segment represents a group of airways or ducts, with common features as defined by the designers of the anatomical model (human and rat: generation; guinea pig: order; rabbit: zone). For a given species, the plotted dots represent a predicted dose that corresponds to a given segment. The dots have been joined by lines for ease of interpreting the plots; these lines do not represent predicted values except where they intercept the dots. TB = tracheobronchial region; P = pulmonary region.

(3) *Effect of respiratory tract inhomogeneity*. Models have predicted that the further the proximal alveolar region is from the trachea, the less the O_3 tissue dose (mass of O_3 absorbed per unit surface area) to the proximal alveolar region. (For modeling purposes, the proximal alveolar region has been defined as the first pulmonary generation or the first pulmonary region model segment along a path; this region is a part of the CAR.) Overton et al. (1989) predicted a threefold greater proximal alveolar region dose for the shortest path relative to the longest path in rats. Ultman and Anjilvel (1990) simulated O_3 distribution in a small segment ($<1\%$) of the distal airways of an asymmetric anatomic model of the human lung. They found that the O_3 tissue dose (mass per square centimeter) in the proximal alveolar region along the shortest path was approximately 50% greater than that along the longest path. Mercer et al. (1991) found that path distance and ventilatory unit size affect dose: predicted doses in the proximal segments (essentially, the proximal alveolar region) of the larger ventilatory units (with the smallest relative dead space) are significantly larger than the average proximal segment doses. Further, for the small sample of ventilatory units modeled (43), Mercer et al. (1991) predicted a range of proximal segment doses of greater than a factor of 6. Because the proximal alveolar regions of Ultman and Anjilvel (1990) and of Mercer et al. (1991) belonged to a "local cluster", and there are many clusters with varying distances from the trachea, a variability greater than 50% and a factor greater than 6, respectively, are expected in proximal alveolar region doses. Mercer and Crapo (1993) illustrated the effect of ventilatory unit volume alone on the distribution of dose, predicting that a 2.3 times larger unit receives 1.9 times the dose (mass per surface area) of the smaller unit at the entrance of the unit.

The variability of predicted proximal alveolar region doses and, by inference, CAR doses suggests that the magnitude of toxicological effects for different CARs are different. This prediction is consistent with the observations of Schwartz et al. (1976) and Boorman et al. (1980) of damage variation among different CARs of the same rat. It is reasonable to assume that variable damage at equivalent but different morphological locations also occurs in humans.

Specific Topics

Effect of Assumptions About Anatomical Dimensions. For rats and guinea pigs, Overton et al. (1987) used two morphometrically based anatomical models (rat anatomical models: Kliment, 1973, and Yeh et al., 1979; guinea pig anatomical models: Kliment, 1973, and Schreider and Hutchens, 1980) to investigate the influence of anatomical model formulation on predicted uptake. Results with all four anatomical models in combination with different ventilatory parameters showed a qualitative similarity in the shapes of the dose profiles, but the two anatomical models for the same species resulted in considerable differences in predicted percent RT and pulmonary region uptakes.

Respiratory Tract Uptake in Human Adults and Children. Overton and Graham (1989) used several sources of data on age-dependent LRT dimensions and structure to construct theoretical LRT anatomical models for humans from birth to adulthood. The O_3 dosimetry model of Miller et al. (1985) was used to estimate the regional and local uptake of O_3. For the percent uptake (84 to 88%) during quiet breathing, the LRT distribution of

absorbed O_3 and the centriacinar O_3 tissue dose are not very sensitive to age. Regional percent uptakes are more dependent on age during heavy exercise or work than during quiet breathing, and total uptakes range from 87 to 93%. Generally, the total quantity of O_3 absorbed per minute increases with age. For all conditions simulated, the largest O_3 tissue dose is predicted to occur in the CAR. Miller and Overton (1989) present similar results. Because uptake by the URT was not simulated and because this region can be assumed to have an important effect on LRT uptake, a comparison of predictions of LRT uptakes in children and adults should be viewed with caution. On the other hand, URT uptake probably has little effect on the shape of the dose curves.

Dosimetry Modeling Results Compared to Dosimetry Data

Based on the experimental conditions discussed in Gerrity et al. (1988) and using the model and parameters of Miller et al. (1985), Miller et al. (1988) simulated the uptake of O_3 distal to the oropharynx of human subjects. For the target f's of 12 and 24 bpm and V_Ts ranging from 0.4 to >1.6 L, the simulation results were in good agreement with the breath-by-breath experimental data. The average experimental LRT uptake efficiency was 0.91 as compared to the 0.89 prediction given by Miller et al. (1985) for the region distal to the oropharynx. It should be remembered, however, that values for uptake efficiency from the Gerrity et al. (1988) study were derived from the raw data using a steady-state method, whereas the models of Miller et al. (1985) and Miller et al. (1988) utilize cyclic flow, thus making the predictions more appropriate for comparison with uptake data from non-steady state methods. From an analysis in Gerrity et al. (1994), it appears that total RT uptake computed by either steady-state or non-steady state methods differ by only about 10% in relative terms.

There have been major improvements to the original model, as described by Miller et al. (1985) and Overton et al. (1987), including the addition of the URT and establishing a regional mass transfer coefficient based on experimental data. Table 8-6 summarizes the assumptions that underpin these improvements, as well as other relevant assumptions used for simulating the uptake of several human dosimetry experiments. A discussion of the assumptions is given in Section 8.5.2.

After taking into consideration the assumptions of Table 8-6, Table 8-7 compares experimental total RT uptake efficiency data and model predictions for humans. Use of the rat assumptions in conjunction with the model will be discussed later (Section 8.4.3). The model predictions show good agreement with the total RT uptake efficiency data of Gerrity et al. (1988), Gerrity et al. (1994), and Hu et al. (1992b). In all cases, the predictions are within 10% of the measured values. The agreement with the data of Hu et al. (1992b) is even better, as expected.

The model prediction for the data of Wiester et al. (1996) is less accurate. Comparison of the Wiester et al. (1996) data with the Hu et al. (1994) data (Figure 8-1) shows, however, that the results are in good agreement with each other. Thus, it would appear that the V_T dependence of the model does not necessarily reflect the real world. However, the general agreement between the model predictions and data are quite good.

Although the models are capable of making reasonable predictions of total RT uptake efficiency, their accuracy for specific regions remains uncertain. The O_3 bolus data (Hu et al., 1992b, 1994; Ultman et al., 1993) and the airway uptake efficiency data

Table 8-6. Assumption for Application of Dosimetry Model to Breathing Frequency Responses to Ozone[a]

Species	Mode of Breathing	Respiratory Tract Morphology	Mass Transfer Coefficients
Human	Oral	- LRT structure from Weibel (1963). - Volume of oral cavity through larynx and surface to volume ratio (S/V) from Hu et al. (1992a,b). - Dead space volume (V_d) and FRC from Hart et al. (1963); TB region volume at FRC equals V_d minus oropharyngeal volume. - Proximal alveolar region defined as first respiratory bronchiole. - Pulmonary region expands; TB does not expand.	- Mass transfer coefficients for each oropharyngeal segment and each TB generation defined as Ka/(S/V) where S/V for the TB region is from Weibel (1963) dimensions reduced to FRC; Ka from Hu et al. (1992b). - Pulmonary mass transfer coefficient is 0.10 cm/s (Miller et al., 1985).
Rat	Nasal	- NP dimensions from Schreider and Raabe (1981). - TB region from Yeh et al. (1979). - Volumes and surface areas of LRT isotropically scaled to FRC. - Pulmonary region from Mercer et al. (1991). - TB and pulmonary regions expand uniformly during breathing. - Proximal alveolar region is first generation of pulmonary region.	- NP and TB mass transfer coefficients estimated using data of Hatch et al. (1989). - Mass transfer coefficient of pulmonary region = 0.137 cm/s; inferred from Pinkerton et al. (1992).

[a]See Appendix A for abbreviations and acronyms.

Table 8-7. Comparison of Total Respiratory Tract Uptake Data with Model Predictions[a]

V_T (mL)	f (bpm)	Measured F_t	Predicted F_t	Data Source
832	12	0.97	0.96	Gerrity et al. (1988)
832	24	0.96	0.93	Gerrity et al. (1988)
500	15	0.86	0.89	Hu et al. (1992b)
1,000	7.5	0.93	0.94	Hu et al. (1992b)
1,650	25	0.88	0.95	Gerrity et al. (1994)
1,239	35	0.87	0.93	Gerrity et al. (1994)
631	16	0.76	0.94	Wiester et al. (1996)

[a]See Appendix A for abbreviations and acronyms.

(Gerrity et al., 1995) raise questions about the model predictions of uptake in the conducting airways. These latter data sets suggest that the models of Miller et al. (1985, 1988) may underestimate the O_3 uptake coefficients in the conducting airways. Ultman et al. (1993) show in their analysis of the bolus data that the reactivity of O_3 with the lung liquid lining decreases with increasing depth into the lung. This could imply that more O_3 is taken up in central airways than previously had been thought. However, the predictions presented in Table 8-7, which are based on the assumptions of Table 8-6, represent a revision of the Miller et al. (1985) model in that the mass transfer coefficients are derived from the actual human data of Hu et al. (1992b).

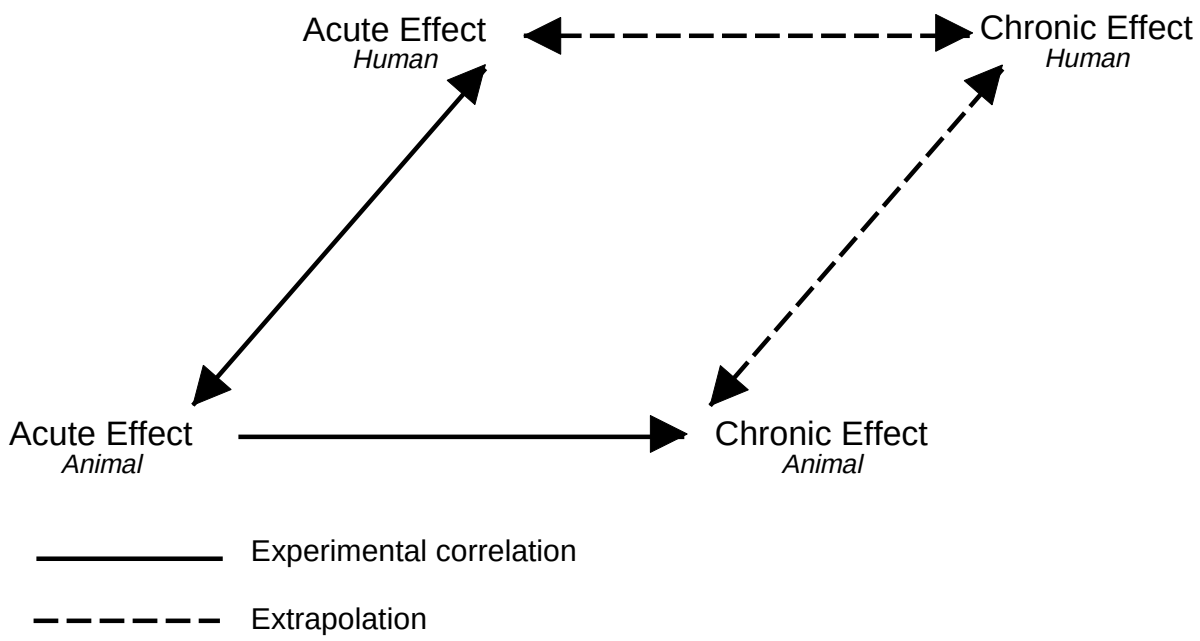
Because the utility of dosimetry models is their usefuleness in facilitating interspecies extrapolation, it is important to compare predictions with animal data as well as with human data. Using the Overton et al. (1987) model formulation and parameters, Overton et al. (1989) developed a formula that can be used to calculate LRT uptakes in rats, given their V_Ts and f's. For comparison purposes, the data of Wiester et al. (1987, 1988) and Hatch et al. (1989) can be used. The average uptake efficiency for the rat from these data is 0.45. Based on the V_Ts and f's of these animals, an average LRT uptake of 0.61 is computed using Overton et al. (1989). If Overton et al. (1989) had included the effects of URT uptake, their model would have predicted more than 0.61.

It is important to note that these results are based on the older model assumptions, not those presented in Table 8-6. If the newer assumptions had been used, the agreement between predictions and actual results would have been much better (i.e., total uptake would have been in the 50 to 60% range, depending on ventilation).

8.3 Species Sensitivity: Lung Function and Inflammatory Endpoints Exemplifying an Approach

8.3.1 Introduction

Quantitative extrapolation of animal-based O_3 toxicity data to the human circumstance requires a paradigm that includes both an estimate of target tissue dose (dosimetry) and an algorithm that relates the responsiveness of the test species to that of the human (species sensitivity). This paradigm can be depicted as an extrapolation parallelogram (Figure 8-6), which conceptualizes a relationship between chronic animal study data and long-term human health effects based on an understanding of acute effects in both species (Graham and Hatch, 1984). Although recent studies have begun to elucidate the underlying mechanisms determining response, the bulk of the present O_3 toxicity database in animals and humans remains largely descriptive. Hence, only a simplified application of this paradigm is feasible at this time. The following section will attempt to harmonize selective literature on acute human and animal responses to O_3 exposure (already reviewed in detail in Chapters 6 and 7) with what is known about the dosimetry of O_3, in an effort to discern relative species sensitivity. To construct an argument that is plausible for this test application, focus is on endpoints for which there are sufficient data in both humans and test animal species and for which exposure scenarios are similar. The endpoints compared include measures of pulmonary function and markers of lung inflammation, most notably BAL protein and cells. When possible, other influencing parameters, such as ventilation augmentation and antioxidants within the lung also will be discussed. The body of in vitro cell studies has not been included because of the



difficulties in interpretation associated with ***Figure 8-6.***

Parallelogram paradigm for utilizing animal data for human health predictions. Acute homologous endpoints serve as the basis for extrapolating chronic effects in humans from animal data.

dosimetry and culturing systems. The reader is referred to Chapter 7, Section 7.2.5, and the recent review by Koren et al. (1994). The goal here is to develop a hypothetical model for the assessment of species-specific sensitivity with acute O_3 exposure that can serve as a framework to better predict human responses, especially with regard to chronic effects. The complex issue of whether controlled human clinical studies accurately reflect population-based responses also will not be considered in this discussion.

8.3.1.1 Dosimetry

This topic has been discussed in detail in Section 8.2. Recent studies by Hatch et al. (1994), utilizing the nonradioactive isotope of oxygen, ^{18}O, to label O_3, have shown that exposure of exercising humans (60 L/min) and resting rats to 0.4 ppm O_3 for 2 h resulted in 4 or 5 times the ^{18}O dose (as adduct) to the BAL constituents of humans as compared to those of F344 male rats. This four- to fivefold difference appeared to be due to the exercise-stimulated hyperventilation of the humans when compared to the rat and compared favorably with indices of effect (i.e., BAL cells and protein at 24 h). Only when the rats were exposed to 2 ppm O_3 for 2 h did the $^{18}O_3$ labeling of BAL constituents approximate that of the human. Thus, on the basis of this study of cellular and protein influx due to O_3 injury, the rat and human appear to have similar sensitivity to O_3 when exercise is considered. Additional related studies with $^{18}O_3$ indicate that deposition in the RT is a cumulative function of ventilation over the initial period of exposure, which would lend support to these findings (Santrock et al.,

1989). Attempts to compare animal data obtained without exercise to human study data with exercise thus would underestimate the dose to the lung and presumably the resultant risk of effect. Studies of O_3 effects (but without assessment of dose) in exercising rodents have confirmed this conclusion (Mautz et al., 1985).

Exercise is only one factor that can alter dose and effect. Studies in laboratory animals that incorporate other factors, such as time of day or diurnal rhythms (Van Bree et al., 1992), animal strain (Pino et al., 1991; Costa et al., 1993), or nutrition (Chapter 6, Section 6.2.5), also show substantial modification of response to O_3, and thus emphasize the need for careful consideration of exogenous factors when attempting to compare or extrapolate study findings. It is likely that a similar range of factor-dependent variability exists within human test subjects.

8.3.2 Homology of Response

The concept of species sensitivity actually consists of two integrated components. The first, homology of response, indicates whether the outcome seen in the animal test species represents the same biological response in the human. In many cases, a measurement of the same endpoint in both species can be presumed to reflect the same toxic phenomenon or mechanism (i.e., the pulmonary irritant-induced tachypnea) (see below). On the other hand, there may be endpoints that, although homologous, are not expressed similarly; for example, the burning discomfort of sensory irritation in the human and the pause on tidal expiration seen in rodents. The second component of species sensitivity relates the dose-response curve to given homologous responses. Alterations in permeability of the air-blood barrier of the lung appear to reflect true species differences in sensitivity to pulmonary irritants such as O_3 (Hatch et al., 1986). Ideally, these elements of species sensitivity should flow directly into extrapolation formulae developed to integrate animal and human research data.

8.3.2.1 Lung Function Endpoints as Homologous Indicators

Lung function studies of small mammals have provided basic physiological information important to the understanding of both normal and diseased lungs (Snider and Sherter, 1977; Harkema et al., 1982; Raub et al., 1982; Mauderly, 1984; Costa, 1985). Animal lung function tests, adapted from those used clinically, have proven useful in describing the nature and severity of lung injury and in distinguishing toxicant-induced effects in the central or peripheral airways from those effects in the parenchyma. In practice, the interpretation of functional changes detected in animals derives from knowledge and experience in human pulmonary medicine. Supporting this view, in theory, is the allometric database for normal mammals, in which the lung function variables associated with ventilation and aerobic metabolism scale systematically to body mass over nearly seven orders of magnitude (Stahl, 1967; Leith, 1976). The lung function studies of O_3 toxicity in animals and humans considered in the present discussion are described in detail in Chapters 6 and 7, Sections 6.2.5 and 7.2, respectively, of this document and in the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986).

8.3.2.2 Inflammatory and Antioxidant Endpoints as Homologous Indicators

Inflammation of pulmonary airways and airspaces is best described as a cascade of events that network infiltrating leukocytes, plasma proteins, and cell-derived mediators, which function presumably to defend or repair (but may further damage) the injured lung (see

Chapters 6 and 7, Sections 6.2.2 and 7.2.4, respectively; Koren et al., 1994). Key markers of the basic inflammatory process include plasma-derived proteins, such as albumin, globulins, and plasmin, and a primary inflammatory cell, the polymorphonuclear leukocyte (PMN). Of these two markers, plasma-derived proteins in the acute phase generally are thought to represent a "leak" from the vasculature to the airspace lumen. Hence, under controlled temporal conditions, plasma protein residing in the airspace and accessible by BAL can be used as a proportional marker of effect that, in turn, should be related to dose. The presence of PMNs in the airspaces is a bit more complex because of the signals involved in recruiting these cells into the lung lumen after injury and the cascade of events apparently involved in their poiesis from the vasculature to the lung lumen. For the purposes of species comparison, plasma-derived protein (nonspecific) and the proportion of PMNs among total cells as sampled by BAL will be emphasized as primary indices of damage and inflammation within the lung.

Antioxidant substances in lung tissue (Slade et al., 1985) and BAL fluid and cells (Slade et al., 1993) have been identified and quantified for humans and several laboratory animal species. The species profiles of these antioxidants in the lung tissue and their respective BAL cells and fluid can differ appreciably (Table 8-8), but collectively they appear to play a significant role in defense of the lung against both endogenous and exogenous oxidant challenge. In particular, ascorbate and vitamin E appear to have major functions in protecting the lung from O_3 challenge (Chapter 6, Section 6.2.1; Slade et al., 1989; Crissman et al., 1993; Koren et al., 1989b; Elsayed et al., 1988), and, when their levels are manipulated in vivo, either can influence the degree of toxic outcome. Hence, the measurement of basal and O_3 response levels of these antioxidants in BAL cells and fluids is useful in assessing the qualitative and quantitative responses among humans and laboratory test species.

8.3.3 Studies of Lung Function

8.3.3.1 Confounding Influences in Lung Function Studies

Ideally, a system for measuring pulmonary function in small animals would be approximately the same as that used in humans for cooperative, unrestrained subjects. However, in animal studies, this is usually not possible. Fortunately, certain measures (e.g., static lung volumes, diffusion capacity) appear to be minimally influenced by sensory reflex or muscular activity in spite of unnatural stresses or blunting of responses caused by anesthetic or physical immobilization. On the other hand, some measurements, typically those involved in the assessment of ventilatory mechanics, can be profoundly influenced by these and other factors, such as ambient and toxicant-altered body core temperature, thus confounding cross-species comparisons. Because a major emphasis of this section is the comparison of lung function data of animals and humans, it is important that the reader realize potentially confounding influences borne by studies of lung function in rodents when compared to analogous measurements in humans. These are discussed briefly below.

Anesthesia

Anesthesia alters pulmonary function measurements in both humans (Rehder et al., 1975) and laboratory animals (Skornick and Brain, 1990; Lamm et al., 1982; Rich et al.,

Table 8-8. Pulmonary Antioxidant Substances in Various Laboratory Animal Species and Humans[a]

Antioxidant	Mouse	Hamster	Rat	Guinea Pig	Rabbit	Human
Ascorbate						
Tissue[b]	41±4	26±2	34±2	39±1	27±3	22±7
BAL cells[c]	—	—	50.3±5.4	17.9±1.4	—	3.5±0.1
BAL fluid[c]	—	—	199.4±9.1	28.8±2.2	—	21.4±2.8
Glutathione						
Tissue[b]	62±3	61±2	50±2	83±3	83±3	7±1
BAL cells[c]	—	—	14.8±2.7	14.6±2.4	—	2.9±0.5
BAL fluid[c]	—	—	12.1±5.0	11.2±1.9	—	20.4±3.8
Tocopherol						
Tissue[b]	1.0±0.1	1.0±0.1	2.1±0.1	2.0±0.2	1.4±0.2	0.8±0.1
BAL cells[d]	—	—	577.7±83.1	454.5±58.2	—	95.1±23.4
BAL fluid[d]	—	—	0.6±0.2	1.4±0.5	—	47.2±3.8
Uric Acid						
Tissue[b]	—	—	0.35±0.05	4.14±0.24	—	—
BAL cells[c]	—	—	<0.01	0.8±0.1	—	0.07±0.03
BAL fluid[c]	—	—	4.3±0.6	2.7±0.4	—	15.9±2.5

[a]See Appendix A for abbreviations and acronyms; data (mean ± SE) extracted and summarized from Slade et al. (1985, 1993).
[b]Data expressed as mg/100 g wet tissue.
[c]Data expresses as nmol/mg protein.
[d]Data expressed as nmol/mg lipid phosphorus.

1979). In general, ventilation is reduced and changes in ventilatory patterns occur (Pavlin and Hornbein, 1986; Bellville et al., 1960; Hunter et al., 1968; Siafakas et al., 1983). In humans, anesthesia can decrease compliance and FRC, and it also can increase airway resistance (R_{aw}) (Rehder et al., 1974, 1975). In small laboratory mammals, an analogous decrease in FRC occurs, although apparently via a different physiological mechanism (Lamm et al., 1982). Additional anesthesia-related effects include a blockade of irritant reflexes (Weissberg et al., 1976) and alteration of ventilatory patterns in response to CO_2 (Martin-Body and Sinclair, 1985). Hence, although not invalidating experimental results, choice of anesthetic agent may affect the measured response and may confound cross-species comparison.

Restraint

Collection of small animal pulmonary function data without the use of anesthesia usually requires some type of physical immobilization. Restraint may range from minimally restrictive, allowing turning and some locomotion, to extremely confining, as occurs when animals are inserted into nose-only exposure tubes. Although restraint reduces movement

artifacts and permits attachment of delicate probes or sensors, immobilization can also produce undesirable physiological disturbances such as changes in body core temperature (T_{co}) (Nagasaka et al., 1979), hypermetabolism (Nagasaka et al., 1980), increased expiratory CO_2 (Jaeger and Gearhart, 1982), changes in ventilation and ventilatory pattern (Lai et al., 1978; Mauderly, 1986), and gastric response (Toraason et al., 1980). Such stress-related responses are poorly understood, and their influence on toxicologic responses may well pass unnoticed unless specifically examined.

Temperature

Although not widely appreciated, toxicant-induced changes in thermoregulatory function can modify the results of toxicological studies (Gordon et al., 1988; Gordon, 1991). Recent studies indicate that exposure to 0.37, 0.50, and 1.00 ppm O_3 also can decrease T_{co}, heart rate, and blood pressure over 2 or more hours in unrestrained, unanesthetized rodents maintained at normal room temperature (Uchiyama et al., 1986; Watkinson et al., 1995). On the other hand, when rats were restrained in a head-out body plethysmograph and exposed to the same concentration of O_3 (1 ppm) as in the Uchiyama et al. (1986) and Watkinson et al. (1995) studies, no change in blood pressure was observed (Tepper et al., 1990). The discordance between these findings may be the result of restraint stress, which has been shown to increase T_{co} (Nagasaka et al., 1979) and, in this circumstance, could have blunted the decrease in T_{co} associated with O_3 exposure.

Although O_3-induced changes in heart rate and T_{co} may be unique to rodents, this phenomenon has not been well studied in humans. It is possible that because of their larger thermal mass and different thermoregulatory mechanisms, humans do not exhibit similar changes in these parameters on exposure. For example, rectal temperature increased by the same amount in both air and 0.4 ppm O_3 groups of humans during a 2-h exposure at 35 °C (Bedi et al., 1982). The effects on T_{co} may have been confounded because the subjects performed moderate exercise during alternate 15-min periods during exposure. On the other hand, women exercising intermittently in moderate (24 °C) and hot (35 °C) ambient conditions showed no change in T_{co} related to O_3 exposure, but did show less of an increase in heart rate (2.7%) than did air-exposed (8.1%) subjects at 35 °C (Gibbons and Adams, 1984). It should be noted, however, that other studies have shown potentiation of human lung function responses associated with increased ambient temperature and O_3 exposure (Folinsbee et al., 1977; Gibbons and Adams, 1984). The full importance of temperature in relating rodent and human responsiveness to O_3 remains to be understood.

Exercise and Ventilation

Exercise has long been employed in human studies to enhance the effects of air pollutants, especially O_3 (Folinsbee and Raven, 1984). Exercise appears to exacerbate functional effects by increasing the inhaled dose (Hatch et al., 1994) and possibly by shifting the deposition of the pollutant to more sensitive pulmonary sites (Gerrity and Wiester, 1987). Although exercise can be used in laboratory animals to enhance deposition of O_3, no direct methods for measuring ventilation or breathing mechanics are available for small animals during exercise. Alternatively in an attempt to mimic the increase in ventilation produced by exercise in humans, studies employing restrained animals have used CO_2 as a ventilatory stimulant. Carbon dioxide (8 to 10%) maximally increases $\dot{V}_E$ three to five times in rodent species; CO_2 in excess of 10% will result in a reduction in ventilation (Wong and Alarie, 1982;

Tepper et al., 1988). This increase in $\dot{V}_E$ is equivalent to light (2 to 3 × resting $\dot{V}_E$) or moderate exercise (4 to 6 × $\dot{V}_E$) in humans (U.S. Environmental Protection Agency, 1986). In many O_3 studies in humans, both heavy (7 to 8 × $\dot{V}_E$) or very heavy (>9 × $\dot{V}_E$) exercise have been used. Similar increases in ventilation cannot be attained in small animals using the CO_2 challenge technique, thus posing a limitation in attempting to make direct comparisons between animal and human studies.

The application of this CO_2-challenge methodology in O_3-exposed rats (0.25 to 1.0 ppm for 2.25 h with 15 min alternating hyperventilation) clearly demonstrates enhanced pulmonary irritation, as reflected in breathing pattern changes during exposure (Tepper et al., 1988, 1990). The breathing pattern alterations typical of O_3 exposure appeared to be larger than would be predicted based solely on increased dose, suggesting that CO_2 challenge during O_3 exposure may have enhanced deposition at critical lung sites (Tepper et al., 1989). This augmented response was reflected clearly in the large increases in protein observed in the BAL fluid (Costa et al., 1988b). In postmortem studies, rats exercised during exposure have been found to have exacerbated lung pathology, thus appearing to confirm this hypothesis (Mautz et al., 1985) and suggesting that exercise may, in fact, enhance toxicity disproportionate to the apparent dose of toxicant.

8.3.3.2 Acute Exposure Data

Two corners of the parallelogram paradigm can be constructed readily from data gathered in empirical studies of acute O_3 exposure in humans and laboratory animals. These studies have the bulk of the data with the highest frequency of common endpoints that can be compared. Hence, the following discussion will focus on several categories of homologous lung function and BAL study data that have been obtained from humans and animals exposed similarly to O_3. The human studies were drawn from the large existing database on lung function and represent typical responses. The corresponding BAL data are more limited and are used to the extent possible. In contrast, the animal studies selected for comparison are highly selective and represent a rather small database involving similar exposure scenarios and homologous endpoints. This approach, of necessity, excludes the large majority of animal studies, not because they do not contain important toxicologic data on O_3, but rather, they are disparate in their exposure parameters or biologic endpoints that readily can be tied to those available in humans.

Tidal Breathing

In humans, O_3 produces pulmonary irritation, a response associated with cough and substernal soreness (Chapter 7). Although these symptoms are difficult to assess in animals, exposure to sufficient concentrations of O_3 produces reflex alterations in tidal breathing that can be measured objectively. Most notably, the response is an increase in f that is usually accompanied by a decrease in V_T (tachypnea), whereas $\dot{V}_E$ may not be altered. Although the magnitude of the tachypneic response is variable, depending on the species and exposure conditions, this endpoint is quite sensitive and consistent across many species (e.g., guinea pigs, cats, dogs, rats, monkeys, humans).

To examine the cross-species response to O_3, data were evaluated from human and animal studies that reporting immediate postexposure alterations in f. Most human studies employed an exercise regimen during O_3 exposure to increase dose. On the other hand, few animal studies have used exercise, relying rather on high exposure concentrations or

CO_2-induced hyperventilation. Three representative human studies were selected because they used a large range of concentrations and ventilation rates. Selected data from these three human studies are compared to the available animal data in Figure 8-7 and discussed further below.

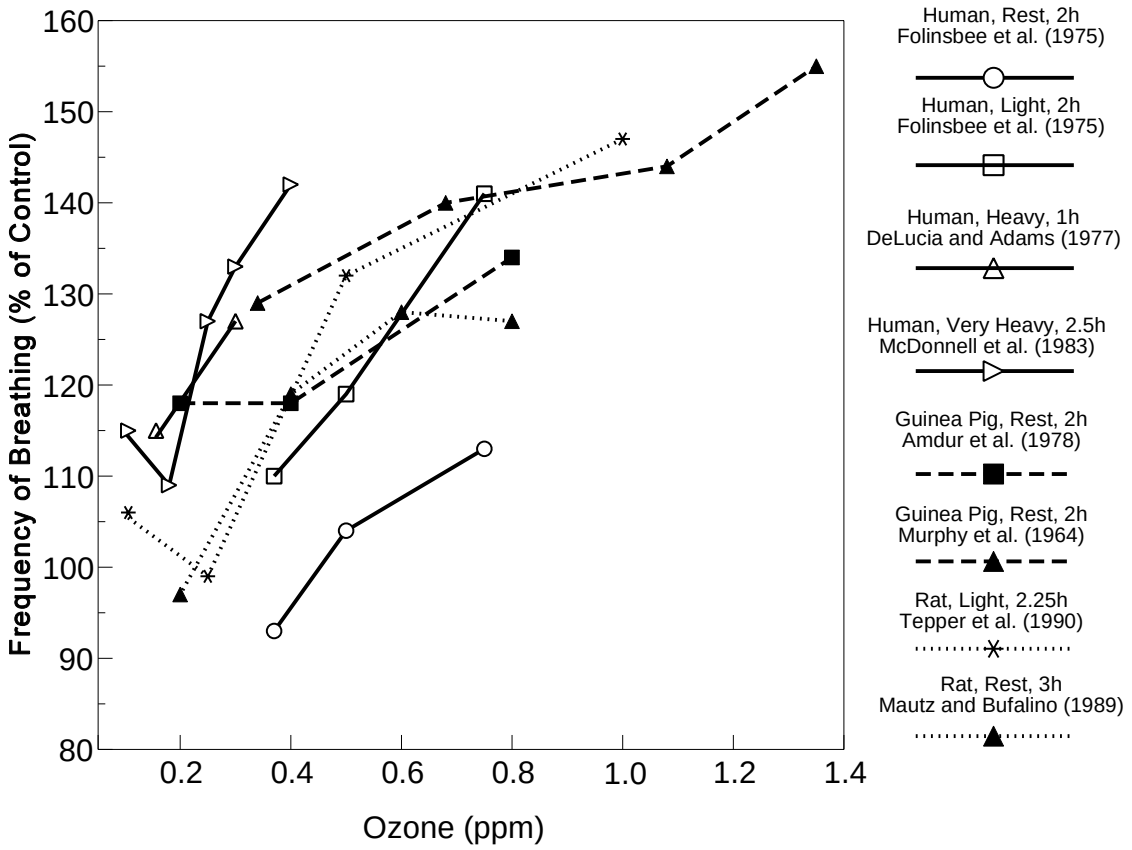


Figure 8-7. Comparison of changes in frequency of breathing after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols, whereas animal data are plotted with broken lines (differentiated by species) and closed symbols.

Folinsbee et al. (1975) examined the upper limits of the O_3 concentration-response curve in human subjects exposed either at rest or while performing light to moderate intermittent exercise (IE; 29 L/min; 5 × resting $\dot{V}_E$). Measurements of f were obtained during exercise following a 2-h exposure to 0.37, 0.50, or 0.75 ppm O_3. Concentration-dependent increases in f were observed in both the resting and exercising group, although the magnitude of the change in the exercising group was greater. The role of exercise in altering

the O_3-induced changes in f was examined further by DeLucia and Adams (1977), who exposed humans to 0.15 or 0.30 ppm O_3 for 1 h while the subjects exercised at one of four ventilation levels (1, 3, 4, or 6 × resting $\dot{V}_E$). The magnitude of the f response increased with concentration and exercise level, but was significant only in the highest exercise group at 0.15 and 0.30 ppm. Lastly, the lower limits of the concentration-response were explored by McDonnell et al. (1983), where subjects performing very heavy exercise (65 L/min; ≥10 × resting $\dot{V}_E$) were exposed to 0.12, 0.18, 0.24, 0.30, or 0.40 ppm O_3 for 2.5 h. Significant changes in f were observed at all exposure levels.

Although several animal studies have evaluated tidal breathing changes during and after O_3 exposure, only four studies have examined multiple concentrations such that comparisons to human data can be made. Unanesthetized, restrained guinea pigs were exposed for 2 h to 0.34, 0.68, 1.08, or 1.34 ppm O_3 via nose cones, while tidal breathing was measured using a constant-volume plethysmograph (Murphy et al., 1964). A similar experimental preparation was used by Amdur et al. (1978) to evaluate the respiratory response of guinea pigs to 0.2, 0.4, and 0.8 ppm O_3. In both of these experiments, a monotonic increase in f was observed; however, the animals studied by Murphy et al. (1964) were uniformly more sensitive to O_3 than those of Amdur et al. (1978). Mautz and Bufalino (1989) measured breathing patterns in awake, restrained rats exposed for 3-h to 0.2, 0.4, 0.6, and 0.8 ppm O_3. Concentration-related increases in f were observed up to 0.6 ppm, but the responses to 0.6 and 0.8 ppm were the same. In another study, awake rats were exposed to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h in head-out pressure plethysmographs where CO_2-stimulated breathing was incorporated to augment ventilation (Tepper et al., 1990). With the added CO_2, rats and guinea pigs appeared to be similarly responsive to O_3. In general, as depicted in Figure 8-7, restrained guinea pigs and rats appeared to be as responsive as the lightly exercising humans, and clearly more responsive than the humans exposed at rest. Only with strenuous exercise does the response of humans appear to exceed that of rodents.

In addition to similar concentration-related effects in humans and animals, the time-related effects of O_3 exposure appear to be similar. To demonstrate this homology, Mauderly (1984) compared the time course of response to O_3 in humans and guinea pigs exposed under somewhat similar conditions. Humans were exposed to 0.75 ppm O_3 for 2 h while engaging in nonstrenuous IE at 15-min intervals (Folinsbee et al., 1975). In another study, respiratory parameters were measured at 30-min intervals during exposure and for 4 h postexposure (Bates and Hazucha, 1973). Similarly, unanesthetized, restrained guinea pigs were exposed to 0.68 ppm O_3 for 2 h as part of a concentration-response study (described above), with respiratory function assessed at 15-min intervals during exposure and for 3.5 h postexposure (Murphy et al., 1964). In both guinea pigs and humans, f increased and V_T decreased; both parameters then returned toward control values during the postexposure period. The percent change from control in f and V_T was nearly the same throughout the exposure and postexposure periods, indicating that a similar concentration of O_3 (≈0.7 ppm) produced similar temporal alterations in ventilation. Again, the guinea pigs would appear to be slightly more responsive than humans because the guinea pigs were exposed to a lower concentration (0.68 ppm) at rest, whereas the humans were exposed to 0.75 ppm with light IE.

Mechanics

Breathing mechanics have been examined in several animal and human-O_3 exposure studies, but there is little similarity between the databases for the concentrations or the specific

techniques used. Bates et al. (1972) examined breathing mechanics in resting and in lightly exercising (2 × resting $\dot{V}_E$) humans exposed for 2 h to 0.75 ppm O_3. Although no concentration-response data were obtained, increased total pulmonary resistance (R_L) and decreased dynamic compliance (Cdyn) were found for both resting (+22 and 12%) and exercising (+67 and 51%) subjects exposed to O_3. In a similar study, Hazucha et al. (1989) exposed men for 2 h to 0.5 ppm O_3 using moderate IE (40 L/min), and found a significant 12.5% increase in R_{aw}, although no concomitant change in Cdyn was detected. McDonnell et al. (1983), using a broad range of O_3 concentrations (0.12, 0.18, 0.24, 0.30, and 0.40 ppm; 2 h) and very heavy exercise (65 L/min), reported concentration-dependent increases in R_{aw}. In humans at rest or performing light exercise, however, a 2-h exposure at near-ambient O_3 concentrations would be expected to induce only modest increases in R_{aw} and no changes in Cdyn (Hazucha, 1987).

Although relatively high O_3 concentrations (1.0 ppm) produced effects on R_L and Cdyn in animals (Murphy et al., 1964), only three studies in animals have evaluated these parameters at lower, more relevant O_3 concentrations. Watanabe et al. (1973) studied anesthetized, paralyzed, and mechanically ventilated cats exposed via a steel tracheal tube to either 0.25, 0.50, or 1.00 ppm O_3 for between 2 and 6.5 h. Measurements of breathing mechanics were recorded every 30 min. With increasing O_3 concentration and exposure duration, R_L increased and, to a lesser extent, Cdyn decreased. Bronchoconstriction at 0.25 ppm O_3 was reversed following atropine (a parasympathetic receptor blocker), but only partially reversed at the two higher concentrations, suggesting the involvement of more than bronchoconstriction in the increase in R_L at these levels. Unfortunately, relating the concentrations used in this study to other animal or human studies is difficult because exposure through a tracheal tube would eliminate scrubbing of O_3 by the nose and oropharynx and likely would exaggerate the pulmonary O_3 dose (Gerrity et al., 1988).

Other studies have attempted to examine breathing mechanics in unanesthetized animals with natural nasal breathing and avoidance of potential anesthesia-related blunting of reflex responses. Murphy et al. (1964) exposed unanesthetized guinea pigs to several concentrations of O_3 for 2 h and measured ventilation, as previously discussed, and R_L. At concentrations less than 1 ppm, O_3 had no effect, but R_L increased 48 and 147% at 1.08 and 1.34 ppm, respectively. Using a similar test system, Amdur et al. (1978) observed no significant alteration of R_L in unanesthetized guinea pigs during a 2-h exposure to 0.2, 0.4, or 0.8 ppm O_3. However, Cdyn decreased significantly at 0.4 and 0.8 ppm O_3. In analogous studies in unanesthetized rats, Tepper et al. (1990) observed no significant changes in R_L or Cdyn after a 2.25 h exposure to 0.12, 0.25, 0.50, or 1.00 ppm O_3, in spite of intermittent 15-min periods of exercise-like hyperventilation induced by CO_2.

Although increased resistance is demonstrable in guinea pigs, cats, dogs, and humans, a comparison of percent change in resistance from control measurements after an acute (2 h) O_3 exposure (Figure 8-8) suggests that humans are more likely to bronchoconstrict due to O_3 exposure than rodents. Neither of the guinea pig studies (Murphy et al., 1964; Amdur et al., 1978) nor the rat study (Tepper et al., 1990) showed a significant increase in R_L at less than 1 ppm. However, closer examination of the human data reveals that the McDonnell et al. (1983) study employed very heavy exercise, and most of the studies included in the Hazucha (1987) model used moderate to heavy exercise. Thus, the inhaled dose likely would be greater than in spontaneously breathing animals. A more comparable study in humans that employed

only light exercise reported that 0.25, 0.37, and 0.50 ppm for 2 h resulted in minimal, nonsignificant 118, 124, and 104% increases in R_{aw} (Hackney

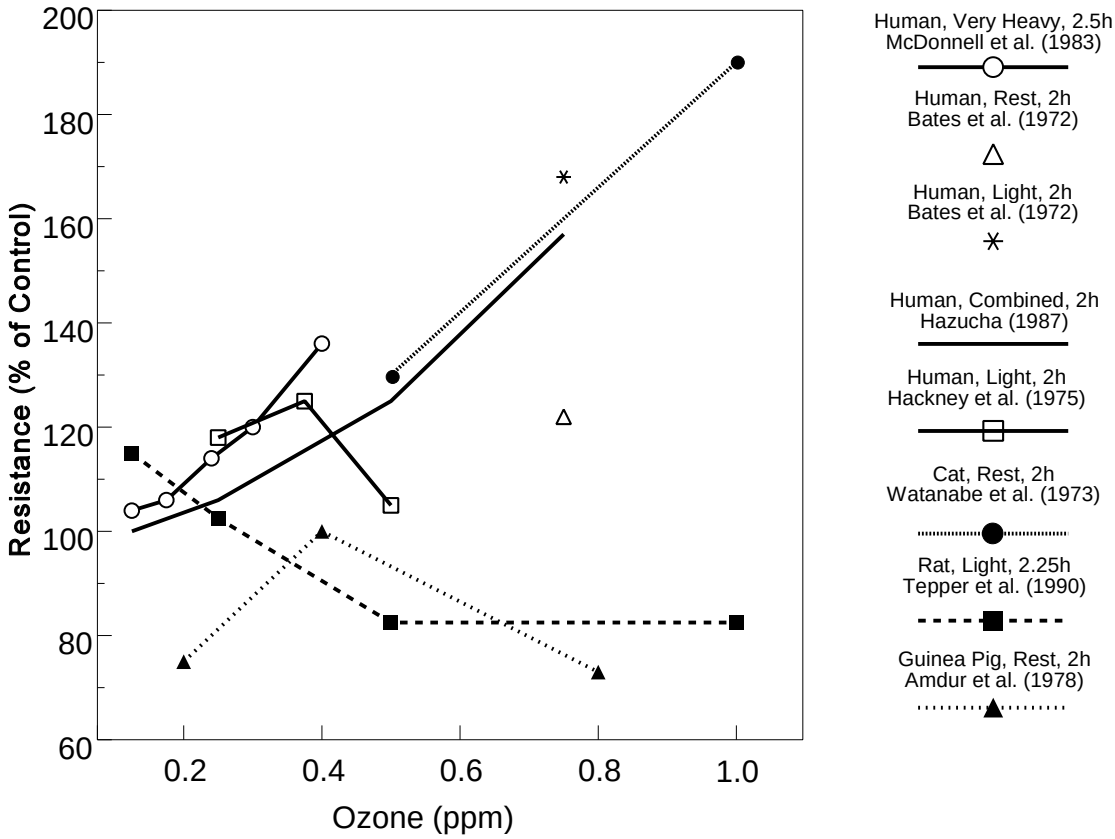


Figure 8-8. Comparison of changes in resistance after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines and open symbols. The line labeled "Hazucha (1987)" is a model of predicted response. Animal data are plotted with dashed lines (differentiated by species) and closed symbols.

et al., 1975). Likewise, the Bates et al. (1972) data obtained in subjects at rest and with light exercise (0.75 ppm O_3) also argue against an unusually high O_3 responsiveness in humans relative to test animals for this endpoint when exercise-related dose is considered.

In general, similar findings have been observed using the measurement of Cdyn; however, the response decrements were more variable and of smaller magnitude. Given the distal deposition of O_3, as indicated by morphological studies (Chapter 6, Section 6.2.4), it is surprising that so little attention has been given to this parameter. Available data suggest that these changes in Cdyn are of little biological significance for ambient exposures.

Airway Responsiveness

The ability of O_3 to increase airway responsiveness to nonspecific bronchoconstricting stimuli in humans and other mammalian species has been known for at least a decade (Chapters 6 and 7, Sections 6.2.5 and 7.2.4, respectively). However, airway responsiveness is perhaps the least understood response to O_3, particularly in the context of species comparisons. Humans clearly exhibit increases in airway responsiveness at environmental O_3 exposure levels (Gong et al., 1986; McDonnell et al., 1987; Folinsbee et al., 1988; Horstman et al., 1990), whereas analogous responses in animals at O_3 concentrations below 1 ppm are controversial. Most studies of airway responsiveness in laboratory animals focus on the development of asthma-like models to elucidate generic mechanisms of airway responsiveness and utilize concentrations as high as 3 ppm for brief periods of time to injure the airways. Hence, anything more than a qualitative comparison between animal species and humans is tenuous and, thus, will not be discussed further in this section. Details of the methodologies of the laboratory animal and human bronchoreactivity studies can be obtained in the reviews of pulmonary function found in Chapters 6 and 7, Sections 6.2.5 and 7.2.3, respectively.

Elasticity and Diffusion

The integrity of the pulmonary air-blood barrier is essential for efficient exchange of oxygen and CO_2. This fragile epithelial interface with matrixed interstitial connective tissues and capillaries possesses inherent elastic properties and presents a finite resistance to oxygen diffusion to the blood. Although the elastic and diffusionary properties of the blood-air barrier are not linked implicitly to one another functionally, both properties can be quantified readily and compared between humans and laboratory animals (Costa, 1985). When combined, assessment of these functional properties is often sufficient to evaluate pathologic or toxic events in the distal reaches of the lung. For this reason and because of the fact that O_3 deposits in the deep lung, the effects of O_3 on these parameters will be discussed together.

Inhaled O_3 is known to penetrate to the depths of the lung and preferentially deposit in the smallest airways and its proximal acini (Section 8.2). Somewhat surprisingly, relatively few studies in humans have sought to characterize potential functional impairments at the air-blood interface. The reasons for this are likely twofold. First, in the early studies of the health effects of O_3 on humans, static compliance and diffusing capacity for carbon monoxide (DL_{CO}) were affected at only very high concentrations, well above what would be considered environmentally relevant. Second, from a practical perspective, these measurements proved to be considerably more tedious to perform than the forced expiratory measurement, which sensitively detects O_3-induced alterations (discussed below). Nevertheless, there are sufficient data on humans exposed acutely to O_3 to allow a reasonable comparison of these endpoints with their more abundant animal homologues.

The earliest studies leave little doubt that O_3 is edemagenic at high concentrations in virtually all mammalian species. In the past, occupational exposures of 2 to 3 ppm O_3 were not uncommon, and a 9-ppm peak exposure has been reported (Kleinfeld et al., 1957; Challen et al., 1958). The resultant worker symptoms and signs, including chest radiograms, were consistent with the manifestations of edema reported in experimental animals (i.e., increased lung weight and stainable edema in the airspaces) (Stokinger, 1965). Lung function, however, typically was not measured in these work-related exposures. In a later study of arc welders exposed to 0.2 to 0.3 ppm O_3, little, if any, convincing evidence of functional impairment, in

terms of altered lung volumes, maximal expiratory flow rates, and DL_{CO} was obtained (Young et al., 1963). To further explore the possible effects of O_3, Young and coworkers (1964) subjected 11 human volunteers at rest to controlled atmospheres of 0.6 to 0.8 ppm O_3 via mouthpiece for 2 h. Small reductions in vital capacity (VC), forced expiratory volume in 0.75 s, dynamic and static lung compliance, and intrapulmonary gas distribution were observed, but only the 25% fall in DL_{CO} proved to be statistically significant. Similar, but considerably more variable effects on lung function were reported by Hallett (1965) in 10 subjects exposed to 1 to 4 ppm O_3 for 30 min. Nonetheless, of 10 exposed subjects, seven showed at least a 20% drop in DL_{CO}. Like Young and coworkers, Hallett (1965) interpreted these changes to indicate lung edema, in agreement with the hypothesis that the deep lung irritant O_3 was having its effect at the alveolar level. Interestingly, additional work from the same laboratory of the Young study (Bates et al., 1972) found that resting subjects receiving nasal exposure to 0.75 ppm O_3 for 2 h resulted in a nonsignificant 3% reduction in DL_{CO}. However, in a limited test group, the co-imposition of light exercise, which doubled ventilation, enhanced this response (12%). It appears that the nasal (Bates et al., 1972) versus mouthpiece (Young et al., 1964) routes of exposure were instrumental in the differential response, because it is likely that the mouthpiece diminished what scrubbing occurs when exposure is via the unencumbered mouth in human test subjects (as reported by Gerrity et al., 1988). Since these early studies, there have been no additional controlled human acute studies that have examined alterations in DL_{CO} at O_3 concentrations below 0.6 ppm.

Analogous animal studies of acute O_3 exposure indicate that the general pattern of functional impairment is similar to that reported in human studies. Anesthetized and ventilated cats showed a general decline in VC, static lung compliance, or DL_{CO} with exposures up to 6.5 h of 0.26 to 1.00 ppm O_3 (Watanabe et al., 1973). The responses of the 20 animals were variable, and these declines, which did not achieve overall statistical significance, were thought to be largely secondary to the substantial (36 to 200%) increases in R_{aw}. In a more complex study design, rats were exposed for 2 or 7 h to 0.5 or 0.8 ppm O_3 with intermittent 8% CO_2 to hyperventilate (2 to 3 × resting $\dot{V}_E$) the animals as an exercise analogue to human exposures (Costa et al., 1988a). The DL_{CO} values were reduced by about 10% at both 0.5 ppm time-points and by about 12% with a 2-h exposure to 0.8 ppm. Exposure to 0.8 ppm for 7 h, however, greatly exacerbated the alveolar effect with a resultant 40% reduction in DL_{CO}. Static compliance, unaffected by the other exposure conditions, was affected only at this latter exposure duration. These O_3-induced effects, particularly the reductions in DL_{CO}, appeared to correlate with the degree of lung edema in affected animals, as had been surmised for the acutely exposed humans. With the multitude of more recent studies of O_3 at ambient levels, alterations in static lung compliance or DL_{CO} rarely are reported in either humans or animals.

Forced Expiration

Reductions in FVC and FEV_1 have become the hallmarks of acute lung dysfunction in humans after O_3 exposure (Chapter 7). These measures are sensitive to O_3 levels as low as 0.12 ppm for as little as 2 h when heavy IE is included during the exposure (McDonnell et al., 1983) and show cumulative dysfunction resulting from 6.6 h of lower levels of this oxidant (0.08 and 0.10 ppm) when nearly continuous, moderate exercise is employed (Horstman et al., 1990). Reductions in FEV_1 and FVC induced by O_3 are believed to be partly the result of pain-mediated interruption of maximal inspiration (Hazucha et al., 1989). Exactly what level

of tissue injury or inflammation correlates with these functional deficits is unclear and is an active area of research (Chapter 7, Section 7.2.4).

Most studies of O_3 in experimental animals make little effort to mimic human study designs, thereby impeding the extrapolation of experimental animal study results to humans. Recently, however, rat studies involving periods of intermittent CO_2-induced hyperventilation to enhance dosimetry have attempted to capitalize on the qualitative similarity of the rat and human maximum expiratory flow volume (MEFV) curves as a potentially sensitive endpoint of toxicity (Costa et al., 1988a; Tepper et al., 1989). In the rat, FVC does indeed decrease with O_3 exposure, although the magnitude of response is apparently less than that observed in humans. As in the human, the reduced FVC in the animal appears not to be the result of a change in residual volume. Total lung capacity may be reduced slightly, but lung compliance does not change. However, it is premature to assume a common mechanism for the FVC reductions in the rat and human. Unlike the human, pain on inspiration in the animal model is likely not an issue because the animal is anesthetized during the procedure and is brought to TLC by a defined airway pressure (30 cm H_2O). Because general anesthesia is known to diminish sensory afferent stimuli, an analogous O_3-induced fall in rat FVC should expectedly have been blunted, if not totally eliminated. To what extent anesthesia mitigates the rat response or that there are inherent species differences in dosimetry or sensitivity is not clear from these studies. Nevertheless, comparison of model-predicted FVC changes in humans (Hazucha, 1987) with analogous rat data (Costa et al., 1988a) would suggest that this response in the anesthetized rat is about half that of the human (Figure 8-9).

Studies of Inflammation and Antioxidant Content of Bronchoalveolar Lavage Fluid

Both humans and animals exhibit a PMN inflammatory response with associated changes in lung permeability after acute exposure to O_3. Recent studies indicate that humans exposed to O_3 concentrations as low as 0.08 ppm for 6.6 h with moderate exercise (40 L/min) exhibit a fourfold increase in the percentage PMNs when BAL is obtained 18 h postexposure (Devlin et al., 1991). To date, animal studies at comparable exposure levels are rare, (Hotchkiss et al., 1989), and exercise enhancement of exposure dose has yet to be incorporated. As noted above, the issue of dosimetry is critical if extrapolation at such levels is to be attempted. Nevertheless, in the one acute rat study at 0.12 ppm O_3 for 6 h, an increase in nasal-lavage-derived PMNs was noted 18 h postexposure, with no similar change in PMN number in the BAL (Hotchkiss et al., 1989). In contrast, in the same study when higher concentrations of O_3 (0.8 and 1.5 ppm) were used, BAL PMNs were elevated, but no changes were observed in the nose washings. Such a "competitive" nasal-pulmonary response has yet to be studied directly in humans. Nevertheless, the data support the general hypothesis that there is comparability between the inflammatory responsiveness of rats and humans. More direct comparison of laboratory animal inflammatory responses with those of humans can be drawn from studies at higher concentrations when the nasal/lung competitive response in the rat is skewed to the lung, and, like in the lung-function comparison, analogous exposure conditions can be more directly compared. These studies are tabulated in Table 8-9 and discussed in more detail below.

Four representative human studies of lung inflammatory responses after acute O_3 exposure can be compared with existing acute animal data from studies of analogous design. Seltzer et al. (1986) exposed moderately exercising (83 to 100 W) subjects to 0.4 or 0.6 ppm O_3 for 2 h, with BAL obtained 3 h postexposure. The BAL fluid from the

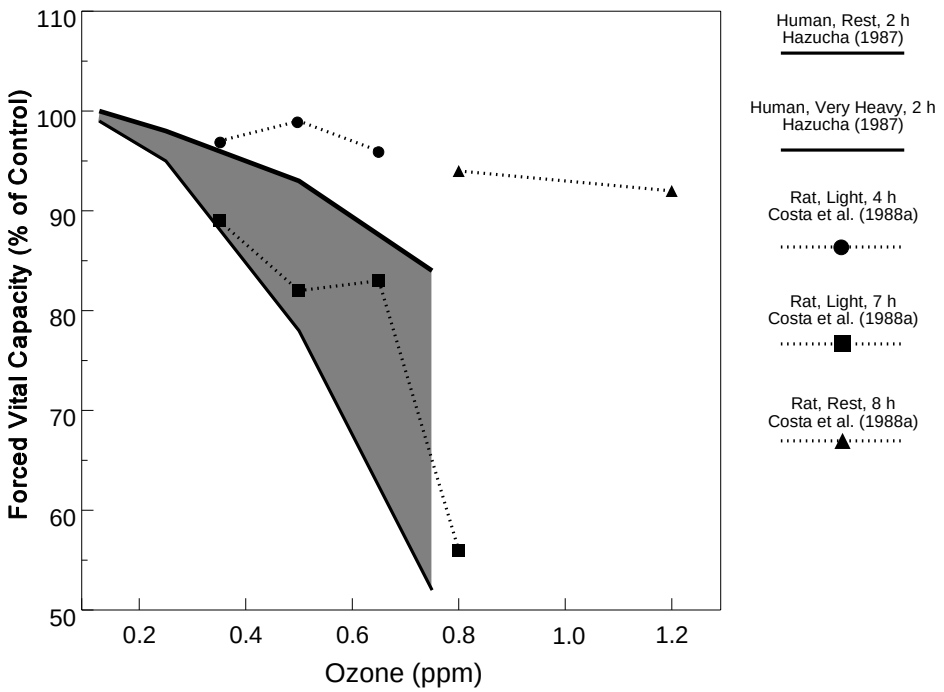


Figure 8-9. Comparison of changes in forced vital capacity after ozone exposure in humans and animals. Data are expressed as percent of the control response. Right-hand legend indicates species, exercise level, exposure duration, and the reference. Human data are plotted with solid lines from the equation of Hazucha (1987), with the shaded area representing the predicted range of decrements expected between light exercise (top line) and very heavy exercise (bottom line). Rat data are plotted with dashed lines and closed symbols.

combined 0.4- and 0.6-ppm O_3 groups contained an average 7.8-fold more PMNs than did BAL fluid after filtered air. Protein levels in the BAL fluid were not assayed in this study. In separate studies, humans were exposed to 0.4 ppm O_3 for 2 h with heavy exercise (60 L/min), with BAL samples collected at 1 and 18 h postexposure (Koren et al., 1989b, 1991). These two studies showed that the inflammatory response is quickly initiated postexposure. The PMN and protein content were elevated at both times (1 h: PMN 18.2×; protein 1.2×), with the 18-h timepoint (PMN 8×; protein 2.2×) being higher for protein, but somewhat less for PMNs. Schelegle et al. (1991) followed the time course of the inflammatory response (1, 6, and 24 h postexposure) after a 1-h exposure of 0.3 ppm O_3 with exercise (60 L/min). The PMN content of the combined airway and alveolar BAL fluid was elevated at 6 h (3×) and 24 h (2.5×) only, with the 6-h point representing the peak. Protein content of the BAL fluid did not change significantly at any point.

The many studies of O_3-induced inflammation in laboratory animals are reviewed in Chapter 6, Section 6.2.2. For the purpose of species comparison, only selected studies

Table 8-9. Polymorphonuclear Leukocyte and Protein Permeability Response to Ozone by Species[a]

Exposure Parameters	Exposure Conditions	Species (Strain)	Postexposure BAL Time	PMN[b] Increase	Protein[b] Increase	Reference
0.4-0.6 2 h	Exercise (15 min/alt; 83-100 W)	Human	3 h	7.8	Not done	Seltzer et al. (1986)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	18 h	8.0	2.2	Koren et al. (1989a,b)
0.4 2 h	Exercise (15 min/alt; 60 L/min)	Human	1 h	18.2	1.2	Koren et al. (1991)
0.3 1 h	Exercise (15 min/alt; 60 L/min)	Human	1, 6, 24 h	3.0 at 6 h 2.5 at 24 h	No change	Schelegle et al. (1991)
0.96 8 h	Daytime; rest	Rhesus monkey	1, 12, 24, 72, 168 h	27 at 1 h 19 at 12 h 24 at 24 h 6 at 72 h 3 at 168 h	3 at 1 h 3 at 12 h 8 at 24 h 3 at 72 h 1 at 168 h	Hyde et al. (1992)
0.2 0.5 1.0 2.0 4 h	Daytime; rest	(a) Mouse (Swiss Albino) (b) Guinea pig (Hartley) (c) Rat (S-D) (d) Hamster (Golden Syrian) (e) Rabbit (NZW) M	16 h	Not done	(a) 1.8 at 1.0 ppm 3.2 at 2.0 ppm (b) 1.4 at 0.2 ppm 2.0 at 0.5 ppm 4.1 at 1.0 ppm 4.5 at 2.0 ppm (c) 2.1 at 1.0 ppm 3.6 at 2.0 ppm (d) 1.5 at 1.0 ppm 2.6 at 2.0 ppm (e) 2.7 at 2.0 ppm	Hatch et al. (1986)
0.5 1.0 4 h	Daytime; rest; Vitamin C (AH_2+/□)	Guinea pig (Hartley) M	16 h	Not done	For 0.5 ppm; 1.1 for AH_2^+, 2.1 for $AH_2^{\square}$. For 1.0 ppm; 2.4 for AH_2^+, 2.7 for $AH_2^{\square}$	Slade et al. (1989)
0.5 0.8 2 or 7 h	Daytime; (15 min/alt for 2 h; 45 min/alt for 7 h); 3-5× V_E	Rat (F344) M	1 h	Not done	For 0.5 ppm; 1.2 at 6 h, 2.1 at 7 h. For 0.8 ppm; 1.5 at 2 h, 3.3 at 7 h	Costa et al. (1988a)

[a]See Appendix A for abbreviations and acronyms.
[b]Ozone response/air response.

(Table 8-9) will be considered. The spectrum of exposure conditions used in the various animal studies makes difficult the direct comparison among laboratory test species. However, one study by Hatch and coworkers (1986) specifically addressed this question by exposing mice, guinea pigs, rats, rabbits, and hamsters under identical conditions (0.2, 0.5, 1.0, and 2.0 ppm O_3 for 4 h), followed at 18 h postexposure with BAL and assay for protein. Guinea pigs were most responsive, responding at 0.2 ppm, whereas mice, hamsters, and rats began responding at 1.0 ppm, and rabbits responded only to 2.0 ppm. Only one study involving BAL assessment of PMNs and protein in exposed monkeys has been published (Hyde et al., 1992). At 0.96 ppm O_3 (8 h), the monkeys had a significant inflammatory response, but it is difficult to assess monkey responsiveness relative to the human for this endpoint. Assuming a linear concentration times duration relationship, the monkey data appear similar to the guinea pig response. However, none of these species showed BAL protein increases approximating those reported in human studies.

In recent studies (0.4 ppm O_3 for 2 h, with BAL 16 to 18 h postexposure) (Slade et al., 1989; Crissman et al., 1993), guinea pigs made vitamin C-deficient exhibited enhanced responsiveness to O_3, this result is comparable to that of the exercised humans of Koren et al. (1989b) (Figure 8-10). Similarly, when rats were exposed to 0.5 ppm O_3 for 2 h with intermittent CO_2-induced hyperventilation (Tepper et al., 1993) to mimic mild/moderate exercise (three- to fivefold $\dot{V}_E$), the BAL protein, as well as PMN responses at 18 h postexposure compared favorably with those data of Koren and coworkers (1989a,b) (Figure 8-11).

Within a given laboratory animal species, responses among strains also can differ appreciably, as demonstrated in rats by Pino et al. (1991) and Costa et al. (1993). These studies indicated that Wistar rats exhibit greater inflammatory responses (protein and PMN) to O_3 than S-D and F344 rats after an 8-h exposure to 0.5, 1.0, and 1.5 ppm with BAL sampled at 2 or 24 h later. Similarly, mouse strains (C3H/HeJ and B6C57/6; Kleeberger et al., 1990) and S-D rat substrains (Costa et al., 1985) have been shown to possess specific genetic susceptibility to high levels of O_3 (2 ppm). In the case of the mice, the responsive strain is seven times (at the 6-h postexposure peak) as susceptible as the databases in animals and, particularly, in humans with regard to these antioxidants are quite limited. Supplementation and deprivation studies with vitamins C and E have shown that these antioxidants have some role in protecting against the effects of O_3 in animals (Elsayed et al., 1988; Slade et al., 1989; Crissman et al., 1993) and in humans (Chatham et al., 1987). Of the animal models, ascorbate-deprived guinea pigs appear to have BAL ascorbate levels most like humans, with a protein permeability response (without exercise in the animal) very similar to the human exposed to the same concentration (0.4 ppm O_3 for 2 h) with exercise. However, Crissman et al. (1993) also resistant strain for the PMN response to 2 ppm O_3; the protein response is twice (at 24 h postexposure) that of the resistant strain. In the S-D substrain, protein extravasation into the alveolar lumen immediately postexposure is 40% higher in the responsive strain than the resistant (no other time points were examined).

Humans have an order of magnitude less ascorbate in BAL fluid as compared to the rat, but they have nearly twice the glutathione, four times the uric acid, and 80 times the vitamin E, as normalized to lipid P-surfactant (Slade et al., 1993) (Table 8-8). However, on a BAL-derived cell/protein basis, the ratios clearly favor the rat for all of these antioxidants, with the exception of uric acid, which is generally not high in rats because of species differences in protein and prime nucleotide catabolism (urea being the major nitrogenous

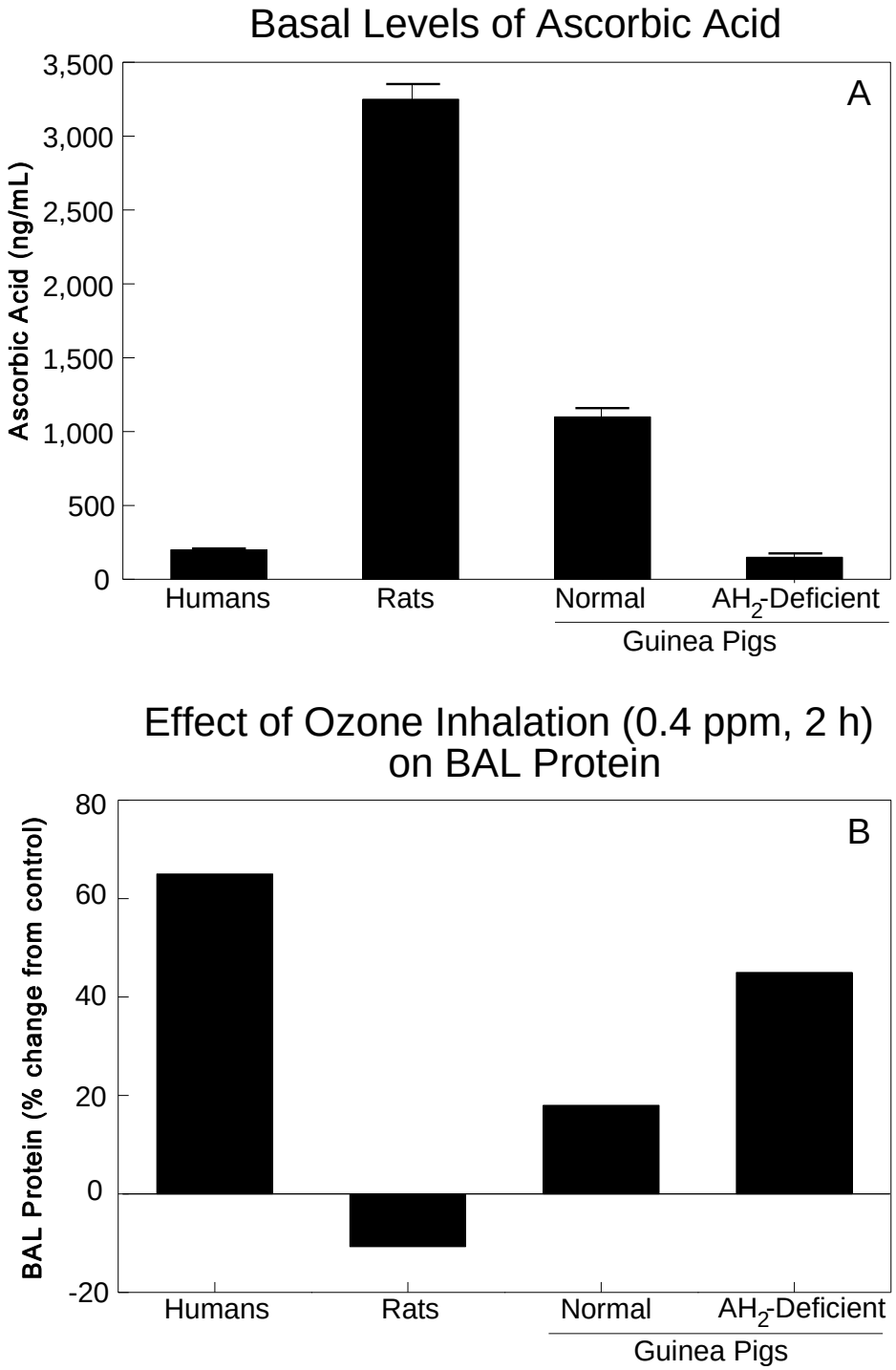


Figure 8-10. Composite of data from Slade et al. (1989), Koren et al. (1989b), and Crissman et al. (1993) comparing basal bronchoalveolar lavage (BAL) ascorbate levels (A) to ozone-induced changes in BAL protein (B). Ozone-exposures (0.4 ppm; 2 h; 16 to 18 h BAL) of humans (exposed with exercise), rats (exposed resting), and guinea pigs (exposed resting) with (ascorbic acid [AH_2]-deficient) and without (normal) AH_2 deficiency.

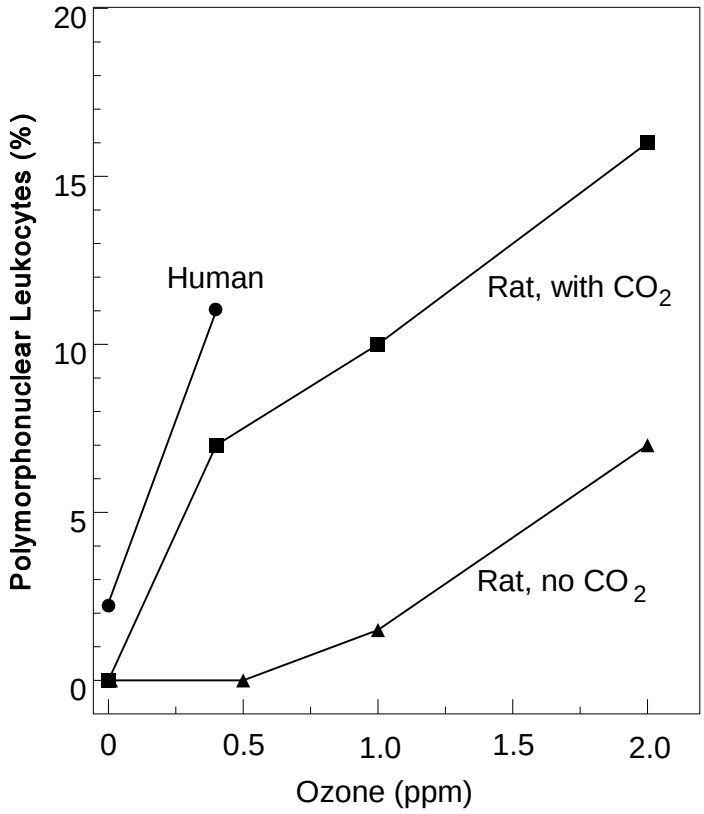


Figure 8-11. Composite of data comparing polymorphonuclear leukocytes obtained by bronchoalveolar lavage 16 to 18 h after ozone (O_3) exposure (0.4 ppm; 2 h) of humans (with exercise) (Koren et al., 1989b) to those of rats exposed to O_3 (0.5 to 2.0 ppm) at rest or hyperventilated with carbon dioxide (CO_2) (Tepper et al., 1993).

by-product for rats). The guinea pig most closely resembles the human for ascorbate. Because these antioxidants are thought to function in the defense against oxidant challenge, it would appear critical to appreciate their presence and function when attempting to interpret data for extrapolation. Unfortunately, the reported that reduced ascorbate levels in BAL fluid (18 h postexposure) increase in the human, whereas those levels decrease in the rat. Whether this relates to the distinctly different basal levels of this vitamin and is associated with the disparate protein responsiveness (ignoring exercise) is unclear because deficiency in animals (guinea pigs) appears more critical to the responsiveness at low (ambient-like) concentrations than at higher concentrations (1.0 ppm). Although it would appear that vitamin C is involved in the interplay between O_3 and the exposed subject (human or animal), there is not full coherence of the data. For example, Hatch et al. (1986) showed that the rabbit was the least responsive to O_3 in terms of BAL protein, but this species has among the lowest tissue levels of vitamin C (Slade et al., 1985). However, rabbits apparently have a low propensity to form lipid

peroxides (Arakawa et al., 1986), an expected product of lung lipid-O_3 interaction (Pryor, 1992). Thus, to interpret interspecies sensitivity only in terms of basal antioxidant levels, however tempting this may be, would be overly simplistic and premature at this time.

8.3.3.3 Prolonged Exposure Studies

The previous sections of this text have utilized lung function responses to acute O_3 exposure in an attempt to elucidate the relative sensitivity among laboratory test species and humans and, thereby, to complete two corners of the parallelogram paradigm (Figure 8-6). Because acute responses represent only part of the extrapolation paradigm, temporal-based exposure responsiveness also will be considered, despite the relative paucity of comparative data between laboratory animals and humans for prolonged exposures. Again, in an effort to best extract species-specific response differences, the criterium for selection of studies was similarity in exposure scenario. The discussion will focus on relative adaptability of acute functional changes and associated BAL-derived findings after repeated exposures and the coherence of the findings from prolonged human and animal exposure studies and epidemiological results.

Lung Function Studies

Reversal ("attenuation") of pulmonary function decrements using a scenario of repeated exposure to O_3 has been reported for both humans and laboratory animals. At least nine studies between 1977 and 1984 have documented that, for repeated exposures between 0.2 and 0.5 ppm O_3 (2 h/day, up to 5 days), spirometric changes were most severe on the first or second day of exposure, waned over the next 3 days of exposure, and, by the fifth day, had returned to control preexposure levels (Chapter 7, Section 7.2.1.4). In the only animal study using a similar exposure protocol and analogous experimental design, Tepper et al. (1989) showed that rats initially displayed a tachypneic response to O_3 that attenuated after 5 consecutive days of exposure, a pattern quite similar to that of humans. Exposures were for 2.25 h and included challenge with CO_2 during alternate 15-min periods to augment ventilation (2 to 3 $\times$ resting $\dot{V}_E$, which is equivalent to light exercise in humans). As in the human studies, the functional changes were largest on Day 1 or 2, depending on the parameter and the O_3 concentration (0.35, 0.50 and 1.00 ppm). Attenuation of the changes in shape constant of the flow-volume curve of the rats also was observed over this period. Thus, under analogous conditions of exposure, both the humans and the rats exhibited similar initial functional responses to O_3 with full and kinetically similar reversal of effects.

More difficult is the direct comparison between human and animal lung function responses to prolonged (several-week) O_3 exposure, largely because of the limited availability of controlled human study data. In the only study of its kind, Bennett (1962) exposed 12 human subjects at rest to 0.2 or 0.5 ppm O_3 for 3 h/day, 6 days/week for 12 consecutive weeks. Although no effects were discernable early in the exposure, there appeared to be small, but significant O_3-induced reductions in FEV_1 (and small, nonsignificant reductions in FVC), particularly in the last weeks of the study. This reduction in FEV_1 more likely would reflect obstructive changes within the lung at these points late in time rather than the pain-mediated reductions that are seen with acute O_3 exposure, which attenuate after a few days of exposure. The lack of concentration-related decrements in FEV_1 and FVC is somewhat unsettling, but, regardless, after 9 postexposure weeks in clean air, all measured effects had reversed.

Unfortunately, there are no directly parallel animal studies to compare to this limited database. But, if the two-fold sensitivity difference between the rat and human FVC response (see Figure 8-12) is assumed, a number of animal studies may be considered comparable for the purposes of this discussion. On the one hand, rats exposed to 0.2 or 0.8 ppm O_3 for 6 h/day, 5 days/week for 12 weeks were reported to exhibit some degree of small airway obstruction based on the MEFV curves, but little, if any, reduction in FVC or DL_{CO} was observed (Costa et al., 1983). Others have reported analogous marginal increases in rat TLC or its component volumes (Bartlett et al., 1974; Costa et al., 1983; Raub et al., 1983) or in regional R_{aw} (Yokoyama et al., 1984) after intermittent or continuous exposures to 0.25 ppm O_3 for 4 to 12 weeks, which would not be unexpected with distal airway or lung damage. Actual pathology in the distal lung tends to be focal and difficult to correlate precisely with the marginal functional impairment.

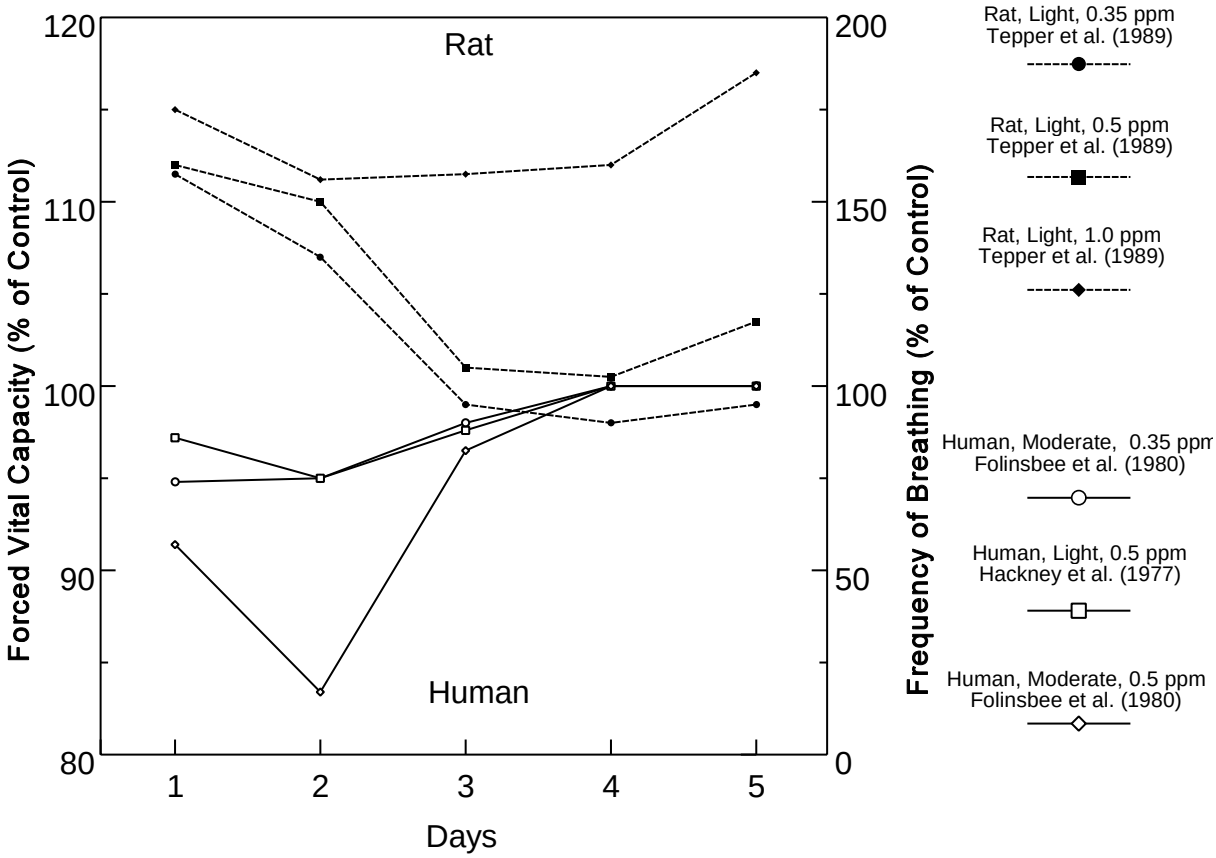


Figure 8-12. Comparison of changes in forced vital capacity in humans (left ordinate) and frequency of breathing in rats (right ordinate) with up to 5 consecutive days of ozone (O_3) exposure. Data are expressed as percent of the control response. Right hand legend indicates species, exercise level, O_3 concentration, and the reference. Human data are plotted with solid lines and open symbols, whereas rat data are plotted with broken lines and closed symbols.

The limited functional data available in monkeys generally agree with the pattern of distal lung pathophysiology reported in rats. When exposed to 0.5 ppm O_3 for 90 days (8 h/day), monkeys exhibited a slight increase in lung distensibility (Eustis et al., 1981). Likewise, monkeys exposed to 0.25 ppm (8 h/day for 18 mo) exhibited increased chest wall (but not lung) compliance and lung volumes, which was most marked in the monkeys exposed to O_3 only in alternate months during that 18-mo period (Tyler et al., 1988). Recall, however, that little or no change in lung elasticity has been associated with controlled O_3 exposures in humans, whether the exposures are acute or repeated. On the other hand, higher concentrations (0.64 ppm for 1 year) resulted in the alteration of distal airway mechanics in exposed monkeys, as gleaned from local resistances measured using oscillatory methods; again, this is in general agreement with the presence of distal lung injury or disease. Morphometric analyses of the end-airways and distal lung regions of O_3 exposed monkeys consistently show altered cell profiles and interstitial restructuring, even when functional changes are marginal, which, like in the rat, likely reflects the large functional reserve of the integrated lung. Thus, although these collective data from subchronic animal studies suggest a reasonably homologous distal lung response to O_3, many of these linkages in functional outcomes remain uncertain in terms of what to anticipate in the human response.

Clearly, the question of potential lung impairment resulting from a near-lifetime exposure to O_3 ranks among the most pressing concerns about this toxicant. The animal data, although demonstrating that chronic O_3 exposure can induce changes in the structure and function of the lung, have yet to provide evidence of potential disease or disability in humans exposed to O_3 over prolonged periods of their lives. The existing epidemiologic studies (Chapter 7, Section 7.4.2), too, merely provide suggestive evidence that persistent or progressive deterioration in lung function may be associated with long-term oxidant pollutant exposure (Detels et al., 1981, 1987). Detels and coworkers (1991) reported decrements in FEV_1 and nitrogen washout across all age groups in areas where oxidant pollution is high. Similarly, analysis of the pulmonary function data from the National Health and Nutrition Examination Survey (NHANES) II showed loss of lung function when annual averages ofambient O_3 exceeded 0.04 ppm (Schwartz, 1989). This pattern of impairment is consistent qualitatively with the chronic animal studies (Costa et al., 1994).

Studies of Inflammation and Antioxidant Content of the Bronchoalveolar Lavage Fluid

The virtual absence of human BAL study data after repeated or prolonged exposures to O_3 hinders the comparison of nonacute human and animal inflammatory responses. However, the recent study of Devlin et al. (1995) suggests that the PMN and protein responses to repeated daily exposures to 0.4 ppm O_3 (2 h with IE for 5 consecutive days) attenuate, much as do the functional responses. Hence, for most of the BAL parameters (with the exception of lactate dehydrogenase activity [a marker of cell injury]), there is indeed an apparent reversal of acute inflammation when exposures are continued over the 5-day exposure period. Rat studies largely appear to show similar attenuation to O_3, but this response seems to be influenced by exposure patterns or conditions (Bassett et al., 1988; Tepper et al., 1989; Van Bree et al., 1989). The study with the most similar design to the human protocol (Tepper et al., 1989) showed some reduction in BAL protein with repeated exposure involving intermittent hyperventilation with CO_2, but over the 5-day period, the protein levels remained significantly elevated; cells were not evaluated in this study. Interestingly, vitamin C and glutathione levels

in the BAL fluid increased over the 5-day course of exposure, a response consistent with an unregulated antioxidant role in the adaptative mechanism. Van Bree and coworkers (1992), on the other hand, reported that 5 consecutive days (12 h/day) of 0.4 ppm O_3 resulted in complete reversal of both BAL albumin and PMN measures to the control values. It should be noted, however, that other biomarkers and mediators within the BAL were not fully recovered, which might suggest a slower reversal time frame or continued O_3-induced pathogenesis, a conclusion of the Tepper et al. (1989) study.

In guinea pigs made deficient in vitamin C and exposed to O_3 (0.2, 0.4, or 0.8 ppm) continuously for 7 days, attenuation of the functional and inflammatory endpoints appeared nearly complete in spite of the deficiency (Kodavanti et al., 1995). Other antioxidants, not altered basally, were unregulated more by the O_3 challenge; the small residual reservoirs of ascorbate, which persisted in the nearly 98% deficiency state of the animals, were apparently mobilized to the site of injury, allowing repair to proceed. Likewise, chronically exposed rats have elevated BAL ascorbate indicative of the oxidant burden and the ongoing repair (Grose et al., 1988). Prolonged exposures up to 18 mo appear to sustain a low-grade interseptal inflammation and evidence of lung matrix remodeling in both rats and monkeys, suggesting that humans would behave similarly. However, such data are not presently available from humans.

8.4 Quantitative Extrapolation of Acute Ozone Effects

8.4.1 Introduction

Advances in dosimetry since the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) fall into five major areas: (1) greater sophistication of model applications (e.g., Overton et al., 1989; Mercer et al., 1991), (2) the appearance of experimental uptake data that can be compared to model predictions (Wiester et al., 1987, 1988, 1996; Hatch et al., 1989; Gerrity et al., 1988, 1994, 1995), (3) experiments specifically designed to estimate model parameters (Hu et al., 1992b), (4) a better understanding of the role of O_3 in the liquid linings and tissues of the RT (Pryor, 1992), and (5) a better understanding of anatomical aspects (Mercer et al., 1991). The role of these advances in interspecies dosimetric extrapolation follows.

With the information available for rats, reasonably reliable predictions of the flux of O_3 to the air-liquid lining interface of toxicologically important regions, such as the CAR, is possible. There are two main investigations that make this feasible: (1) Hatch et al. (1989), who estimated the percent uptake and the fraction of the retained O_3 that is in the URT, trachea, and lung of rats, and (2) Pinkerton et al. (1992) (with elaboration by Miller et al., 1993) who illustrated the basic correctness of modeling assumptions for ventilatory units. (The judgment of basic correctness is based on the assumption that the dose causing the response is proportional to the flux of O_3 to the air-liquid lining interface.) Using this information, regional mass transfer coefficients could be estimated, which would allow the prediction of local respiratory tract O_3 doses in rats exposed under general conditions.

The results of the investigation of Hu et al. (1992b) can be used to estimate URT and TB model parameters, but may not be sensitive enough to determine pulmonary region parameters. If uptake is not confined to the URT and TBs, then their mass transfer coefficients alone would not be sufficient to account for total RT uptake (e.g., as measured by Wiester et al. [1996] or Gerrity et al. [1988]), and the difference in predicted (without pulmonary

region uptake) and experimental uptakes could be used to estimate the pulmonary region mass transfer coefficient. Unfortunately, no dosimetry data for the human pulmonary region are available.

Despite limitations, the O_3 dosimetry data that have been obtained over the past several years, coupled with the advances in modeling, suggest that there has been continual convergence between the model predictions and experimental observations. Given the many areas of consistency between models and experiments, it is valuable to begin to employ these models to provide the dosimetric basis for animal-to-human extrapolation. One of the greatest sources of uncertainty in such an application of dosimetry models is the lack of full understanding of the appropriate target site for O_3 toxicity (e.g., upper or lower airways or pulmonary region) that initiates a particular response, especially the functional changes in the lung. However, reasonable assumptions can be made to narrow the target site.

The first application of dosimetry models given in Section 8.4.2 is an examination of delivered dose versus response within a given species. This is followed in Section 8.4.3 by some interspecies comparisons of delivered dose versus response.

8.4.2 Intraspecies Delivered Dose Response

Assuming that changes in FEV_1 in humans exposed to O_3 mainly are the result of O_3 pulmonary tissue dose, Miller et al. (1988) constructed a dose-response curve. They plotted decrements in FEV_1 versus predicted cumulative pulmonary region O_3 tissue dose scaled to body mass (Figure 8-13). The concentration-response data are from McDonnell et al. (1983), in which 135 healthy subjects were exposed to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm O_3. Exposure was for 2.5 h with heavy IE. Miller et al. (1988) used the average weight and height of the subjects to estimate the FRC that was used in the model to simulate O_3 dose. The exercise breathing parameter data were used along with an estimate of resting breathing parameters. Figure 8-13 is similar in shape to the concentration-response curve of McDonnell et al. (1983). Differences between these two curves, however, are accounted for by the translation between exposure concentration and O_3 dose.

As another example, Pinkerton et al. (1992) examined the relation between actual tissue response of rats chronically exposed to O_3 and a prediction of O_3 dose as a function of distance from the bronchiole-alveolar duct junction (BADJ) to ventilatory units. Using these data, Miller and Conolly (1995) plotted the predicted O_3 dose and the observed change in wall thickness due to the exposure versus distance from the BADJ (Figure 8-14). Even though considerable variability in the thickness change can be inferred from the data, the two curves, scaled to their values at the junction, show a remarkable similarity and suggest a basic correctness in regards to the ventilatory unit model parameters.

8.4.3 Interspecies Delivered Dose Response

The illustrations presented in this section are based on dosimetric estimates for humans and rats using existing or modified theoretical models (Miller et al., 1985; Miller et al., 1988; Overton et al., 1987). One functional and one inflammatory endpoint will be provided drawing from the f and BAL protein data described in Section 8.3. Because the diversity of exposure scenarios across species is so great, the window of exposure parameters has been narrowed, minimizing exposure-based differences in relating species responsiveness.

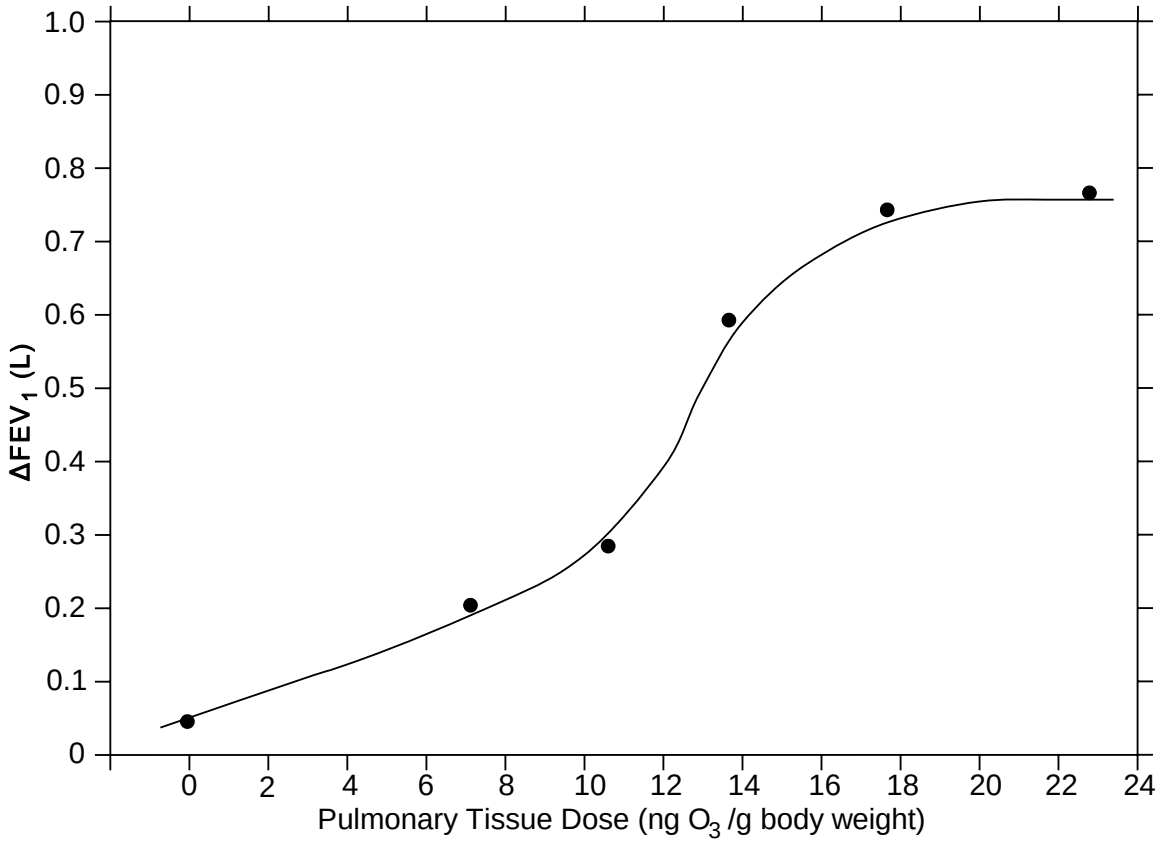


Figure 8-13. Changes in forced expiratory volume in 1 s (ΔFEV_1) versus pulmonary tissue dose. Plotted are decrements in FEV_1 (liters) for human subjects against predicted pulmonary tissue dose normalized to body weight. In order, from left to right, the dose values correspond to 0, 0.12, 0.18, 0.24, 0.30, and 0.40 ppm ozone (O_3) exposure concentrations. The continuous curve was an "eye fit".

Source: Miller et al. (1988)

Because the theoretical models developed by Miller et al. (1985, 1988) and Overton et al. (1987) estimate the dose distribution to the RT on a per breath basis, a minimum quantitative description of tidal breathing (V_T and f) is needed to utilize the theoretical models of O_3 deposition. The need for detailed breathing parameters, therefore, severely restricts the application of the model to studies providing such data. Unfortunately, breath-by-breath parameters over the course of an exposure normally are not measured or reported in most publications. Two studies each in humans and rats, providing adequate detail over the course of the exposure, allowed the model computations to be performed for this illustration (Figure 8-15). The human studies examined were DeLucia and Adams (1977), who exposed the subjects to 0.15 and 0.30 ppm O_3 for 1 h with continuous exercise (65% oxygen uptake to the body) periods, and Beckett et al. (1985), who exposed the

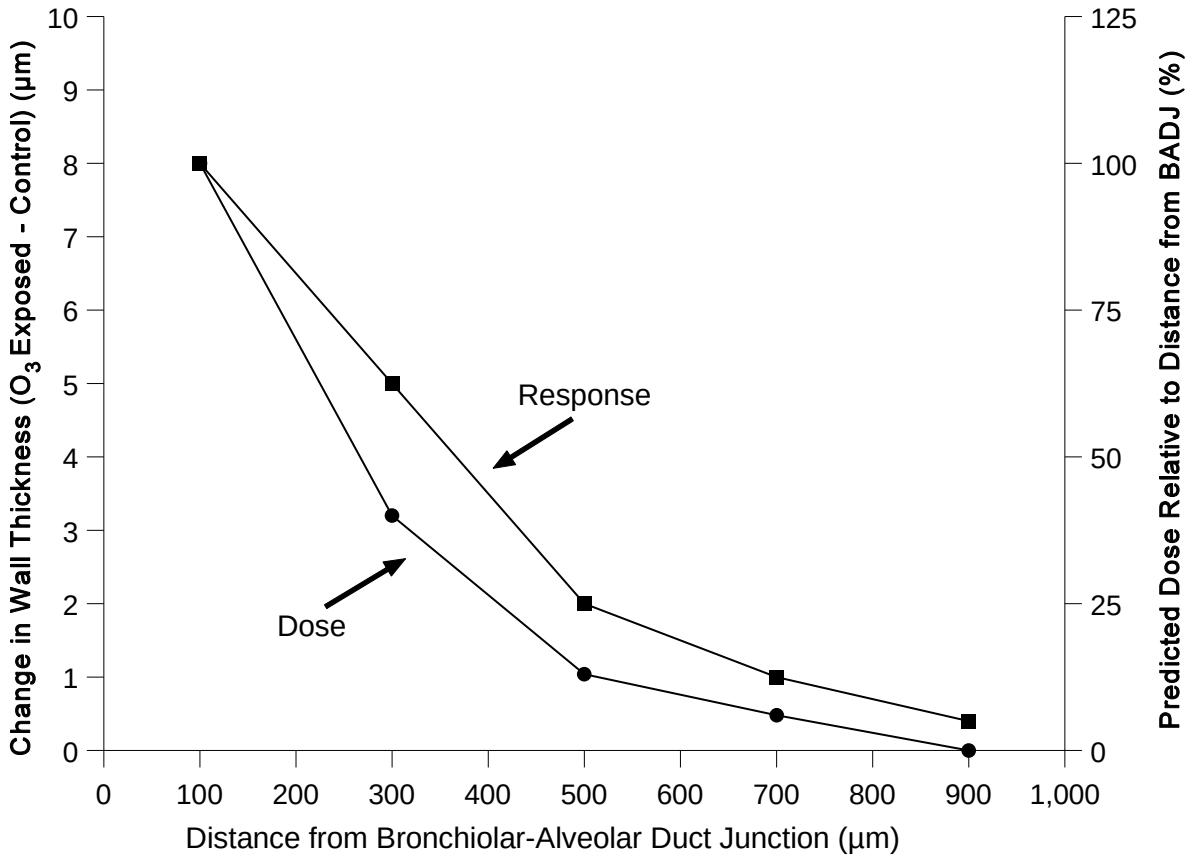


Figure 8-14. Relationship between change in alveolar wall thickness and predicted ozone (O_3) dose as a function of distance from the bronchiole-alveolar duct junction (BADJ). Rats were exposed to 0.98 ppm O_3 for 8 h/day for 90 days.

Source: Miller and Conolly (1995).

subjects to 0.4 ppm O_3 with alternating 15 min exercise (70 L/min). Three rat studies were evaluated: (1) Tepper et al. (1989), who exposed rats to 0.35, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2-induced hyperventilation (2 to 3 × resting $\dot{V}_E$); (2) Tepper et al. (1990), who exposed rats to 0.12, 0.25, 0.50, and 1.00 ppm O_3 for 2.25 h with alternating 15-min periods of CO_2, such that subsequent periods of CO_2 exposure had higher CO_2 concentrations than previous periods; and (3) Mautz and Bufalino (1989), who exposed rats to 0.8 ppm O_3 for 3 h (at rest). The response parameter was the ratio of O_3-altered f (f_{O_3}) to control f (f_{cnt}). Dose rate was the average dose to the proximal alveolar region (PAR) computed from time 0 to the time of the maximum f_{O_3}/f_{cnt}. The PAR was chosen as the target based on the perception that O_3 acts on deep lung stretch receptors

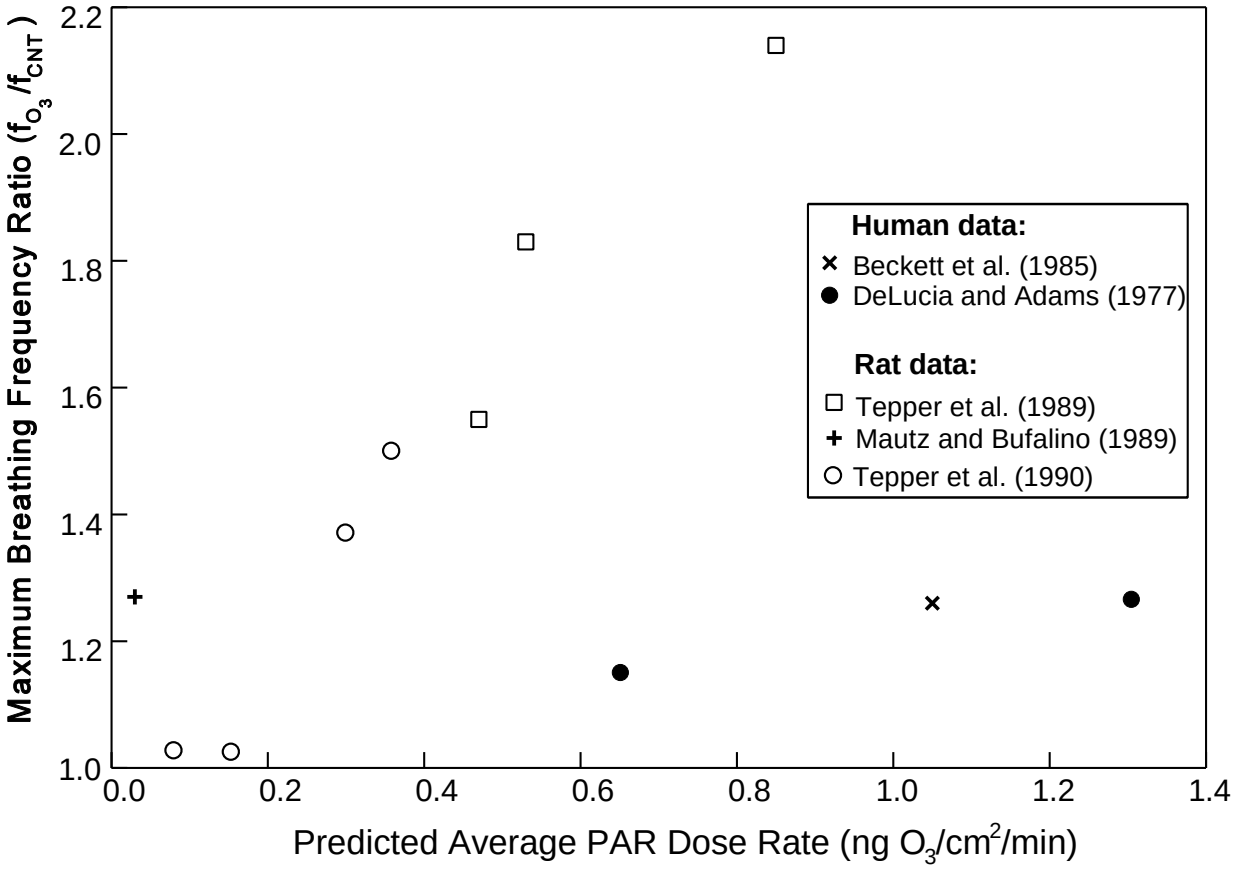


Figure 8-15. The maximum ratio of ozone (O_3)-altered breathing frequency (f_{O_3}) to control breathing frequency (f_{cnt}) at various O_3 concentrations versus predicted average dose rate to the proximal alveolar region (PAR; first generation distal to the terminal bronchioles). The average is for the time from the initiation of exposure to the time of occurrence of the maximum ratio. Beckett et al. (1985) and Mautz and Bufalino (1989) provided sufficient ventilatory data for only one O_3 concentration.

directly or indirectly (via tissue fluid pressure [edema]) as the stimulus for tachypnea. A number of assumptions were necessary to implement the deposition model for comparison of the human and the rat in this context; these are noted in Table 8-6. Although admittedly not validated, Figure 8-15 suggests that the response of the rats not only exceeded that of humans, but was initiated at a lower dose rate to the targeted lung region. This conclusion is in general agreement with that emanating from Figure 8-7, but the dose-to-target approach appears to accentuate the apparent difference in sensitivity for this endpoint in favor of the rat.

Using an analysis similar to that applied above for f, Miller et al. (1988) related pulmonary tissue dose (normalized to body weight) to BAL protein from rats, guinea pigs, and rabbits (Hatch et al., 1986) for O_3 concentrations of 0 to 2.0 ppm for 4 h. These values have

been supplemented in Figure 8-16 to include the results of the human study of Koren et al. (1989b). As can be seen in the illustration, there appears to be a log-normal relationship between BAL protein and dose to the pulmonary region, the purported site of plasma leakage to the airspace lumen. This relationship would support the contention that there is a mechanistic consistency in response across species that may exhibit a quantitative sensitivity factor for use in further quantitative interspecies extrapolation. This sensitivity factor is evident from the clustering of data from different animal species.

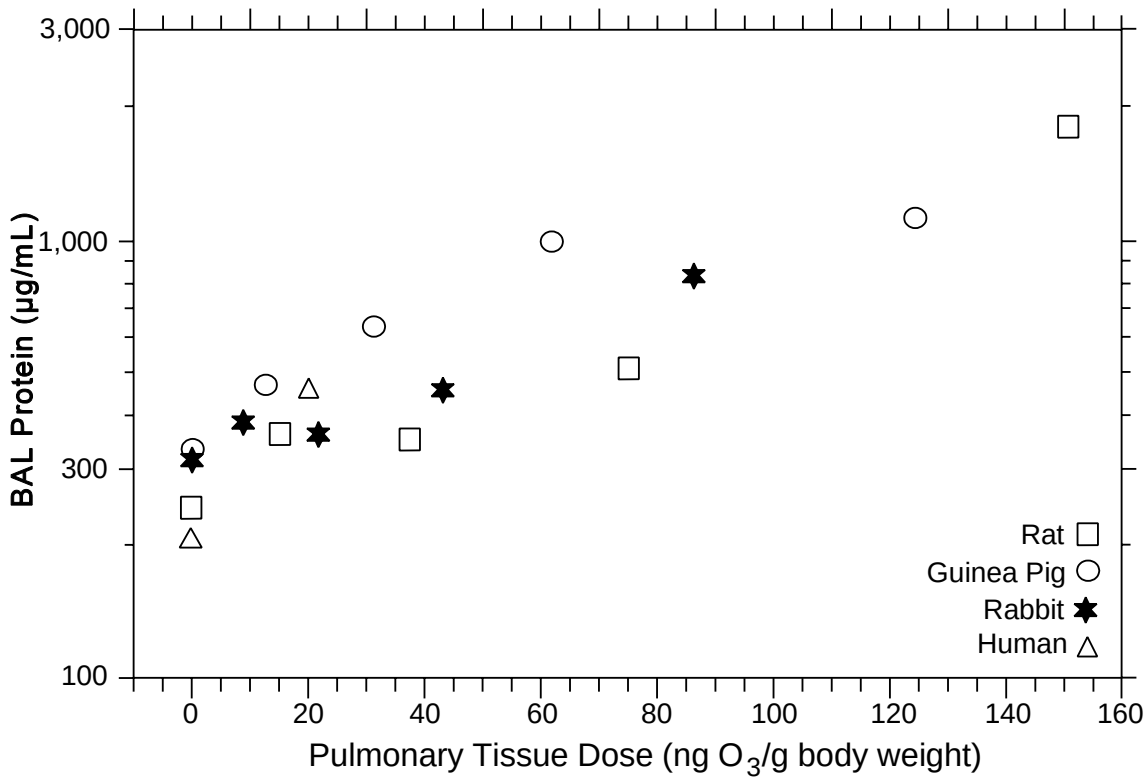


Figure 8-16. Protein in the bronchoalveolar lavage (BAL) for several laboratory animal species and humans, as related to the estimated pulmonary dose (normalized per gram of body weight).

Source: Modified from Miller et al. (1988).

8.5 Quantitative Extrapolation of Chronic Ozone Effects

8.5.1 Introduction

In previous sections, the homology between laboratory animal responses to acute O_3 exposure and effects observed in human subjects was demonstrated. Most of these responses were related to lung function and, to a more limited extent, recovery of extravasated

protein in BAL fluid, because these effects are measurable in both human and nonhuman species. Of laboratory species commonly used to address questions of acute toxicity, rats have the strongest and most compatible database for such comparisons. Studies in nonhuman primates typically are limited to morphology endpoints and to longer exposure periods, thereby limiting the practical utility of these studies in this same context. A conclusion drawn from the discussion of acute effects is that there is reasonable semi-quantitative agreement and homology between species with regard to their functional and permeability responses to short-term O_3 exposure. Where data from long-term exposures exist, cross-species relationships among similar endpoints (usually functional) are considerably weaker, although there is the suggestion that long-term exposure to O_3 can alter the distal lung (that zone where theoretical models would predict greatest O_3 deposition).

This section continues the rationale founded in the extrapolation paradigm illustrated in Figure 8-6 in an attempt to quantitatively address the question of potential chronic alteration to the lungs of O_3-exposed people. Direct data from epidemiological studies remain suggestive at best (see Chapter 7). On the other hand, the extensive database of morphometric effects on the distal lung of exposed animals (rodents and nonhuman primates) reveals the sensitivity of such endpoints and clearly relates to the site of the lung associated with incipient chronic lung diseases associated with known toxic inhalants (e.g., tobacco smoke). Unfortunately, there is a lack of reliable morphometric data on the human lung that are associated with O_3 or other air pollutants. Thus, the goal here is to draw from the extensive and reliable animal database, which demonstrates chronic effects of long-term exposures to O_3, and project from it the potential for chronic effects in humans utilizing the linkage provided by newer, refined O_3 dosimetry estimates.

In the long-term studies selected for detailed analysis, great importance was placed on the relevance of the exposure concentrations, the site specificity of the morphometric analysis, and the consistency of analysis within species. Two rat studies were selected that represent near-lifetime exposures to O_3 over a range of concentrations and scenarios (Chang et al., 1992; Chang et al., 1995). The former study was conducted in conjunction with the U.S. Environmental Protection Agency (EPA) and involved a weekday urban pattern of exposure represented by a 9-h spike (5 days/week) slowly rising to 0.25 ppm from a near-continuous baseline of 0.06 ppm O_3 for 78 weeks. The latter study was conducted by the same morphometrists but on rats exposed weekdays (6 h/day) to 0, 0.12, 0.50, or 1.00 ppm O_3 for 87 weeks. Both studies utilized F344 rats. Likewise, the primate studies were conducted by the same investigators, but involved two strains of monkeys. In one study, the bonnet monkey was exposed for 90 days (8 h/day) to 0, 0.15, or 0.30 ppm O_3 (Harkema et al., 1993), whereas the second study consisted of daily (8 h/day) exposures to the smaller fascicularis monkey of 0 or 0.25 ppm O_3 for 18 consecutive mo or 9 mo with alternating months of clean air (Tyler et al., 1988). The assumptions needed to model the specific species dosimetry of each study are provided in the following section. Growth was compensated where appropriate (for rats), and allometric anatomic adjustments or assumptions were made to estimate unavailable anatomic data (as in the case of the monkey) for the dosimetry models. Ventilation was assumed to be unaffected by O_3 after the first 2 days of exposure because adaptive events are known to occur in that time frame. This simplified the model formulations for the animal studies, although varied activity and exposure profiles were considered throughout for the human dose estimates. Allometric equivalent life-span estimates also were made in an effort to relate the duration of the exposure period relative to life-span and cumulative dose.

In each experiment, a subregion of the CAR (where major lesions have been observed) was chosen by the investigators for study. For the two rat studies, the site was the PAR, which was defined by Chang et al. (1992) as "the alveolar tissue surrounding the alveolar ducts beginning at the bronchiolar-alveolar duct junction and ending at the second alveolar duct bifurcation." For modeling purposes, the first generation distal to the terminal bronchioles corresponded to the PAR. The monkey studies focused on an analogous airway region, the respiratory bronchioles. Thus, in three of the four experiments, the first generation distal to terminal bronchioles was the explicit site of the observed effects. For simplicity, similar assumptions were made for the Tyler et al. (1988) monkey study that came from the same laboratory as Harkema. For discussion purposes, the term PAR has been used for the monkey sites, even though the airway morphology of this site differs between the rat and monkey. The simulated dose of the PAR was chosen for comparison to the reported effect.

Because the PAR generally is thought to be the site of incipient lung disease, it is of particular interest with regard to the potential role of O_3-induced chronic lung disease. Effects in the PAR can be evaluated specifically using morphometric techniques with an electron microscope, and, likewise, dosimetric models can estimate surface-area-normalized focal tissue doses within the same region. The dose-response relationships constructed in this extrapolation focus on the theoretical dose to the PAR of rats and monkeys, with their corresponding impact on the PAR interstitial structure (as total and acellular thickness). Analogous estimated cumulative doses to the PAR of a 9-year-old child and of an outdoor worker exposed to a New York City summer O_3 season then are interpolated from the effects in the experimental animals at nominally similar doses.

In this attempt to extrapolate chronic O_3 effects from the experimental animals to humans, the two foremost assumptions are that (1) there exists coherence of analogous dose-response data across species with regard to acute exposure effects (as represented by one side of the parallelogram model [Figure 8-6]), and (2) there exists some relationship of effect to the total cumulative dose to the target tissue. The essential hypothesis is that the rate of change of interstitial thickness is related to the rate of O_3 uptake. Clearly, the latter assumption ignores potential adaptive or reparative processes, but rather assumes that continuing exposure of any scenario imparts irreversible or slowly reversible changes within the constitutive structure of the target area. There is little to substantiate this assumption other than the commonly believed irreversibility of fibrogenesis and degenerative lung disease.

8.5.2 Factors Considered in Estimating Dose

For each of the animals for which doses were simulated, Table 8-10 lists information or parameters needed for the dosimetry simulations and the source of the information. Also, details are provided as to how this information was modified, scaled, or used to correspond to the experimental or hypothetical animals for which dose was estimated. An expanded discussion follows.

8.5.2.1 Human

The dose simulations were for a hypothetical New York City adult outdoor worker and a hypothetical 9-year-old New York City child, who is active out of doors. Their

Table 8-10. The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Characteristics of simulated animal	Chang et al. (1992); Tepper et al. (1991): 0.21-0.47 kg, 10-88 weeks old, males.	Chang et al. (1995); Last et al. (1994): 0.13-0.53 kg, 7-93 weeks old, males.	Harkema et al. (1993): 2.3-9.7 kg (used 6-kg), 22-35 weeks old, males and females.	Tyler et al. (1988): 1-2 kg (used 1.6-kg), 30-107 weeks old, males.	Present simulation: 31 kg, 9 years, 132-cm height, male or female.	Present simulation: 73 kg, 181-cm height, adult male.
Exposure regime	Chang et al. (1992); 0.06 ppm continuous background, 22 h/day, 7 days/week; 0.25 ppm ramped peak over 9 h, 5 days/week. 3-, 13-, and 78-week exposures.	Chang et al. (1995); 0, 0.12, 0.50, and 1.00 ppm 6 h/day, 5 days/week for 87 weeks.	Harkema et al. (1993); 8 h/day for 90 days; 0, 0.15, and 0.30 ppm.	Tyler et al. (1988); 8 h/day for 18 mo; 0 ppm, 0.25 ppm (daily), and 0.25 ppm (daily, alternating months).	Johnson (1994); continuous exposure pattern for a New York outdoor child; April-October, 1991. Avg. exposure 22 ppb; range, 0-238 ppb.	Johnson (1994); continuous exposure pattern for a New York outdoor worker. April-October, 1991. Avg. exposure, 23 ppb; range, 0-227 ppb.
LRT structure	PUL: Mercer et al., 1991 (0.283-kg S-D rat). TB: Yeh et al., 1979 (0.33-kg Long-Evans rat).		Weibel (1963): adult human LRT.			
Dead space volume at FRC	Dead space volume = URT + TB volumes.		Dead space volume = URT + TB volumes.		Hart et al. (1963): height: 92-198 cm, age: 4-42 years, weight: 16-115 kg.	
FRC	Mercer et al., 1987 (0.291-kg S-D rat). FRC $\propto$ weight$^{0.55}$ (Takezawa et al., 1980).		Kosch et al., 1979 (radiata monkeys, FRC = 52.8 mL/kg).	Tyler et al., 1988 (2-kg fascicularis). FRC $\propto$ weight$^{0.86}$ (relation for combined rodent species, Takezawa et al. (1980).		
URT volume (V) and surface area (S)	Patra et al., 1987 (F344 rat, 0.012-0.366 kg, 1-63 weeks old). Graphical interpolation and extrapolation with respect to age.		Schreider and Raabe, 1981 (7-kg rhesus monkey). V $\propto$ weight; S $\propto$ V$^{2/3}$.		V: The ratio of URT and dead space volumes are the same as in the adult human. S $\propto$ V$^{2/3}$	Hu et al. (1992a).
TB volume (V) and surface area (S)	V: Mercer et al., 1994a (0.293-kg S-D rat). S: Yeh et al., 1979 (0.33-kg Long-Evans rat). S $\propto$ V$^{2/3}$.		V: Pulmonary and TB volumes in same ratio as human. S $\propto$ V$^{2/3}$.		V: volume = dead space volume minus URT volume. S $\propto$ V$^{2/3}$.	
PUL region volume (V) and surface area (S)	V: FRC minus TB volume. S $\propto$ V$^{2/3}$.		Pulmonary and TB volumes in same ratio as human. S: Mercer et al. (1994b), interspecies interpolation.	V: Pulmonary and TB volumes in same ratio as human. S: Tyler et al., 1988 (2-kg fascicularis). S $\propto$ V$^{2/3}$.	V: volume = FRC volume minus dead space volume. S $\propto$ V$^{2/3}$.	

Table 8-10 (cont'd). The Basis of Information for Model Parameters[a]

Information Needed	F344 Rat (EPA Chronic Study)	F344 Rat (NTP/HEI Chronic Study)	Bonnet Monkey (*Macaca radiata*)	Monkey (*Macaca fascicularis*)	Human Child	Human Adult
Tidal volume and breathing frequency	Costa (1994): measurements made at 1, 3, 13, 52, and 78 weeks of exposure (interpolation for other time points).	None reported. Assumed similar to that of the EPA chronic study.	None reported. A range of parameters used. See Table 8-11 and Section 8.5.2.2.		Johnson (1994): activity pattern. Hofmann et al. (1989): tidal volumes and breathing frequences.	Johnson (1994): activity pattern. ICRP (1975): tidal volumes and breathing frequences.
Mass transfer coefficients	Parameter estimation using the rat data of Hatch et al. (1989) and an assumed pulmonary region coefficient. See Table 8-6 and Section 8.5.2.3.		For corresponding generations or model segments, the same as for the adult human. See Table 8-6 and Section 8.5.2.1.			Hu et al. (1992b); Miller et al. (1985); Weibel (1963). See Table 8-6.

[a]See Appendix A for abbreviations and acronyms. PUL, V, and S indicate pulmonary, volume, and surface area, respectively. In some cells of this table, the information is ordered as follows: characteristics of the species and the reference to the basis of the information, followed by an indication as to how the reference information was used or modified for the present simulations. The proportion symbol $\propto$ indicates that one parameter is proportional to another (e.g., $S \propto V^{2/3}$ implies that $S = S_0 (V/V_0)^{2/3}$, where the subscripted parameters are known; V is the new or desired volume and S is the estimated surface area of the new volume.

residences were not air-conditioned, and they lived, worked, or played in the same area. These two groups of people would have tended to experience higher outdoor O_3 concentrations than other New York City people because the chosen area had higher outdoor O_3 concentrations than other areas, and O_3 levels are higher in homes without air-conditioning (Johnson, 1994). These scenarios were selected because they involve the same at-risk population groups used for the O_3 risk analysis in the staff paper (U.S. Environmental Protection Agency, 1995).

The activity patterns and O_3 exposure estimates were generated by the probabilistic National Ambient Air Quality Standards Exposure Model for O_3 (pNEM/O_3) (Johnson, 1994) for April through October 1991. The modeling approach for outdoor workers can be described as follows. Outdoor workers residing in New York City were divided into nonoverlapping sets of cohorts, such that each cohort could be identified by a residential location, a work location, and a residential air conditioning system. The pNEM/O_3 model generated an "exposure event sequence" (EES) for each cohort based on data obtained from activity diary studies involving outdoor workers. Each exposure event in the EES assigned the cohort to a geographic location, a microenvironment, and an exertion level. Algorithms within the pNEM/O_3 model provided estimates of O_3 exposure and equivalent ventilation rate (EVR) for each event that lasted from 1 to 60 min. The EVR is ventilation rate (liters per minute) divided by body surface area (square meters). Exposures were calculated on a minute-by-minute basis; however, the concentration and EVR values of all minutes within a given exposure event were held constant during the event. The pNEM/O_3 model used for outdoor children was generally consistent with the model for outdoor workers but relied on human activity data from children. Another difference was that the cohorts of children were identified by residential location and air conditioning system only; workplace location was not specified.

Figures 8-17A and 8-17B give an idea of the exposure concentrations for the outdoor worker and child, respectively, with plots of the average hourly and monthly O_3 concentrations. The exposure concentrations of the adult and child differ because their daily activities and locations are different. Note that in a few cases, the hourly average exceeds the 0.12 ppm (120 ppb) standard.

Anatomical Aspects

The adult New York City worker was assumed to have characteristics similar to the subjects of an investigation by McDonnell et al. (1983). The body surface area of the worker (needed to convert EVR to minute volume) was assumed to be 2 m^2 (Koren et al.,1989b 1.90 and 1.98 m^2; Johnson, 1994: 1.90 to 1.95 m^2). The New York City child had a body surface area of 1.07 m^2, which was consistent with both 9-year-old males and females (Johnson, 1994). The assumed values for the child's height (132 cm) and weight (31 kg) were based on Phalen et al. (1985) and Johnson (1994).

For the simulations, no distinction was made between mouth and nose breathing with respect to URT uptake. The significance of this assumption is not clear. If both modes have approximately the same uptake efficiency, then the assumption of no distinction between mouth and nose breathing is appropriate for predicting PAR doses. Whether this is a valid assumption has not been settled (see Section 8.2.3.4). The same URT anatomical model was used for both ages, but was isotropically scaled as necessary. Surface areas and lengths were assumed to be proportional to the volume to the two-thirds and one-third powers for adults and children, respectively (e.g., if x is proportional to volume V to the power p, then

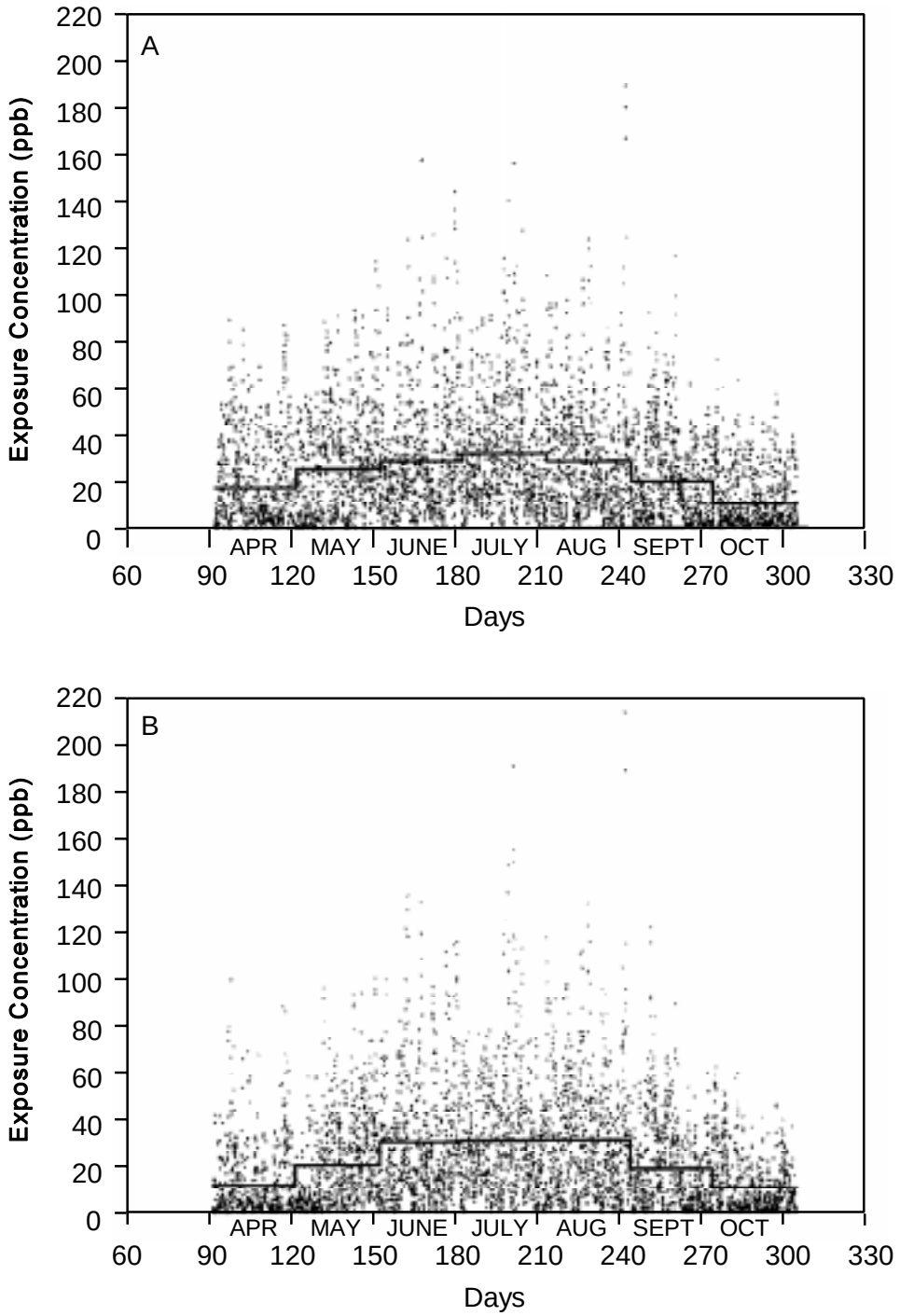


Figure 8-17. The variation in exposure concentration for the New York City adult and child. Plotted are the hourly and monthly O_3 averages for the New York City outdoor worker (A) and the 9-year-old child (B). The hourly averages are represented by the "dots" and the monthly average by the continuous solid line.

$x_n = x_0(V_n/V_0)^p$, where the subscripts n and o indicate the new and original values, respectively). Dimensions used for the adult were from Hu et al. (1992b), who based their values on the acoustic reflection measurements of Fredberg et al. (1980) that corresponded to the oropharyngeal (mouth through larynx) region. The LRT model developed by Weibel (1963) was used for LRT structure and dimensions, but was scaled isotropically to FRC and dead-space volumes, as appropriate.

Mass Transfer Coefficients

This parameter is necessary to determine the local dose, expressed in terms of the flux of O_3 to respiratory tract surfaces. Specifically, this dose is related to the product of the mass transfer coefficient and gas-phase concentration of O_3 integrated over the time of interest. Good estimates of mass transfer coefficients are needed to predict a reliable dose at specific respiratory tract sites.

A brief description of how the human URT and TB region mass transfer coefficients were derived from Hu et al. (1992b) and Weibel (1963) is presented in Table 8-6. These coefficients were "validated" by simulating the bolus-response experiment of Hu et al. (1992b). Simulation results of the bolus uptakes overpredicted by 5%; results for the other two measured variables deviated from the experimental values by 30%.

As a result, simulations with inhaled and exhaled flow rates of 250 mL/s are expected to overpredict total uptake by no more than 5%. The significance of the poor results in simulating the other two variables is not clear. Possibly, predictions of the distribution of absorbed O_3 within the RT are questionable; also, predicted total uptakes for flow rates different from the experimental value of 250 mL/s may be suspect. For very different reasons, this latter speculation is probably valid. Measurements by Hu et al. (1994) at rates higher than 250 mL/s indicate that local mass transfer coefficients increase with increasing flow rates. In addition, naive subjects were used for the bolus experiments. Chronic exposure to O_3, which is more appropriate to the present simulation scenarios, could alter mass transfer coefficients due to chemical reactions changing the properties of biochemical constituents. See Section 8.2.4.2 and Table 8-7 for a comparison of dosimetry modeling results with dosimetry data, using coefficients based on Hu et al. (1992b).

The experimental data of Hu et al. (1992b) were not sensitive enough to allow an estimate, with confidence, for the pulmonary region or for the PAR mass transfer coefficient. For this reason, these coefficients were assumed to be the same as used by Miller et al. (1985). The value of this parameter is approximately the same as used for the rat. Although the value of the pulmonary region mass transfer coefficient of rats also is unknown, a comparison by Pinkerton et al. (1992) of morphometric effects and dosimetry model predictions suggests that the rat value is not unreasonable.

Ventilation

Activity patterns for the adult and the child (Johnson, 1994) were used to estimate $\dot{V}_E$s. The set of estimates had over 300,000 minute-by-minute cases, each case consisting of the day of the year, hour, minute, and equivalent ventilatory rate ($\dot{V}_E$ divided by body surface area). The generated patterns were for 214 sequential days, from the beginning of April through October 1991.

For the adult, V_Ts were estimated by interpolation from a plot of V_T versus $\dot{V}_E$ that was drawn using information from Table 120 in International Commission on Radiological

Protection (1975). Tidal volumes and f's for the child were estimated using a relationship between V_T and $\dot{V}_E$ developed by Hofmann et al. (1989). For both humans, f's were defined as $\dot{V}_E$ divided by the estimated V_T.

Estimating Proximal Alveolar Region Dose

The PAR doses were estimated by simulation for each of the minute-by-minute equivalent $\dot{V}_E$s and personal concentration estimates; these doses were averaged.

8.5.2.2 Monkeys

Anatomical Aspects

Because there were no data on the uptake of O_3 by the URT of monkeys, no distinction was made between oral and nasal breathing. The URT dimensions were based on Schreider and Raabe (1981) for the nasopharyngeal region of a 7-kg rhesus monkey. Volumes were proportional to body weight and dimensions were scaled isotropically. For the nasopharyngeal region, the dimensions used may have been too large because the casting procedure may have resulted in a larger volume (Yeh et al., 1989) than in a live animal, and air flow streamlining (Morgan et al., 1991) may have resulted in a smaller effective volume. The structure of the LRT was based on Weibel (1963) for humans (the same as used for the human simulations), which was isotropically scaled to monkey FRC values, assuming that TB and pulmonary region volumes were in the same proportion as the human FRC values. The use of the human LRT structure for the monkey simulations is considered to be of less importance to reasonable predictions than the correct specification of volumes and surface areas of the different regions.

Mass Transfer Coefficients

The human mass transfer coefficient values were assumed because there were no reported values nor uptake data that would have allowed estimates of these coefficients. For corresponding model segments or model generations, the monkey coefficients were the same as those used for humans.

Ventilation

No ventilation parameters were reported for the monkey experiments. For this reason, four states of ventilation were used to calculate the doses (see Table 8-11). The first three in the table were extrapolated from human values of V_Ts and f's. These human parameters were consistent with the algorithm used for the human simulations and correspond approximately to sedentary, low, and light activity as categorized by Hofmann et al. (1989) for an adult human. The last set of parameters correspond to measurements made by Moss (1995) on five awake female adult cynomolgus monkeys (*Macaca fascicularis)*.

Estimating Proximal Alveolar Region Doses

For the two monkey experiments and corresponding ventilatory parameters, scaled PAR doses were estimated and multiplied by the average exposure concentrations to obtain estimated average PAR doses.

8.5.2.3 Rats

Anatomical Aspects

Rats are nasal breathers; therefore, the URT model corresponds to and is based on

Table 8-11. Estimating Monkey Ventilatory Parameters[a]

Human Activity Level[b]	Extrapolation from a 73-kg Human with the Following Parameters[c]			To a 1.6-kg Monkey			To a 6-kg Monkey		
	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)	$\dot{V}_E$ (L/min)	V_T (mL)	f (breaths/min)
Sedentary	6	500	12.0	0.34	11.0	31.2	0.92	41.1	22.4
Low	12	900	13.3	0.68	19.7	34.7	1.84	74.0	24.9
Light	20	1,300	15.4	1.14	28.5	40.0	3.07	107.0	28.7
	Extrapolation from a 4.4-kg *Macaca fascicularis* with the following parameters[d]								
	1.78	52.9	33.6	0.83	19.3	43.2	2.25	72.3	31.1

[a]See Appendix A for abbreviations and acronyms. For extrapolation: $(V_T)_1 = (V_T)_2 \times (\text{weight}_1/\text{weight}_2)$; $f_1 = f_2 \times (\text{weight}_1/\text{weight}_2)^{0.25}$; $\dot{V}_E = V_T \times f$. Subscript 1 corresponds to the 1.6- or 6-kg monkey, and subscript 2 corresponds to either the human or the 4.4-kg monkey.

[b]These characterizations for the human parameters are consistent with those of the International Commission on Radiological Protection (1975) and Hofmann et al. (1989).

[c]For a given human $\dot{V}_E$, the human V_T and f are based on the International Commission on Radiological Protection (1975). For the selected $\dot{V}_E$, V_T and f are the same as used for the adult human.

[d]Moss (1995); parameters are the average for five monkeys.

the F344 rat nasopharyngeal region. The dimensions may be too large, however, because

the casting procedure can result in a larger volume than in a live animal (Yeh et al., 1989), and air flow streamlining (Morgan et al., 1991) may result in a smaller effective volume. The structure of the LRT is a composite of the TB model of the Long-Evans rat (Yeh et al., 1979) and a pulmonary region based on the Mercer et al. (1991) ventilatory unit model of the S-D rat. Given the dosimetry model used, the volumes and surface areas of the different regions are more important than the structural differences of the three strains used to construct the rat RT.

Mass Transfer Coefficients

Mass transfer coefficients for the nasopharyngeal region and TB of the rat were estimated using the uptake data of Hatch et al. (1989) and an assumed pulmonary region mass transfer coefficient. Hatch et al. (1989) reported the average uptake of O_3 for eight F344 rats and the average fraction of $^{18}O_3$ in the nasopharyngeal region, the trachea, and the lungs. (Hatch et al. [1989 and 1994] discuss issues related to using $^{18}O_3$ dose as a measure of O_3 dose.) Based on a discussion in Miller et al. (1993) concerning the investigation of Pinkerton et al. (1992), the pulmonary region mass transfer coefficient was defined as that used by Mercer et al. (1991). This, combined with information from Hatch et al. (1989), allowed an estimate of mass transfer coefficients for the nasopharyngeal region and the TB. A common estimate of mass transfer coefficients also was made using the data for the individual rats. For these coefficients, whose values were essentially the same as the first set, the individual rat simulations deviated from the experimental data by an average of 23%.

Ventilation

For the EPA chronic study, V_T and f were measured at Exposure Weeks 1, 3, 13, 52, and 78. For intermediate weeks, these parameters were estimated by interpolation and assumed constant during each of those weeks. Because ventilatory parameters were not reported for the National Toxicology Program/Health Effects Institite (NTP/HEI) chronic study, parameters similar to those of the EPA chronic study were assumed (see below).

Estimating Proximal Alveolar Region Doses

For the EPA chronic study, the exposure pattern was variable during the week, but repeated each week (Tepper et al., 1991). The PAR doses were simulated for each week and averaged. The ventilatory, physiological, and anatomical characteristics of the NTP/HEI study rats with respect to time were assumed to be similar to the EPA chronic study rats, and the average scaled PAR dose ([g/cm^2-min]/[g/m^3 ambient O_3]) was defined as the same that was predicted for the EPA study rats. Given this and the average exposure concentration, the average PAR doses were estimated.

8.5.3 Results and Discussion

8.5.3.1 Simulation Results

The simulation results are presented in Table 8-12. The first and second columns identify the laboratory experiments and the hypothetical human exposures that were simulated. Column 4 applies only to the monkeys and is discussed above (see Section 8.5.2.2) in the discussion of monkey ventilation. The "Average PAR Dose" (column 5) is the predicted average flux of O_3 to the surfaces of the alveoli or respiratory

Table 8-12. Summary of Simulation Results[a]

Simulation of	Source for Simulation	Average Weight (kg)	Minute Volume[b] (L/min)	Average PAR Dose ($\mu g/cm^2$-min)	Cumulative PAR Dose ($\mu g/cm^2$)	Duration of Experiment (weeks)	Equivalent Human Child Time[c] (weeks)	Equivalent Human Adult Time[c] (weeks)
EPA rat (3 weeks)	Chang et al. (1992) Tepper et al.(1991)	0.24		4.11e-5	1.24	3	8.9	11
EPA rat (13 weeks)		0.29		3.85e-5	5.1	13	39	48
EPA rat (78 weeks)		0.4		3.43e-5	27	78	232	286
NTP/HEI rat (0.12 ppm)[d]	Chang et al. (1995)	0.4		0.828e-5	7.3	87	259	319
NTP/HEI rat (0.5 ppm)[d]		0.4		3.45e-5	30.3	87	259	319
NTP/HEI rat (1 ppm)[d]		0.4		6.89e-5	60.5	87	259	319
Bonnet monkey (0.15 ppm)	Harkema et al. (1993)	6		1/2 below	[e] 1/2 below[e]	12.9	19.5	24
Bonnet monkey (0.3 ppm)		6	0.92	0.61e-5	0.79	12.9	19.5	24
			1.84	3.99e-5	5.17	12.9	19.5	24
			3.07	9.92e-5	12	12.9	19.5	24
			2.25	5.65e-5	7.3	12.9	19.5	24
Fascicularis monkey (daily; 0.25 ppm)	Tyler et al. (1988)	1.6		2 × below	2 × below[f]	78	[f] 164	203
Fascicularis monkey ("seasonal"; 0.25 ppm)		1.6	0.34	0.197e-5	1.55	78	164	203
			0.68	1.26e-5	9.91	78	164	203
			1.14	3.25e-5	25.6	78	164	203
			0.83	1.81e-5	14.3	78	164	203
9-year-old NYC[g] child	U.S. Environmental Protection Agency (1995)	31		3.2e-5	9.9	30	30	NA
Adult NYC[g] outdoor supervisor		73		2.78e-5	8.6	30	NA	30

[a]See Appendix A for abbreviations and acronyms.
[b]Only relevant for the monkeys. See Table 8-11.
[c]Given the nonhuman exposure time, the number of weeks the human would have to be exposed for equal human and nonhuman physiological times: $weeks_{HUMAN} = weeks_{NONHUMAN} \times (weight_{HUMAN}/weight_{NONHUMAN})^{0.25}$.
[d]The relative PAR dose [$(g/cm^2\text{-min})/(g/m^3\ O_3)$] was assumed to be the same as the average for the EPA rat.
[e]For each minute volume, the exposure concentration for these bonnet monkeys was one-half those listed below.
[f]For each minute volume, the fascicularis monkeys exposed "daily" were exposed to O_3 twice as long as the "seasonal" fascicularis monkeys.
[g]NYC = New York City.

bronchioles in the first model generation distal to the terminal bronchioles. For each experiment, the average is over the total time or duration of the experiment (given in column 7, "Duration"); the average includes times of no exposure. Column 6, "Cumulative PAR Dose," is the total quantity of O_3 predicted to be absorbed by the tissue and liquids of the PAR during the experiment; these dose values are the same as the duration (in minutes) multiplied by the average PAR dose (column 5).

The entries in columns 8 and 9 are the total times a human would have to be exposed to reach the equivalent physiologic times of a specific laboratory animal species. Basically, the concept of physiological time is that the time courses of equivalent processes (e.g., a breath, a heartbeat) across species are approximately the same with respect to the time span of the process divided by the body mass raised to the one-fourth power (Travis et al., 1990). If this concept is applicable to the time course of O_3-induced biological effects, then the entries in the two columns are the human exposure durations necessary to obtain the same cumulative PAR dose biological effect as that of the corresponding laboratory animal duration. Note that for only two or three of the laboratory animal experiments are the human durations greater than the laboratory animals' equivalent or real (duration) times.

8.5.3.2 Interpretation of Chronic Site-Specific Dose-Effect Estimates

Because the PAR is considered the primary site of O_3 injury and represents that region of the lung from which most chronic lung diseases originate, it was selected as the most appropriate target to develop cross-species dose-response extrapolations. The selected "effect" relates to the thickness of the interstitium at the PAR, which is indicative of fundamental structural remodeling. The indices of thickness provided in the noted studies include both cellular and acellular constituents, a distinction that was not always clear; however, interstitial arithmetic thickness, areal volume (cubic micrograms per square microgram), or volume density was available and could be represented in terms of percent change from control.

Dose-response curves for O_3-induced thickening of the PAR are represented in Figures 8-18A (rats) and 8-18B (monkeys). The cumulative PAR dose for the specific exposure scenario (from Table 8-12; Cumulative PAR Dose) for each study is provided on the abscissa as is its corresponding percent change in total and acellular interstitial (PAR) thickness on the ordinate (detailed in Table 8-13). The rat studies are plotted separately from the monkey studies because of an approximate three- to fivefold difference in their responses, with the monkey being more responsive than the rat. Although this difference could represent innate sensitivity differences between the species, it should be noted that estimates of daytime rat exposures (a period of quiescence for the rat), in contrast to the daytime-active monkeys, may have been substantially lower in terms of dose than that predicated on the basis of rat ventilatory measurements that were derived when they were in an aroused, awakened state. Recent studies comparing rat and human $^{18}O_3$ dosimetry indicate that exercise can account for up to a fivefold difference in acute responsiveness between species (Hatch et al., 1994). These explanations remain speculative, however, in the absence of direct data. Ventilation values for the monkeys were selected a priori as light activity to be similar to the human model being used, which had ventilation values only slightly higher than the empirically derived values (see Table 8-11) from comparable awake, resting monkeys (when adjusted for size).

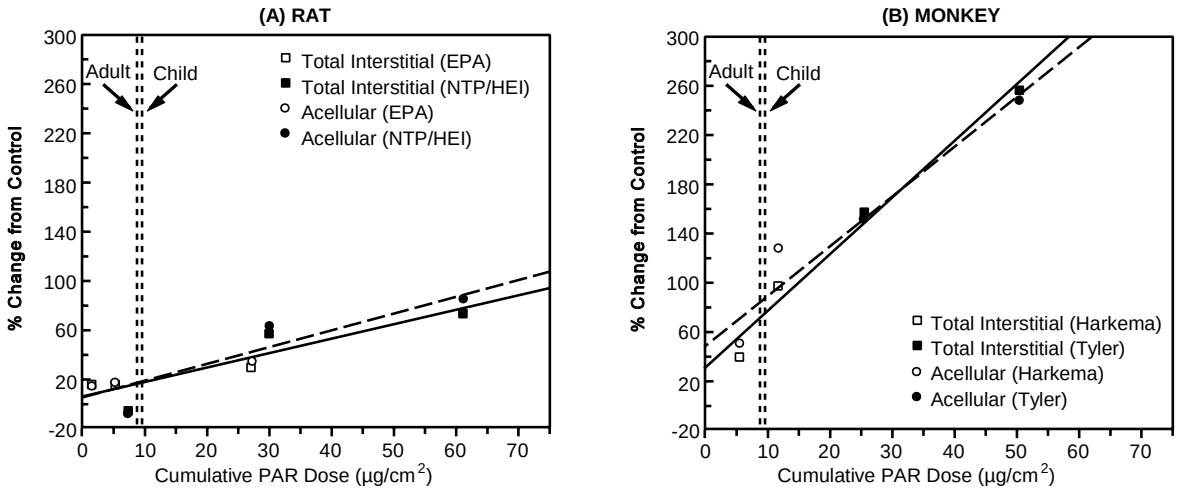


Figure 8-18. (A) Change from control of total interstitial and acellular thickness for the rats exposed to ozone (O_3) in the U.S. Environmental Protection Agency (Chang et al., 1992) and National Toxicology Program/Health Effects Institite (Chang et al., 1995) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.84 and 0.80. (B) Change from control of total interstitial and acellular thickness for the monkeys exposed to O_3 in the Tyler et al. (1988) and Harkema et al. (1993) studies. Solid line represents the linear regression for the total interstitial thickness, and the dashed line represents the linear regression for the acellular thickness across the various cumulative dose estimates for both studies. The respective correlation coefficients (r^2) are 0.97 and 0.93. Vertical dashed line with arrows for child and adult denote proximal alveolar region (PAR) dose level for interpolation of PAR thickness effect from the monkey upper estimate of response.

What is remarkable in both Figures 8-18A and 8-18B is the apparent linear dose-response relationships within species. This may be due, in part, to the fact that there was consistency in the methods of the respective investigatory team studying each species. It is interesting to note that the estimated total cumulative PAR dose for the EPA chronic study (27 μg O_3/cm^2) is quite similar to that estimated for the NTP/HEI study group of 0.5 ppm O_3 (30.3 μg O_3/cm^2). An examination of the respective PAR thickness response from each study indicates an apparent similarity between the studies, with the NTP/HEI results being a bit larger in value. At 0.5 ppm O_3, the NTP/HEI investigators also reported the observation of bronchiolarization into the PAR, whereas this process was absent or not significant at 0.12 ppm O_3 or in the EPA chronic study. It is not clear whether this indicates qualitatively, rather

than quantitatively, different responses initiated at 0.12 and 0.50 ppm O_3. However, it is important to note that the correlation coefficients (r^2) for PAR dose to response across

Table 8-13. Summaries of Study Data Used in Extrapolation of Chronic Ozone Effects[a]

Study Species	Total Interstitial Volume (mean value — $\mu m^3/\mu m^2$)	Cellular Volume ($\mu m^3/\mu m^2$)	Acellular Volume ($\mu m^3/\mu m^2$)
F344 Male Rat; Chang et al., 1992 (EPA) —78 weeks (urban profile)			
Profile: 0.06-ppm continuous background, 22 h/day, 7 days/week; 0.25-ppm ramped peak over 9 h (avg. 0.19 ppm for peak; 0.09 ppm daily), 5 days/week			
3-week exposure (from Figure 4)	(+16% estimate from figure)	(+12%)	(+15%)
13-week exposure (from Table 4)	0.398[b] 0.462 (+16%)[c]	0.153[b] 0.174 (+14%)	0.245[b] 0.288 (+18%)
78-week exposure (from Table 4)	0.473[b] 0.619 (+31%)	0.149[b] 0.179 (+20%)	0.325[b] 0.440 (+35%)
16-week recovery after 78 weeks air	0.531[b]	0.171[b]	0.360[b]
16-week recovery after 78 weeks O_3	0.580 (+9%)	0.168 (0%)	0.410 (+14%)
F344 Male Rat; Chang et al., 1995 (NTP/HEI)—87 weeks (6h/day, 5 days/week)	From Appendix D1—HEI Report		
0 ppm	0.520	0.161	0.360
0.12 ppm	0.497 (5%)	0.162 (0%)	0.335 (7%)
0.50 ppm	0.826 (+58%)	0.239 (+48%)	0.587 (+63%)
1.00 ppm	0.902 (+73%)	0.233 (+45%)	0.669 (+85%)
Fascicularis Monkey; Tyler et al., 1988 —18 mo (8 h/day)	Volume fraction of lung occupied by respiratory bronchiolar wall ($\times 10^{-3}$)—from Table II		
0 ppm	0.716	0.110	0.606
0.25 ppm (daily)	2.550 (+256%)	0.437 (+297%)	2.113 (+248%)
0.25 ppm (daily; alternating months)	1.830 (+156%)	0.297 (+170%)	1.533 (+152%)
Bonnet Monkey; Harkema et al., 1993—90 days (8 h/day)	Arithmetic mean thickness (μm)—from Figure 3 and text		
0 ppm	6.5	3.2	3.3
0.15 ppm	9.2 (+41%)	4.2 (+30%)	5.0 (+51%)
0.30 ppm	13.1 (+102%)	5.6 (+74%)	7.5 (+128%)

[a]See Appendix A for abbreviations and acronyms.
[b]Control value.
[c]Parenthetical values indicate percent difference from respective control.

both studies as illustrated in Figure 8-18A are 0.80 and 0.84 for the total and acelluar interstitial changes, respectively. In the case of the monkey data represented in Figure 8-17B, the analogous correlation coefficients (r^2) were 0.97 and 0.93 for the total and acellular interstitial effects, respectively. These highly significant correlations suggest that interstitial injury is indeed a cumulative process throughout the exposure history. Moreover,in the case of monkeys with seasonal (intermittent monthly) exposures, there was no loss of effect (i.e., reversal) during the air periods. The response was strictly a function of cumulative dose.

The availability of exposure/activity data for a 9-year-old child and adult outdoor worker over an "exposure-season" of 214 days in New York City provides the opportunity to estimate an analogous PAR dose in these human individuals for comparison to the animal dose-response relationships. Because the rat exposure scenario extends over the majority of a lifetime, an attempt was made to determine the equivalent human exposure time using standard, accepted algorithms for lifetime transformations (see Table 8-12). However, the extrapolation of human biologic exposure time from that of the rat did not yield reasonable results. The primate-based extrapolation of exposure-time fared slightly better but also was less than adequate for the purposes of this exercise. Estimates of accumulated dose, however, were in general agreement (see Table 8-12) and served as the basis for the cross-species extrapolation of effects described below.

Unfortunately, there are no hard data to substantiate whether the rat or monkey better represents the human in the context of the endpoint being addressed herein. The monkey could be favored on the basis of responsiveness at low levels of O_3, corresponding to those in human spirometric tests following exposures involving exercise; however, the dose-adjusted BAL protein data are comparable for humans and rodents. The apparent differences in sensitivity between the rat and monkey may, on the other hand, reflect more rapid repair in the rat than in the primate. This concept would be consistent with the algorithms of Travis et al. (1990), which are based on intrinsic metabolic rates and thus may reasonably apply to repair processes after injury or damage from oxidant-lung surface interactions. Thus, the responses for the rat and monkey may best be considered as bracketing the human response. The linearity of the dose-response relationships in both the rat and monkey models lends credibility to interpolation (Figures 8-18A and 8-18B) of the estimated dose to the human PAR to its corresponding response. For example, in the case of the child, the predicted seasonal response could range from about a 20 to 75% increase in PAR thickness; the adult human response would be slightly less with a 15 to 70% increase. Although the actual changes in the human may not be as large as the monkey, the graphical data from both species suggest that recovery may not be complete (in fact, there was no reversal) when exposure is interrupted during alternating months. Likewise, in rats exposed long-term, reversal was incomplete (□66%; see Table 8-13) after a 4-mo postexposure period in clean air. In the case of the rat, 4 mo represents a larger proportion of its life span than a similar period in either the monkey or human, thereby suggesting that even 6 to 8 mo of background "off season" levels of ozone would not be sufficient for complete recovery in primates.

This attempt to extrapolate results of animal studies for the chronic effects of O_3 to the human obviously must be considered preliminary because of lingering questions regarding relative dosimetry across species and the uncertainties associated with episodic (typical human scenario) versus continual, repeated exposures (typical of animal studies). Yet, this extrapolation provides a foundation from which additional questions can be derived and addressed to reduce these uncertainties. The coherent evidence in hand suggests that there is a

real possibility that chronic exposure to O_3 can lead to interstitial thickening at the PAR, that region of the lung involved in chronic diseases such as chronic bronchiolitis associated with cigarette smoke or occupational fibrogenesis. The apparent lack of reversal of effects during periods of clean air raises concern that seasonal exposures have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, but the issue of dosimetry may be explanatory, in part. It would appear that the rat probably represents no less than the lower limit of response and the monkey the upper limit, if not a direct 1:1 correlate as could be speculated on the basis of relative equivalent lifetime estimates and their phylogenetic relationship.

8.6 Summary and Conclusions

8.6.1 Ozone Dosimetry

There have been significant advances in O_3 dosimetry since publication of the previous O_3 criteria document (U.S. Environmental Protection Agency, 1986) that better enable quantitative extrapolation with marked reductions in uncertainty. Prior to 1986, there were limited data on O_3 uptake in laboratory animals (Yokoyama and Frank, 1972; Miller et al., 1979), essentially no reliable data in humans (Clamann and Bancroft, 1959; Hallett, 1965), only one realistic model of O_3 dose (Miller et al., 1978, 1985), and no data on O_3 reaction kinetics in lung lining fluids. At the present time, data gaps in all of these areas have begun to fill in. Experiments and models describing the uptake efficiency and delivered dose of O_3 in the RT of animals and humans are beginning to present a clearer picture than previously has existed.

The total RT uptake efficiency of rats at rest is approximately 0.50 (Wiester et al., 1987, 1988; Hatch et al., 1989). Data from excised rat lungs support these in vivo findings, and further indicate that O_3 uptake efficiency is chemical reaction dependent (Postlethwait et al., 1994). Of the O_3 taken up by the total RT of the rat, 0.50 is removed in the head, 0.07 in the larynx/trachea, and 0.43 in the lungs (Hatch et al., 1989). The regional uptake efficiency data from the rat have been useful in estimating O_3 mass transfer coefficients for the rat.

Ozone dosimetry models require input of regional mass transfer coefficients. Limited studies have been conducted to quantitate the mass transfer coefficients of lung tissue directly using excised animal tissue. In pig and sheep tracheae, mass transfer coefficients were determined for unidirectional flow conditions and were found to be independent of flow, suggesting a lack of dependence of O_3 uptake on gas-diffusion processes (Ben-Jebria et al., 1991). These findings contrast with Aharonson et al. (1974), who found that the mass transfer coefficient in dog NP increased as a function of increasing flow.

In humans at rest, the total RT uptake efficiency is between 0.80 and 0.95 (Gerrity et al., 1988; Hu et al., 1992b; Wiester et al., 1996). At V_Ts around 500 mL, total RT uptake efficiency falls from about 0.9 to 0.75 as flow increases from 250 to 1,000 mL/s. As V_T increases, uptake efficiency increases and flow dependence lessens, suggesting that, at high V_T, uptake may be gas diffusion limited. At a V_T around 1,500 mL, total RT uptake falls from 0.96 at a flow of 250 mL/s to 0.92 at a flow of 1,000 mL/s. The studies of Gerrity et al. (1988) and Wiester et al. (1996) indicate that the mode of breathing (oral versus nasal versus

oronasal) has little effect on URT or on total RT uptake efficiency. This observation is supported by experiments comparing pulmonary function response as a function of mode of breathing (Adams et al., 1989; Hynes et al., 1988). Kabel et al. (1994), however, found that URT uptake efficiency was lower with mouthpiece breathing as compared with nasal breathing. One possible explanation of the discrepancy among the studies is that a mouthpiece may decrease URT uptake efficiency in comparison with unencumbered breathing. The enhanced physiologic response to O_3 with mouthpiece breathing, shown by Adams et al. (1989), supports this concept.

To obtain data on regional O_3 uptake efficiency in humans, Gerrity et al. (1995) measured O_3 concentrations at various anatomical sites (from the mouth to bronchus intermedius) in spontaneously breathing humans. They found that the unidirectional uptake efficiency of the human trachea was similar to that of the sheep and pig trachea (Ben-Jebria et al., 1991), suggesting a similar mass transfer coefficient behavior in the human trachea. Gerrity et al. (1995) also found that the uptake efficiencies between the mouth and various anatomical sites in the total RT agreed well with the O_3 bolus data of Hu et al. (1992). Both the Hu et al. (1992b) and Gerrity et al. (1995) data indicate that the mass transfer coefficients of the large conducting airways are larger than had been thought previously.

When all of the animal and human in vivo O_3 uptake efficiency data are compared, there is a good degree of consistency across data sets. This agreement raises the level of confidence with which these data sets can be used to support dosimetric model formulations.

In the area of mathematical model formulation, there have been several models developed since 1986. They can be grouped according to how transport and chemical reactions are modeled: instantaneous reactions or quasi-steady, first-order reactions. The models (Overton et al., 1987; Miller et al., 1988; Overton et al., 1989; Hanna et al., 1989; Grotberg et al., 1990) predict that net O_3 dose to lung lining fluid plus tissue gradually decreases distally from the trachea toward the end of the TB, and then rapidly decreases in the pulmonary region. When the theoretical dose of O_3 to lung tissue is computed, it is low in the trachea, increases to a maximum in the terminal bronchioles of the first generation of the pulmonary region, and then decreases rapidly distally into the pulmonary region. The models also provide insight into the role that increased ventilation plays in enhancing O_3-induced responses. The increased V_T and flow, associated with exercise in humans or CO_2-stimulated ventilation increases in rats, shifts O_3 dose further into the periphery of the lung, causing a disproportionate increase in distal lung dose. This prediction is supported by the data of Postlethwait et al. (1994) in excised rat lungs and of Hu et al. (1992b) and Gerrity et al. (1995) in human lungs.

Ozone dosimetry models also have enabled examination of regional dosimetry among parallel and serial anatomical structures. When asymmetric lung morphology is used in dosimetric models, the variation of O_3 dose among anatomically equivalent ventilatory units as a function of path length from the trachea has been predicted to vary as much as sixfold (Overton et al., 1989; Mercer et al., 1991; Mercer and Crapo, 1993); units with the shorter paths are expected to have the greater damage. This could have significant implications for regional or localized damage to lung tissue. Whereas the average lung dose might be at a level that would be considered insignificant, local regions of the lung may receive significantly higher than average doses and therefore be at greater risk for chronic effects.

Theoretical models also have been applied to make predictions about delivered doses from exposure scenarios that are not necessarily achievable experimentally. Overton and

Graham (1989) and Miller and Overton (1989) have scaled the human lung dimensions to account for age variations. They predicted that LRT uptake efficiency is not sensitive to age at resting ventilation, but is age dependent when exercise conditions are invoked. The total quantity of O_3 absorbed per minute is predicted to increase with age during heavy work or exercise.

8.6.2 Species Homology and Sensitivity

Examining functional parameters measured analogously in humans and various animal species discloses remarkable similarity in acute O_3-induced effects. The tachypneic response to this oxidant is clearly concentration-dependent in both humans and animals and shows parallel exacerbation when hyperventilation (e.g., exercise or CO_2) is superimposed. Indeed, rodents appear to be slightly more responsive than humans in this regard. What is not known is whether this is evidence of pulmonary irritant sensitivity, perhaps as a prelude to toxicity, or whether tachypnea is a defensive posture taken by the respiratory system to minimize distal lung O_3 deposition. Airway or lung resistance in humans is not affected appreciably by acute exposure to O_3, except under conditions of heavy exercise; animals appear to need high-level exposures or special preparations that bypass nasal scrubbing. Dynamic lung compliance, on the other hand, tends to decrease across species. However, the evidence in both animals and humans is not as strong as one might expect, given the distal lung deposition of this poorly soluble oxidant.

Ozone-induced spirometric changes, the hallmark of response in humans, also occur in exposed rats, although the relative responsiveness of these alterations in the rodent appears to be about half that of the human. It is unclear, however, the degree to which anesthesia (rat) and the comparability of hyperventilation induced by CO_2 (rat) or exercise (human) may influence this difference in responsiveness. Collectively, the acute functional response of laboratory animals to O_3 appears quite homologous to that of the human. Likewise, the studies of BAL constituents indicate that the influx of inflammatory cells and protein from the serum is influenced by species, but perhaps to a less extent than by ventilation and antioxidant status, because adjustment for these factors can modulate responses to approximate animal responses to those of humans. Unfortunately, these influential factors are rarely measured and, even less often, controlled.

When humans are exposed repeatedly for several consecutive days, lung function decrements subside, and normal spirometric parameters are regained. This phenomenon of functional attenuation also has been demonstrated in rats, not only in terms of spirometry, but also in terms of the classic tachypneic ventilatory response. Full or partial attenuation of the BAL parameters also appears to occur in both rats and humans, but exposure scenario appears to play a role; other cellular changes in animals do not attenuate. Existing epidemiologic studies provide only suggestive evidence that persistent or progressive deterioration in lung function is associated with long-term oxidant-pollutant exposure. These long-term effects are thought to be expressed in the form of maximum airflow or spirometric abnormalities, but the foundation for this conclusion remains weak and hypothetical. Animal study data, although suggesting that O_3 has effects on lung function at near-ambient levels, present a variable picture of response that may or may not relate to technical conditions of exposure or some other, yet undiscovered variable of response. Thus, a cogent interpretation of the animal findings as definitive evidence of chronic deterioration of lung function would be difficult at this time. However, the subtle functional defects apparent after 12 to 18 mo of exposure and

the detailed morphometric assessments of the O_3-induced lesions do appear consistent with the modicum of studies focusing on long-term effects in human populations. Based on the apparent homology of these responses between humans and laboratory animals, animal studies provide a means to more directly assess such chronic health concerns.

8.6.3 Quantitative Extrapolation

The agreement between theoretical models of O_3 uptake and experimental determinations of O_3 uptake efficiency now provide a basis on which responses may be examined as a function of delivered O_3 dose instead of O_3 exposure concentration. By examining responses as a function of delivered dose, the goal of quantitative extrapolation between species can be approached.

The use of delivered dose to investigate responses has been examined in two contexts: (1) intraspecies comparisons and (2) interspecies comparisons. With respect to intraspecies comparisons, Miller et al. (1988) assumed that the relevant dose mediating the human pulmonary function response was the pulmonary tissue dose. They then utilized the breathing patterns, exposure concentrations, and pulmonary function responses from the human studies of McDonnell et al. (1983) to predict the dose-response. They found that there was general agreement between the shapes of the concentration-response curves and the dose-response curves and that differences could be accounted for by the translation between exposure concentration and O_3 dose. In another example dealing with intraspecies comparisons, Miller and Conolly (1995) compared the distribution of predicted O_3 tissue dose to a ventilatory unit in a rat as a function of distance from the BADJ, with the distribution of alveolar wall thickening as a function of the same distance measure. Miller and Conolly (1995) found remarkable consistency between the predicted dose distribution and the response distribution (i.e., as predicted delivered dose declined, response declined).

In an attempt to make an interspecies comparison of dose and response, existing or modified models of Miller et al. (1985), Miller et al. (1988), and Overton et al. (1987) were used to predict doses among species for two different types of responses. In the first case, the tachypneic response to O_3 as a function of dose was analyzed. The maximum ratio of O_3-altered f to control f was plotted as a function of the average centriacinar dose over the period from the beginning of exposure to the point of maximum f ratio. Rat and human data were used for this comparison. It was found that, at comparable O_3 doses, the responses of rats greatly exceeded that of humans and were initiated at lower doses. By examining the dose response instead of the concentration response, the difference in tachypneic response between rats and humans is magnified. In another example, an analysis similar to Miller et al. (1988) was performed to examine recovered BAL protein as a function of O_3 dose to the pulmonary region. The species considered were the rat, guinea pig, rabbit, and human. In all cases, the BAL protein response followed a log-linear relationship, suggesting a consistency of response across species. Yet the data from different species tended to cluster together, suggesting species-specific sensitivity factors.

An attempt was made to address quantitatively the question of potential chronic alteration of the lungs of people exposed to O_3 by integrating dosimetry model predictions and biological effects observed in laboratory animals. In the long-term exposure studies selected for analysis, importance was placed on the relevance of exposure concentrations, the site of specificity of the morphometric analysis, and the consistency of analysis within species. Two rat (F344) studies were selected that represented near-lifetime exposures to O_3 over a range of

concentrations and scenarios (Chang et al., 1992, 1995). Two monkey studies were also considered: (1) bonnet monkeys exposed for 90 days (Harkema et al., 1993) and (2) fascicularis monkeys exposed daily for 18 mo or daily every other month for 18 mo (Tyler et al., 1988). The biological effect chosen for extrapolation was the increased thickness of the acellular and total interstitial volumes in the PAR region of the lung; measurements were made in all four of these investigations. The quantity of O_3 predicted to be absorbed per square centimeter of PAR surface area was chosen as the dose.

Generally, the information needed to carry out the dosimetry predictions was not provided by the studies. This required assumptions such as the scaling of ventilation parameters, volumes, and surface areas from one species or strain to another. The assumption that had the greatest impact on the modeling results dealt with the pulmonary region mass transfer coefficient. The value used for this parameter has very little experimental justification and was chosen to be approximately the same for all species (i.e., the values calculated by Miller et al. [1985] for humans and by Overton et al. [1987] for rats). If the value of this coefficient is in fact approximately the same for all the species, the extrapolation of effects is not expected to be affected by the value itself. For the human simulations, a 9-year-old child and an adult outdoor supervisor living in New York City were considered. The activity and exposure patterns for these hypothetical people were generated by an exposure model (Johnson, 1994) for April through October 1991. The laboratory animal dose-response curves showed an apparent linear relationship within species with relatively high correlation coefficients, from 0.80 to 0.98 depending on species and effect. Assuming the relationships depicted in Figure 8-18, the predicted dose for the hypothetical humans indicated a seasonal response for the child of a 20 to 75% increase in PAR tissue thickness and, for the adult, a 15 to 70% increase, depending on the laboratory species used for the prediction (the higher range corresponds to the monkey). For the monkeys, there seemed to be little reversal with postexposure to air, which was consistent with the cumulative dose hypothesis. Although the reader should note the number of assumptions that underlie these predictions, this exercise, nevertheless, suggests that long-term O_3 exposure could impart a chronic effect in humans.

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9

Integrative Summary of Ozone Health Effects

9.1 Introduction

Characterization of the health risks associated with pollutant exposure requires an integrative interpretation of the continuum from air quality to exposure to dose to effects, with full consideration of the actual exposure and susceptibility of different subgroups in the population. The currently published information on population exposure to ozone (O_3) (see Chapter 4) is superseded by recent analyses of the U.S. Environmental Protection Agency (EPA) in a staff paper prepared by the Office of Air Quality Planning and Standards (U.S. Environmental Protection Agency, 1996). Thus, the staff paper contains the risk assessment for O_3.

This chapter characterizes the hazard and dose-response components of risk assessment by integrating the animal toxicological, human clinical and epidemiological, and extrapolation studies of O_3 that were discussed in Chapters 6, 7, and 8, respectively, of this criteria document. Because each of these approaches has different strengths and weaknesses, they were evaluated separately in the respective base chapters; however, a combined evaluation can better describe the full array of effects that are known to occur with exposure to O_3.

The chapter begins with an overview of the relationship between exposure and dose, as this lays a foundation for inter- and intraspecies extrapolation. The chapter then is organized according to biological outcomes, beginning with the effects of short- and long-term exposures of O_3 alone or in ambient air and ending with experimental exposures to binary mixtures with O_3. The section on short-term exposures (i.e., <8 h) presents descriptively symptoms and effects on lung function, exacerbation of existing disease, and cellular-biochemical responses. Quantitative exposure-response relationships for the effects of O_3 on pulmonary function (e.g., changes in lung volume) are summarized separately because the large number of studies allows more complex evaluation and modeling. For the other classes of effects, the more limited exposure-response information is integrated with the description of the effects. The section on long-term exposures encompasses repeated exposures (i.e., 1 to 5 days), prolonged exposures (i.e., months), and genotoxicity and carcinogenicity. Because the available database on binary exposure studies has little predictive value of effects in populations exposed to complex pollutant mixtures, the emphasis is placed on the principles of interaction. The conclusions section is organized according to key questions about the health consequences of O_3 exposure and the population groups that are most likely to be affected.

Because this chapter integrates the results of a large number of studies from the current and earlier O_3 criteria documents (U.S. Environmental Protection Agency, 1978, 1986), it is not practical to provide experimental details or cite specific references. Rather, emphasis is given to main findings that are supported by published, confirmatory studies, unless noted otherwise. Comprehensive details and references are provided in the base chapters (Chapters 6 through 8). A discussion of selective, key references can be found in the summary and conclusion sections of those chapters.

9.2 Exposure-Dose Relationships

Qualitative and quantitative health assessments require, among other things, the ability to relate exposure to dose and dose to effect. In the case of O_3 health assessment, this ability is necessary for two major reasons: (1) to develop unified predictive models of human population responses based on exposure, and (2) to enable extrapolation from animals to humans for chronic effects. Physically and biologically based models of dose simplify the methods of predicting population responses and, in turn, significantly reduce the uncertainty of these predictions. For animal-to-human extrapolations, splitting the problem of exposure and response into an exposure-dose problem and a dose-response problem separates the issue of interspecies sensitivity from purely dosimetric considerations. Responses in animals may be homologous with responses in humans but follow different dose-response curves. By measuring or computing delivered O_3 dose to relevant tissues in animals and humans, transfer functions can, in principle, be developed relating dose-response curves among different species, assuming tissues from different species react in identical fashion. This section discusses the understanding of exposure-dose relationships and how they improve the ability to interpret and predict O_3 responses.

Historically, the first step beyond describing responses solely in terms of exposure concentration was the use of the product of concentration × time × minute ventilation ($C \times T \times \dot{V}_E$), yielding what often has been referred to as an "effective dose". Response modeling has examined the interaction of individual pairs of variables. However, no single model has been able to simply unify any response in terms of the product of $C \times T \times \dot{V}_E$. This is due to the fact that $C \times T \times \dot{V}_E$ is a metric of exposure dose rather than delivered dose and, furthermore, does not account for the mediation of responses in localized regions of the lung that would be responding to local O_3 doses. Advances in O_3 dosimetry modeling and experimental determinations of regional O_3 dose in animals and humans have enabled extensions beyond simple $C \times T \times \dot{V}_E$ modeling to interpret responses.

Ozone dosimetry models provide predictions of the dose distribution of O_3 in the respiratory tract from the trachea to the alveolar spaces of the lung. These models utilize the best available anatomical, physiological, and biochemical data available for animals and humans. These data are incorporated into mathematical formulations of convection, diffusion, and chemical reaction processes in the lung. The models predict that, under resting ventilatory conditions, the O_3 dose per airway generation to all respiratory tract constituents (tissue plus fluid) slowly decreases from the trachea to the terminal and respiratory bronchioles and then declines in the alveolated generations. When dose of O_3 to tissue alone is considered (accounting for reaction and diffusion kinetics in the liquid lining layer), there is a three order of magnitude increase in tissue dose from the trachea to the proximal alveolar regions (PARs), after which the tissue and total dose are virtually equal and fall rapidly in the

alveolated generations. Currently, relationships between delivered regional dose and response are derived assuming that O_3 is the active agent directly responsible for effects; however, there is uncertainty as to the correctness of this assumption. Reactive intermediates, such as peroxides and aldehydes formed when O_3 interacts with constituents of lung lining fluid, may be the agents mediating responses. Thus, the dose of the reactive intermediates may be relevant. Even in the presence of uncertainty over the relevant dose agent, the histopathological findings from chronic O_3 exposures in animals match the predicted distribution of O_3 dose (i.e., the sites of the highest predicted O_3 doses correspond with those regions of the lung with the greatest tissue alterations).

Experimental studies in humans have revealed some important features needed for health assessment. Among these is the observation that the dose of O_3 delivered to the lower respiratory tract is independent of the mode of breathing (i.e., oral versus nasal versus oronasal). This observation simplifies health assessment by eliminating the need for precise information on modes of breathing when considering population responses. Experimental studies in humans also have shown that increasing $\dot{V}_E$ with exercise (increasing both breathing frequency and tidal volume) causes only a small decrease in O_3 uptake efficiency by the total respiratory tract. Based on models of O_3 dose, it appears that the increased $\dot{V}_E$ in exercise, although having little effect on uptake efficiency by the total respiratory tract, causes the distribution of delivered O_3 dose to shift deeper into the respiratory tract. The shift in O_3 dose as a function of $\dot{V}_E$ could help explain the complex relationships seen between response and C, T, and $\dot{V}_E$. An important observation from the human experimental dosimetry studies is the general agreement between O_3 dosimetry models and the measured data.

Experiments in laboratory animals (particularly rats) have been valuable in providing, in conjunction with human experimental data and mathematical dosimetry models, the basis for dosimetric extrapolation. Whereas the human total respiratory tract has an O_3 uptake efficiency between 70 and 100%, the respiratory tract of the rat takes up only about 50% of the inhaled O_3. Unlike the case with humans, the dosimetry models overestimate the uptake efficiency of the rat respiratory tract by approximately 25 to 50% (i.e., the predicted uptake efficiency is between 65 and 75%), but the models are still highly valuable for extrapolation purposes. An important finding has been that the models correctly relate the regional dose of O_3 to the increase in alveolar wall thickness, both of which decline with distance from the junction of the conducting airways and the alveolar regions of the lung.

Experimental O_3 dosimetry and predictive O_3 dosimetry models are informative about the feasibility of extrapolating animal responses to humans. Some acute responses to O_3 can be compared across species on a strict dose-response basis. For example, both animals and humans respond to O_3 in a dose-dependent manner by increasing breathing frequency and decreasing tidal volume (tachypnea). A qualitative comparison between rat and human tachypneic responses at a variety of O_3 concentrations and exercise levels indicates that when exercising, rats and humans have a similar response, but rats are somewhat more responsive at rest. However, when dose to the proximal alveolar region of the lung (normalized to body weight) is considered as the dose metric for tachypneic responses, rats appear to be much more responsive than humans. Another example is influx of protein into the alveolar spaces following O_3 exposure as measured in bronchoalveolar lavage (BAL) fluid. When BAL protein is plotted as a function of pulmonary tissue dose, the rat, guinea pig, rabbit, and human all respond with a similar dose-response pattern, suggesting a common mechanism of response. However, each curve is offset from the other, reflecting overall

sensitivity differences among the species, with the human and guinea pig being more responsive than the rat and rabbit.

Available data on chronic responses to O_3 are considerably more difficult to compare across species. Specific assumptions are required to model exposure-dose relationships. For example, allometric anatomic adjustments provide estimates of unavailable dosimetric data for nonhuman primates, and allometric equivalent life-span estimates better relate the duration of exposure to life-span and cumulative dose. Studies of long-term exposure in monkeys and rats show a near-linear dose-response pattern when alveolar interstitial thickness was related to cumulative dose estimates for the PAR of the lung. Analogous estimates of PAR dose in humans predict similar increases in interstitial thickness at the PAR, with the monkey being more responsive, and the rat less responsive.

9.3 Effects of Short-Term Ozone Exposures

9.3.1 Physiological Responses to Ozone Exposure

Typical acute physiological responses to O_3 exposure observed in both human clinical and field studies include a reduction in forced vital capacity (FVC), decreased expiratory flow rates, and increased respiratory symptoms. The most common symptoms include cough, airway irritation, and chest discomfort associated with deep inhalation. These responses are often accompanied by increased airway resistance and tachypnea. The voluntary spirometry and symptom responses cannot be elicited from animals, but their tachypneic response is well documented. Ozone exposure also increases airway responsiveness to nonallergenic airway stimuli (e.g., histamine) in humans and animals. There is a large range of physiological responses among humans, with at least a 10-fold difference between the most and least responsive individuals.

9.3.1.1 Respiratory Symptom Responses

An association between O_3 exposure and the presence of symptoms has been shown in human clinical, field, and epidemiological studies. Prevalent symptoms include cough, irritation of the airways (described as a scratchy throat or discomfort under the sternum), and discomfort when taking a deep breath (described as chest tightness or pain in the chest). Eye irritation, sometimes reported as a symptom in field or epidemiological studies with exposure to oxidant mixtures including peroxyacyl nitrates, is not associated with exposure to O_3 alone. The most prevalent respiratory symptoms have a much higher incidence in young adults than in older adults and generally are not reported in children or adolescents. Asthmatics have symptoms similar to nonasthmatics but also report a higher incidence of wheezing. The receptors responsible for cough may be unmyelinated C-fibers or rapidly adapting receptors located in the larynx and the largest conducting airways. Thus, there appears to be a potential mechanistic linkage between coughing and changes in spirometry. Field and epidemiological studies also indicate an association between hourly or daily ambient O_3 levels and the presence of respiratory symptoms, particularly cough. Such associations may be most evident in asthmatic children. Although symptoms cannot be elicited from animals, indirect measures of symptom responses in animals include behavioral responses (e.g., decreased wheel-running activity, decreased activity associated with obtaining food) indicative of aversion to O_3 exposure.

Symptom responses to O_3 exposure follow a monotonic exposure-response relationship that has a similar form to that for spirometry responses. Increasing exposure levels elicit increasingly more severe symptoms that persist for longer periods. Symptom and spirometry responses follow a similar time course during an acute exposure and the subsequent recovery, as well as over the course of several days in a repeated exposure study. Furthermore, medication interventions that block or reduce spirometry responses have a similar effect on symptom responses. Levels at which symptoms occur under various exposure conditions are discussed in Section 9.3.4.2. As with spirometry responses, symptom responses vary considerably among subjects, although the individual correlations between spirometry and symptom responses are relatively low. Ozone induces interference with exercise performance, either by reducing maximal sustainable levels of activity or by reducing the duration of activity that can be tolerated at a particular work level; this is likely related to symptoms. In several heavy or severe exercise studies of athletes exposed to O_3, the discomfort associated with the respiratory symptoms caused by O_3 concentrations in excess of 0.18 ppm was of sufficient severity that the athletes reported that they would have been unable to perform maximally if the conditions of the exposure were present during athletic competition. In workers or active people exposed to ambient O_3, respiratory symptoms may cause reduced productivity or may curb the desire to pursue certain leisure activities.

9.3.1.2 Lung Function Responses

Epidemiological, field, and chamber studies have demonstrated that acute exposure to O_3 decreases FVC and forced expiratory volume in 1 s (FEV_1). In humans, O_3 exposure reduces FVC primarily by decreasing inspiratory capacity. This is believed to be the result of neurogenic inhibition of maximal inspiration, possibly caused by stimulation of C-fiber afferents, either directly or from O_3-induced products of inflammation. C-fibers are also thought to be the receptors responsible for the cough reflex in humans. After exposure to O_3, coughing frequently is elicited during the deep inspiration prior to the forced expiratory maneuver used in dynamic spirometry tests such as FVC, FEV_1, and forced expiratory flow at 25 to 75% of FVC. The observation that nonsteroidal anti-inflammatory drugs (e.g., indomethacin, ibuprofen) reduce or block spirometric responses to O_3 exposure and reduce levels of prostaglandin E_2 (PGE_2) within the lung suggests that mediators released by damaged epithelial cells and alveolar macrophages may play a role in the inhibition of maximal inspiration. Although it seems clear that the reduction in total lung capacity is not attributable to reduced static compliance (i.e., a stiffer lung) or inspiratory muscle weakness, other mechanisms may be involved. Increased interstitial fluid in patients with heart disease causes a decrease in vital capacity and frequency-dependent decreases in lung compliance. The O_3-induced tachypneic response, seen in many animal species and in exercising humans, may be related to the decrease in vital capacity. In humans, the pulmonary reflexes that inhibit maximal inspiration may also limit tidal volume during exercise, which leads to a compensatory tachypnea. The tachypneic response in humans may not be entirely involuntary because it has been reported that O_3-exposed subjects may consciously modify their breathing pattern to relieve discomfort.

The time course of the spirometry responses to O_3 exposure depends on the exposure conditions. At low levels of exposure (e.g., light exercise and O_3 concentration <0.18 ppm), responses are induced slowly and progressively and they may or may not reach a plateau of response, depending on the duration of the exposure. At higher levels of exposure (e.g., very heavy exercise and O_3 concentration >0.25 ppm), responses occur rapidly (within

15 min), and the largest portion of the response tends to occur early in exposure, indicative of a plateau of response that typically is not achieved because of termination of the exposure within 1 to 2 h. The quantitative exposure-response relationships are discussed more extensively in Section 9.3.4.

In both chamber and field studies, the responses of healthy children to acute O_3 exposure are similar to those seen in adults. Responses of children and adolescents exposed to ambient O_3 (and other copollutants) at summer camps in at least six different locations in the northeastern United States, southeastern Canada, and Southern California indicate changes in spirometry similar to those found in individuals exposed to O_3 under controlled experimental conditions. There is a substantial range of response among individuals in camp studies and between various locations; however, the average FEV_1 was lower when ambient O_3 was higher. Although direct comparisons cannot be made because of incompatible differences in experimental design and analytical approach, this range of response is comparable to the range of response seen in chamber studies at low O_3 concentrations. In the "camp studies", a key measurement is the slope of the relationship between FEV_1 and the measured O_3 concentration during the previous hour, without consideration of the background O_3 levels (even though exposures occurred over multiple hours). The average slope from six studies was −0.50 mL/ppb within an O_3 concentration range of 0.01 to 0.16 ppm (see Chapter 7). For an exposure to 0.12 ppm O_3, this corresponds to a decrease in FEV_1 of 60 mL from a base level of approximately 2,000 to 2,500 mL, or a 2.4 to 3.0% decrease in FEV_1. This is comparable to the findings of McDonnell et al. (1985) for 8- to 11-year-old boys who experienced a 3.4% decrease in FEV_1 after being exposed to 0.12 ppm O_3 for 2 h. Recent studies in adults performing outdoor exercise also have shown an association between decreased spirometric responses and increased ambient O_3 levels.

A consistent finding across many animal species is that O_3 causes rapid shallow breathing (O_3-induced tachypnea), which, in humans, may be related to a sensation of discomfort associated with taking large tidal breaths. Of particular interest for comparing interspecies responses is that the responses of rats and guinea pigs fall within the same range as seen for humans from rest to heavy exercise.

Common pulmonary function tests do not measure changes in the small airways of the centriacinar region of the lung (that segment between the last conducting airway and the gas exchange region), which is highly susceptible to damage by O_3 and is the site of epithelial cell necrosis and remodeling of respiratory bronchioles. Numerous pulmonary function tests reputed to measure responses in small airways (e.g., closing volume, aerosol bolus) have been used in O_3 studies. Responses have been demonstrated, but it is not clear that these tests correlate with the morphological lesion observed in the small airways of experimental animals (see Section 9.4), which is predicted to occur in humans but has not been confirmed reliably through comparable morphologic data from humans residing in O_3-polluted areas. Even if small airways disease is demonstrated in humans, there is as yet no compelling evidence that it will progress to chronic lung disease.

An increase in airway resistance is an indication of the response of large airways to O_3 exposure and is mechanistically different from lung volume responses. Also, higher O_3 concentrations are required to change airway resistance compared to FEV_1. Changes in specific airway resistance (SR_{aw}) of healthy subjects following O_3 exposure are small relative to those seen in asthmatics with an inhalation exposure to a bronchoconstricting drug (methacholine), a specific antigen, or sulfur dioxide (SO_2). In rats exposed to O_3, changes in

resistance also tend to be small. The observation that changes in airway resistance are modest clearly indicates that reductions in maximum expiratory flow are not caused primarily by narrowing of large airways. The increase in airway resistance appears to be vagally mediated because it is sensitive to inhibition by atropine.

9.3.1.3 Changes in Airway Responsiveness

Ozone exposure also causes increased responsiveness of the pulmonary airways to subsequent challenge with bronchoconstrictor drugs such as histamine or methacholine. This phenomenon is seen even after recovery from spirometric changes, but it typically is no longer present after 24 h. Although changes in airway responsiveness tend to resolve somewhat more slowly and appear to be less likely to be attenuated with repeated exposure, the evidence for a persistent increase in responsiveness from animal studies is inconsistent. Changes in airway responsiveness in rats and guinea pigs tend to occur at higher O_3 concentrations and, as in humans, tend to be most pronounced shortly after the exposure and less so 24 h postexposure. Changes in airway responsiveness appear to occur independently of changes in pulmonary function. This response may not be due to the presence of polymorphonuclear leukocytes (PMNs) in the airway or to the release of arachidonic acid metabolites, but could possibly be due to epithelial damage and the consequent increased access of these chemicals to smooth muscle in the airways or to the receptors in the airways responsible for reflex bronchoconstriction. The clinical relevance of this observation is that, after O_3 exposure, human airways may be more susceptible to a variety of stimuli, including antigens, chemicals, and particles. One animal study has demonstrated decreased antigen-induced bronchoconstriction after O_3 exposure, and a human study in allergic asthmatics is suggestive of an increase in such a response. An increased response to inhalation of a specific antigen to which a human is sensitized is a plausible outcome of O_3 exposure. However, ongoing studies of this phenomenon will need to be evaluated in order to determine the exposure-response relationship for alterations in responses to inhaled antigens, especially with regard to sensitive asthmatics. Enhanced response to antigens in asthmatics could lead to increased morbidity (i.e., medical treatment, emergency room visits, hospital admissions) or to more persistent alterations in airway responsiveness.

9.3.2 Exacerbation of Respiratory Disease

People with preexisting pulmonary disease may be at increased risk from O_3 exposure. Because of their existing functional limitations, any further decrease in function would lead to a greater overall functional decline. Furthermore, some individuals with pulmonary disease may have an inherently greater sensitivity to O_3. Asthmatics, by definition, have inherently greater bronchial responsiveness, but, depending on the severity of their disease and its clinical status, their FEV_1 can be within the normal range (100 ± 20% predicted) or may be less than 50% predicted. Patients with chronic obstructive pulmonary disease (COPD) can have FEVs ranging from 30 to 80% of predicted, again depending on disease severity. Because of their depressed functional state, small absolute changes in lung function have a larger relative impact. For example, a 500-mL FEV_1 decrease in a healthy young man with an FEV_1 of 4,000 mL causes only a 12% decline. In a 55-year-old COPD patient with an FEV_1 that is 50% of predicted, or about 1,670 mL, a 500-mL decline in FEV_1 would result in a 30% decline in FEV_1. Asthmatics with depressed baseline function would have similarly magnified relative responses and, because of increased bronchial

responsiveness, may also experience larger changes in airway resistance. Evaluating the intersection of risk factors and exposures is more complex. For example, an individual with more severe lung disease is unable to exercise heavily and thus would be less likely to encounter an effective exposure.

About 10 million people in the United States (4% of the population) are estimated to have asthma (National Institutes of Health, 1991). The prevalence is higher among African Americans, older (8- to 11-year-old) children, and urban residents (Schwartz et al., 1990). Death due to asthma is an infrequent event; on an annual basis, about one death occurs per 10,000 asthmatic individuals. Mortality rates are higher among males and are at least 100% higher among nonwhites. In two large urban centers (New York and Chicago), mortality rates from asthma among nonwhites may exceed the city average by up to fivefold (Sly, 1988; Evans et al., 1987; National Institutes of Health, 1991; Weiss and Wagener, 1990; Carr et al., 1992). Although some innercity areas may have lower O_3 concentrations than some suburban areas, the concentrations are much higher than those in most rural areas. The impact of ambient O_3 on asthma morbidity and mortality in this apparently susceptible population is not well understood. The few epidemiological studies are subject to confounding factors and have rarely focused on innercity nonwhite asthmatics. Furthermore, controlled human exposure studies of asthmatics typically include mild to moderate asthmatics and have not dealt specifically with nonwhite asthmatics.

A number of epidemiological studies have shown a consistent relationship between ambient oxidant exposure and acute respiratory morbidity in the population. Small decreases in forced expiratory volumes and increased respiratory symptoms, including exacerbation of asthma, occur with increasing ambient O_3, especially in children. Modifying factors, such as ambient temperature, aeroallergens, and other copollutants (e.g., particles) also can contribute to this relationship. Ozone air pollution can account for a portion of summertime hospital admissions and emergency department visits for respiratory causes; studies conducted in various locations in the eastern United States and Canada consistently have shown a relationship with increased incidence of visits and admissions, even after controlling for modifying factors, as well as when considering only concentrations <0.12 ppm O_3. It has been estimated from these studies that O_3 may account for roughly one to three excess summertime respiratory hospital admissions per hundred parts per billion O_3, per million persons.

The association between elevated ambient O_3 concentrations during the summer months and increased hospital visits and admissions has a plausible biologic basis in the physiologic, symptomatic, and field study evidence discussed earlier. Specifically, increased airway resistance, airway permeability, and incidence of asthma attacks and airway inflammation suggest that ambient O_3 exposure could be a cause of the increased hospital admissions, particularly for asthmatics.

The associations found in the epidemiological studies are supported by chamber studies. Asthmatics and nonasthmatics have qualitatively similar responses to chamber O_3 exposures. Although symptom and volume-related responses (i.e., decreased FVC) tend to be similar, airway resistance increases relatively more, from an already higher baseline, in asthmatics exposed to O_3. Ozone-induced alterations in responsiveness to bronchoconstrictor drugs show similar changes in asthmatics and nonasthmatics. There is no evidence at this time that O_3 induces a persistent increase in airway responsiveness or that O_3-exposed asthmatics are more likely to have a late-phase response to specific antigen challenge. Symptom responses also have been reported in asthmatics exposed to O_3. In contrast to

nonasthmatics, wheezing, a typical finding in asthma, is a prevalent symptom in addition to the cough, chest tightness, and shortness of breath that are reported by subjects without asthma.

9.3.3 Morphological and Biochemical Abnormalities

9.3.3.1 Inflammation and Cell Damage

Ozone-induced cell injury may lead to effects including inflammation, altered permeability of the epithelial barrier, impaired host defense and particle clearance, irreversible structural alterations in the lung, exacerbation of preexisting disease (e.g., asthma), and increased sensitivity to biocontaminants (e.g., allergens). Of these, O_3-induced inflammation of the respiratory tract has been best documented and occurs in all species that have been studied. The mechanisms leading to the observed inflammatory responses induced by O_3 are just beginning to be understood. Both animal morphological studies and in vitro studies indicate that airway ciliated epithelial cells and Type 1 cells are the most O_3-sensitive cells and are initial targets of O_3. These cells are damaged by O_3 and produce a number of proinflammatory mediators (e.g., interleukins [IL-6, IL-8], PGE_2) capable of initiating a cascade of events leading to PMN influx into the lung, activation of alveolar macrophages, inflammation, and increased permeability across the epithelial barrier.

Ozone-Induced Inflammation

Ozone causes inflammatory changes throughout the respiratory tract, including the nose. Humans and laboratory animals exposed to O_3 develop inflammation and increased permeability in the nasal passages. A recent study reported a positive correlation between nasal inflammation in children and measured ambient O_3 concentrations. Studies with rats suggest a potential competing mechanism between the nose and lung, with inflammation occurring preferentially in the nose at low O_3 concentrations and shifting to the lung at higher concentrations. It is unclear if this represents a specialization restricted to rats or is a more general phenomenon.

In general, inflammation can be considered as the host response to injury and the induction of inflammation as evidence that injury has occurred. Inflammation induced by exposure of humans to O_3 can have several potential outcomes: (1) inflammation induced by a single exposure (or several exposures over the course of a summer) can resolve entirely; (2) continued acute inflammation can evolve into a chronic inflammatory state; (3) continued inflammation can alter the structure and function of other pulmonary tissue, leading to diseases such as fibrosis; (4) inflammation can alter the body's host defense response to inhaled microorganisms, particularly in potentially vulnerable populations such as the very young and old; and (5) inflammation can alter the lung's response to other agents such as allergens or toxins. Except for outcome (1), the possible chronic responses have not been identified with inflammation induced by exposure of humans to O_3. It is also possible that the profile of response can be altered in persons with preexisting pulmonary disease (e.g., asthma, COPD) or smokers.

The recent use of BAL as a research tool in humans has afforded the opportunity to sample cells and fluid from the lung and lower airways of humans exposed to O_3 and to ascertain the extent and course of inflammation and its constitutive elements. Several studies have shown that humans exposed acutely (1 to 3 h) to 0.2 to 0.6 ppm O_3 had O_3-induced inflammation, cell damage, and altered permeability of epithelial cells lining the respiratory

tract (allowing components from plasma to enter the lung). For individuals acutely exposed to 0.4 to 0.6 ppm O_3, PMNs (the hallmark cells of inflammation) make up 8 to 10% of the recovered BAL cells. This represents a five- to eightfold increase in PMNs compared with similar individuals exposed to clean air, who typically have 1 to 2% PMNs in their BAL fluid. The lowest concentration of O_3 tested, 0.08 ppm for 6.6 h with moderate exercise, also induced small but statistically significant increases in a number of inflammatory mediators, including PMNs.

The percent of PMNs in BAL fluid taken from individuals exposed to 0.4 ppm O_3 for 2 h equals or exceeds those found in individuals exposed to other environmental toxicants, such as asbestos or silica, or in individuals with idiopathic pulmonary fibrosis (IPF) or connective tissue disorders (CTD) (Cherniak et al., 1990). For example, individuals with a history of occupational exposure to asbestos (>10 years) have 3.3 ± 1.3% BAL PMNs, and individuals with a history of occupational exposure to silica (>2 years) have 1.4 ± 0.4% PMNs. Untreated patients newly diagnosed with IPF have 6.7 ± 2.5% PMNs, whereas those with CTD have 16 ± 11.6% PMNs. Baseline levels of PMNs in patients with asthma do not differ significantly from healthy individuals, although PMN levels can increase following allergen bronchoprovocation (Smith and Deshazo, 1993). In contrast, PMNs can make up as much as 80% of BAL cells in patients with acute bacterial infections (Stanley, 1991).

Short-term (<8 h) exposure of animals to O_3 also results in cell damage, inflammation, and altered permeability, although, in general, higher O_3 concentrations are required to elicit a response equivalent to that of humans. Because humans were exposed to O_3 while exercising and most animal studies were done at rest, differences in ventilation likely play a significant role in the different response of humans and rodents to the same O_3 concentration. Studies in which animals were exposed at night (during their active period) or in which ventilation was increased with CO_2 tend to support this idea.

Studies utilizing BAL techniques sample only free or loosely adherent cells in the lung; thus, it is possible that cellular changes have occurred in the interstitium that are not reflected in BAL studies, or that BAL changes exist in the absence of interstitial changes. However, morphometric analyses of inflammatory cells present in lung and airway tissue sections of animals exposed to O_3 are in general agreement with BAL studies. Short-term O_3 exposure (<8 h) causes similar types of alterations in lung morphology in all laboratory animal species studied. The most affected cells are the ciliated epithelial cells of the airways and Type 1 cells in the alveolar region. The centriacinar region (the junction of the conducting airways and gas exchange region) is a primary target, possibly because it receives the greatest dose of O_3 delivered to the lower respiratory tract. Sloughing of ciliated epithelial and Type 1 cells occurs within 2 to 4 h of exposure of rats to 0.5 ppm O_3.

Time Course of Ozone-Induced Inflammatory Response

Findings from human and animal studies agree that the O_3-induced inflammatory response occurs rapidly and persists for at least 24 h. Increased levels of PMNs and protein are observed in the BAL fluid within 1 h following a 2-h exposure of humans to O_3 and continue for at least 20 h. The kinetics of response during this time have not been well studied in humans, although a single study shows that PMN levels are higher at 6 h postexposure than at 1 or 20 h in different individuals. Several animal studies suggest that BAL PMN and protein levels peak 12 to 16 h after an acute O_3 exposure and begin to decline by 24 h, although some studies report detectable BAL PMNs even 36 h after exposure. It is also clear that in humans the pattern of response differs for different

inflammatory mediators. Mediators of acute inflammation, such as IL-6 and PGE_2, are more elevated immediately after exposure; whereas mediators that potentially could play a role in resolving inflammation, such as fibronectin and plasminogen activator, are preferentially elevated 18 h after exposure. The rapidity with which cellular and biochemical mediators are induced by O_3 makes it conceivable that some of them may play a role in O_3-induced changes in lung function—indeed there is some evidence that BAL PGE_2 levels are correlated with decrements in FEV_1, and anti-inflammatory medications that block PGE_2 production also reduce or block the spirometric responses to O_3. Although earlier studies suggested that O_3-induced PMN influx might contribute to the observed increase in airway hyperreactivity, animal studies show that when PMNs are prevented from entering the lung, O_3-induced hyperreactivity or increases in many inflammatory mediators still occur. In addition, studies in which anti-inflammatory drugs are used to block O_3-induced lung function decrements still show increases in PMNs and most other inflammatory mediators (although PGE_2 is not increased).

Individuals and Populations Susceptible to Ozone

To date, there have been no studies that have examined the cellular/biochemical response to O_3 of potentially susceptible subpopulations, such as asthmatics, nor are there any data in humans addressing whether age, gender, or racial differences can modify the inflammatory response to O_3. Increased susceptibility of asthmatics or chronic bronchitics could be hypothesized on the basis that they have an underlying inflammatory disease that may be exacerbated with an otherwise small magnitude of change. Inflammation is not induced to the same extent in all individuals. In moderately exercising humans exposed to 0.08 ppm O_3 for 6.6 h, the mean changes in inflammatory indices were low, but some individuals had increases comparable to those reported in heavily exercising subjects exposed to 0.4 ppm O_3 for 2 h, suggesting that some segments of the population may be more responsive to low levels of O_3. It has not yet been studied whether intersubject differences in inflammatory response to O_3 are reproducible over time for the same subject, as has been shown for intersubject differences in lung function. There seems to be no strong correlation between the various mediators of inflammation, cell damage, and permeability (i.e., those individuals with the greatest PMN response are not necessarily those with the greatest BAL protein, PGE_2, or IL-6 response). Furthermore, the magnitude of lung function decrements and respiratory symptoms has not yet been shown to be correlated with mediators of inflammation, with the possible exception of PGE_2.

Animal studies also show large interspecies and interstrain differences in response to O_3 and suggest that genetic factors may play a role in susceptibility to O_3. Different rat strains respond to O_3 differently; for example, Wistar rats have the greatest PMN influx, whereas Fischer rats demonstrate the most epithelial cell damage. In addition, limited data suggest that dietary antioxidant levels may affect the response of rodents to O_3 and that very young rats produce more PGE_2 in response to O_3 than do older rats. Taken as a whole, the human and animal studies suggest that the inflammatory response to O_3 is complex and that determinants of susceptibility may occur at several different genetic loci.

9.3.3.2 Host Defense

The mammalian respiratory tract has a number of closely integrated defense mechanisms that, when functioning normally, provide protection from the adverse effects of a wide variety of inhaled particles and microbes. Impaired mucociliary clearance can result in unwanted accumulation of cellular secretions and increased numbers of particles and microorganisms in the lung, leading to increased infections and bronchitis.

Mucociliary Clearance of Inhaled Particles

Animal studies show that clearance of inhaled insoluble particles is slowed after acute exposure to O_3. Ozone-induced damage to cilia and increased mucus secretion likely contribute to a slowing of mucociliary transport rates. Interestingly, retarded mucociliary clearance is not observed in animals exposed repeatedly to O_3. The effects of O_3 on mucociliary clearance in humans have not been well studied, and the results are somewhat conflicting; one study reports an O_3-induced increase in particle clearance in subjects exposed to 0.4 ppm O_3 for 2 h, and another study reports no O_3-induced change in particle clearance with a similar exposure regimen.

Alveolar Macrophage Function

Macrophages represent the first line of defense against inhaled microorganisms and particles that reach the lower airways and alveoli. Studies in both humans and animals have shown that there is an immediate decrease in the number of BAL macrophages following O_3 exposure. Alveolar macrophages also have been shown to be crucial to the clearance of certain gram-positive bacteria from the lung. Several studies in both humans and laboratory animals also have shown that O_3 impairs the phagocytic capacity of alveolar macrophages, and some studies suggest that mice may be more impaired than rats. The production of superoxide anion (an oxygen radical used in bacterial killing) by alveolar macrophages also is depressed in both humans and animals exposed to O_3, and the ability of alveolar macrophages to kill bacteria directly is impaired. Decrements in alveolar macrophage function have been observed in moderately exercising humans exposed to the lowest concentration tested, 0.08 ppm O_3 for 6.6 h.

Interaction with Infectious Agents

Concern about the effect of O_3 on susceptibility to respiratory infection derives primarily from animal studies in which O_3-exposed mice die following a subsequent challenge with aerosolized bacteria. Increased mortality of experimental laboratory animals has been shown to be concentration-dependent, and exposure to as little as 0.08 ppm O_3 for 3 h can increase mortality of mice to a subsequent challenge with streptococcus bacteria. In addition, younger mice are more susceptible to infection than older mice; this has been related to increased PGE_2 production in these animals, which likely decreases alveolar macrophage activity.

It has been suggested that impaired alveolar macrophage function is the mechanism likely responsible for enhanced susceptibility to bacteria. However, mortality is not observed with other rodent species, raising the question of whether this phenomenon is restricted to mice. Although both mice and rats show impaired macrophage killing of inhaled bacteria following O_3 exposure, rats mount a faster PMN response to O_3 to compensate for the deficit in alveolar macrophage function. The slower clearance time in mice allows the streptococcus strain to persist in lung tissue and, subsequently, to elaborate a number of virulence factors

that evade secondary host defense and lead to bacterial multiplication and death of the host. Although increased mortality in laboratory animals is not directly relevant to humans, laboratory animals and humans share many host defense mechanisms being measured by mortality in the mouse model. Thus, the category of effect (i.e., decrement in antibacterial defenses) can be qualitatively extrapolated to humans.

There is no compelling evidence from animal toxicological, human clinical, or epidemiological studies that O_3 increases the incidence of respiratory viral infection in humans. A study of experimental rhinovirus infection in susceptible volunteers failed to show any effect of 5 consecutive days of O_3 exposure (0.3 ppm, 8 h/day) on the clinical picture or on host response. Studies in which O_3-exposed mice were challenged with influenza virus have conflicting results: some studies show increased mortality, some show decreased mortality, and still others show no change at all. However, even when increased mortality was demonstrated, there was no difference in viral titers in the lung, suggesting virus-specific immune functions were not altered. One animal study found that, although subchronic O_3 exposure did not affect the acute course of a viral infection, it did enhance postinfluenzal alveolitis.

Taken as a whole, the data clearly indicate that an acute O_3 exposure impairs the host defense capability of both humans and animals, primarily by depressing alveolar macrophage function and perhaps also by decreasing mucociliary clearance of inhaled particles and microorganisms. This suggests that humans exposed to O_3 could be predisposed to bacterial infections in the lower respiratory tract. The seriousness of such infections may depend on how quickly bacteria develop virulence factors and how rapidly PMNs are mobilized to compensate for the deficit in alveolar macrophage function.

Ozone also has been reported to suppress natural killer cell activity in the lung, to suppress proliferative responses to bacterial antigen (Listeria) in both spleen and bronchial lymph nodes, and to induce delayed hypersensitivity responses to Listeria antigen. However, these effects occur at higher exposure levels (0.75 to 1.0 ppm O_3) than those that affect macrophage function.

9.3.4 Quantitative Ozone Exposure-Response Relationships

A quantitative understanding of the relationship between O_3 exposure and subsequent response is useful both for a better understanding of the processes underlying outcomes of interest and for purposes of prediction. Examples of the utility of the latter include identification of exposures unlikely to produce effects, risk and benefits assessment, and prediction of responses based on exposures for which empirical data do not exist. In general, exposure-response relationships have been better characterized for populations than for individuals, and, although the form of the relationships may differ, those for individuals are likely to be qualitatively similar to those of populations. On the other hand, because of large differences in responsiveness among individuals, exposure-response relationships for the population may not reflect quantitatively the experience of a given individual.

Relationships between short-term exposure and acute response have been described for lung function changes, induction of symptoms, and BAL outcomes in experimental exposure studies and for lung function, symptoms, hospital and emergency room admissions, and mortality in epidemiologic studies. Exposure in the experimental studies can be defined in terms of concentration, dose rate of exposure, total inhaled dose, and dose at the active site. The limiting factor in modeling exposure-response with experimental data has been that

no single study has included a wide enough range of the three exposure variables of interest (i.e., C, $\dot{V}_E$, T) to choose between models or to identify the appropriate method of describing exposure.

Exposure-response models in the epidemiologic studies generally have included only O_3 concentration measured at a central monitoring site in the study area as the exposure variable. With some exceptions, characterization of exposure-response relationships in these studies has been limited by little information on activity level or duration of exposure, a generally narrow range of exposure concentrations, the need for complex models to control for potential confounding by other pollutants and extraneous variables, and outcomes for which only a small fraction of the variance is explained by exposure to pollutants. These factors make selection among various models of response difficult.

A number of exposure-response functions have been proposed to describe the results of experimental studies. No single exposure-response model form, however, has been adequately tested and identified as providing an accurate, precise description of the relationship between exposure and response in both humans and laboratory animals for lung function or BAL endpoints. Rather, for a given study, a particular model may have been selected a priori to describe the exposure-response data or may have been identified as providing the best fit among several competing models. In many cases, models have been found to be deficient, but rarely has the performance of a number of possible models been systematically compared.

From the individual studies, several important observations have been made that are qualitatively true for describing BAL and pulmonary function responses in both humans and laboratory animals and that should be considered in the selection of a model to describe population response as a function of exposure. Response increases monotonically with C, $\dot{V}_E$, and T, with C generally being a stronger predictor of response than $\dot{V}_E$ or T. The relationship between response and one of the exposure variables is dependent on the level of the other two variables. The relationship between response and each of the exposure variables is curvilinear over a wide range of exposure conditions, although it may appear linear over certain narrow ranges of exposure. With increasing duration of response (and possibly with increasing concentration), the FEV_1 response may approach a plateau in humans. Some evidence exists suggesting that the level of the plateau with T is a function of C. This plateau has not been observed in animal studies or for BAL endpoints. Respiratory symptom responses generally follow a pattern similar to that observed for spirometry (e.g., mean responses increase with increasing C, $\dot{V}_E$, and T). As with spirometry responses, large individual differences in symptom responses occur. Little analytical work, however, has been performed that mathematically describes either the mean or individual responses as multivariable functions of C, $\dot{V}_E$, and T.

Exposure-response models of FEV_1 and BAL responses in laboratory animals that have been proposed and that fit to varying degrees include linear and polynomial models of C, $\dot{V}_E$, and T, with and without cross-product terms (e.g., C × T); exponential models utilizing C × T as the exposure variable at constant $\dot{V}_E$; and cumulative normal probability or logistic models utilizing C^y × T as the exposure variable at constant $\dot{V}_E$. Models of these types have been found under some circumstances to describe the relationship between exposure and response for a particular data set. Most single data sets, however, do not include a wide enough range of data to test adequately the performance of a particular model across a wide range of exposure conditions or to identify an appropriate exposure metric. In particular, recent efforts have focused on the relationship between response and C and T at

constant $\dot{V}_E$. No definitive work has addressed the modeling of response and $\dot{V}_E$ for a given endpoint or for consideration of $\dot{V}_E$ changing as a function of T. Because animal and human studies often are conducted at different relative levels of $\dot{V}_E$, and because techniques to adjust mathematically for these differences only now are being developed, efforts to compare responses across species or to develop extrapolation models have been hampered. As noted earlier, quantitative models currently do not exist for respiratory symptoms.

Evidence indicates that, for humans and animals, the exposure-response relationship of BAL and pulmonary function outcomes may be modified by previous recent exposure to O_3, and the relationship for FEV_1 changes in humans may be modified by age. Previous exposure to O_3 has not been included in any exposure-response models. For young adults, the modification of the exposure-response relationship by age has been modeled.

Exposure-response models of lung function in epidemiologic studies generally have been limited to linear models of response as functions of various O_3 exposure metrics (e.g., 1-h or 8-h daily maximum, etc.). A number of studies have demonstrated significant negative mean linear relationships between O_3 exposure and lung function. Exposure-response models of respiratory symptoms in epidemiologic studies generally have employed logistic regression techniques with O_3 or total oxidant concentration as the exposure variable. These latter models generally have been chosen a priori reflecting the categorical nature of the outcome variable rather than by comparison of the performance of several candidates.

Ecological studies of the relationship between daily rates of emergency room or hospital admissions or mortality and O_3 exposure have utilized a variety of complex exposure-response models with some metric of daily O_3 concentration as the exposure variable. The complexity of the models results from, among other things, the need to control for potentially confounding long-wave patterns in the health outcomes in relationship to other potential confounders, such as other air pollutants, seasonal and meteorological factors, holidays, and day-of-week effects. In various studies, both linear and nonlinear functions have been used to describe the relationship between adjusted health outcome and concentration.

In summary, O_3 is no exception to the general problems encountered in all studies of environmental epidemiology. No single universal model form has been identified that accurately and precisely describes the relationship between population exposure and response under all circumstances. In general, the ability of a predictive model based on one study to predict responses from an independent study has not been studied adequately. For purposes of prediction or risk estimation, the adequacy of fit of a given model in a given data set and the size and representativeness of the sample should be assessed. Extrapolation beyond the range of observed data introduces additional uncertainty into predictions or risk estimates.

9.3.4.1 Prediction and Summary of Mean Responses

A selection of published reports in which models of population or mean responses have been developed is listed below, along with figures summarizing examples of predicted quantitative exposure-response relationships. Reports numbered 5 and 11 are epidemiological studies, and the remainder are experimental studies. Because no currently available single model is sufficient to accurately describe all major scenarios, the key models are presented without weighting. Following this section is a further section that describes models of individual responses within the population.

1. Hazucha (1987) predicts mean FEV_1 decrements in humans as a function of C (0.0 to 0.75 ppm O_3) for four levels of $\dot{V}_E$ for 2-h exposures (Figure 9-1).

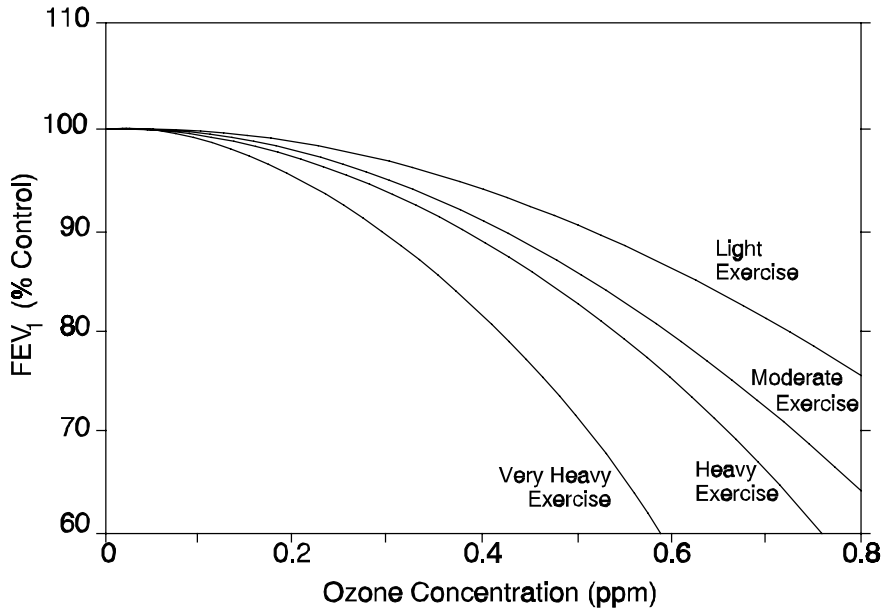

Figure 9-1. Mean predicted changes in forced expiratory volume in 1 s following 2-h exposures to ozone with increasing levels of intermittent exercise.

Source: Hazucha (1987).

2. McDonnell and Smith (1994) predict mean FEV_1 decrements in heavily exercising humans as a function of C (0.0 to 0.4 ppm O_3) and T (1.0 to 6.6 h) (Figure 9-2).
3. Highfill et al. (1992) predict the BAL responses of resting rats and guinea pigs as a function of C (0.0 to 0.8 ppm O_3) and T (2 to 8 h) (Figure 9-3).
4. Tepper et al. (1994) predict the FVC changes as a function of C (0.0 to 0.8 ppm O_3) and T (2 to 7 h) for exposures conducted with rats breathing at three times resting $\dot{V}_E$ (Figure 9-4).
5. Burnett et al. (1994) predict the frequency of hospital admissions (adjusted for covariates) as a linear function of C (previous day 1-h O_3 maximum) for Ontario hospitals (Figure 9-5).

Other reports in which models are developed or that contain data potentially useful for further development or testing of models are listed below.

6. Seal et al. (1993) present data that would allow modeling of FEV_1 decrements in humans as a function of C (0.0 to 0.4 ppm O_3) for 2-h exposures with moderate exercise.
7. Folinsbee et al. (1978) predict lung function changes in humans as a function of C (0.0 to 0.50 ppm O_3) and $\dot{V}_E$ (10 to 65 L/min) for 2-h exposures.

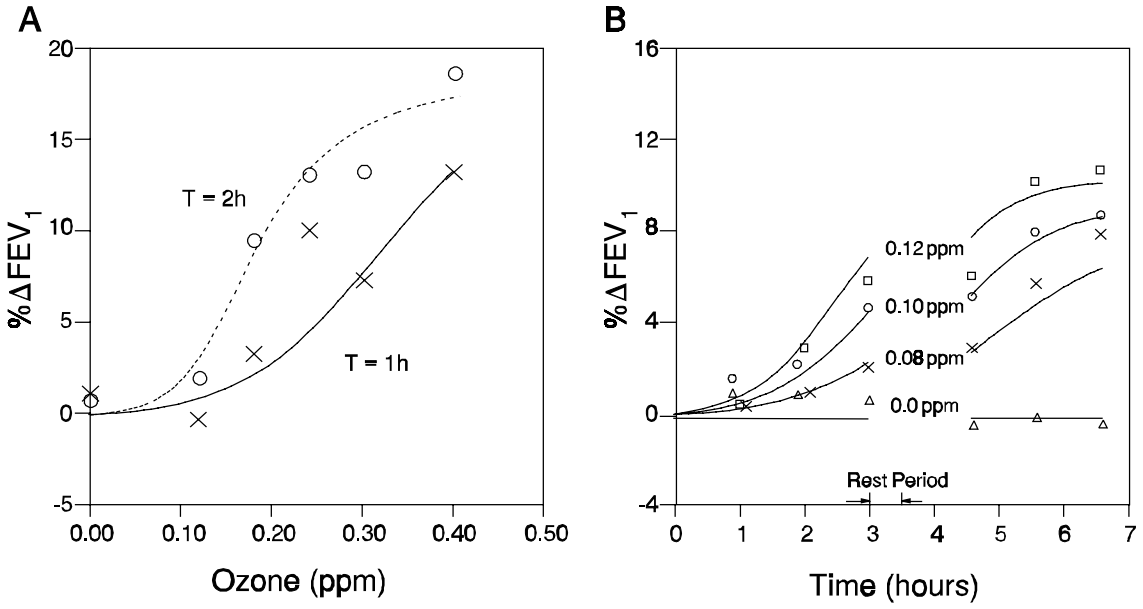

Figure 9-2. Predicted mean decrements in forced expiratory volume in 1 s for 1- and 2-h exposures to ozone with intermittent heavy exercise (A) and 6.6-h exposures with moderate prolonged exercise (B).

Source: McDonnell and Smith (1994).

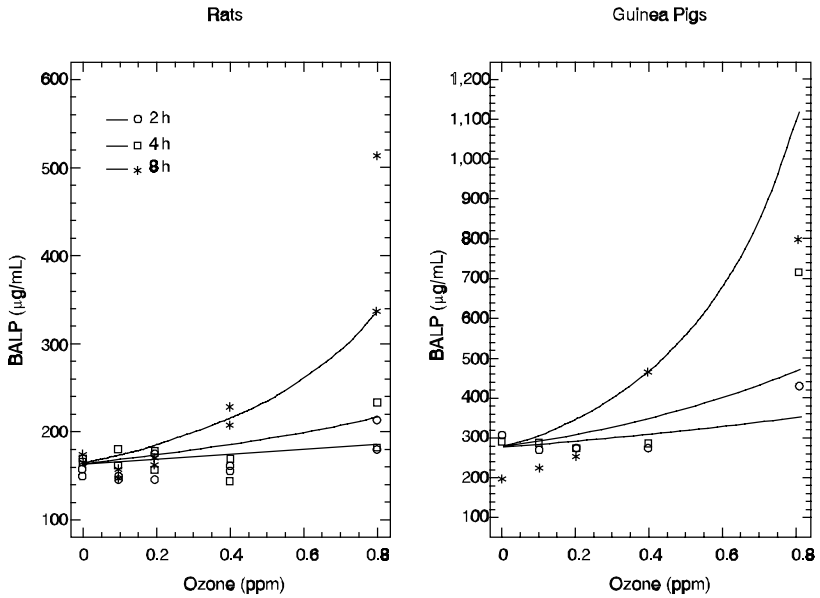

Figure 9-3. Derived means of BAL protein (BALP) denoted by symbols and the exponential model shown by lines as time of exposure varies from 2 to 8 h.

Source: Highfill et al. (1992).

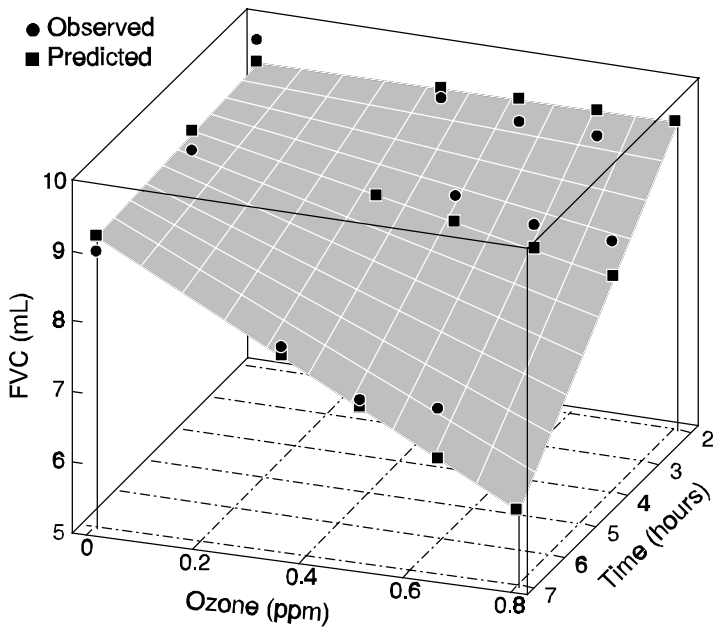

Figure 9-4. Predicted mean forced vital capacity for rats exposed to ozone while undergoing intermittent carbon dioxide-induced hyperpnea.

Source: Tepper et al. (1994).

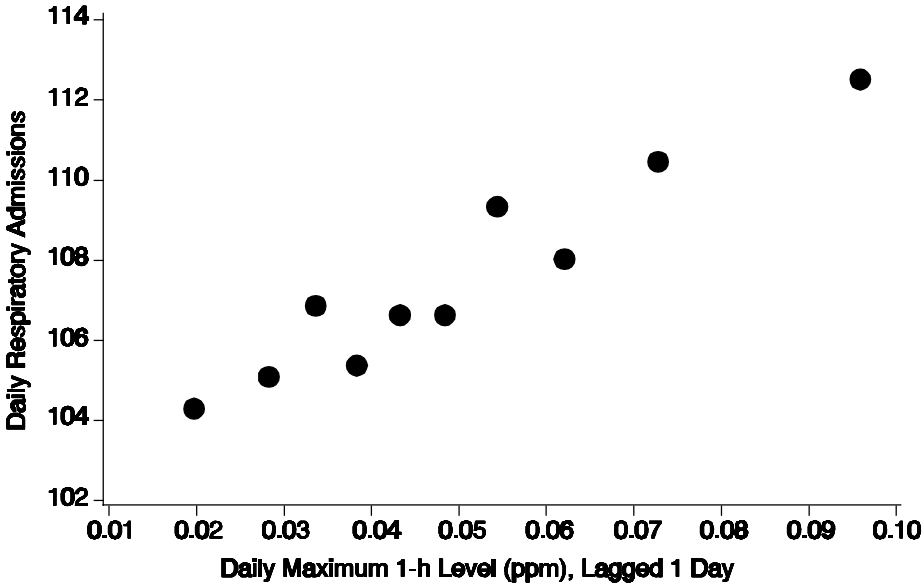

Figure 9-5. Average number of adjusted respiratory admissions among all 168 hospitals by decile of the daily 1-h maximum ozone level (ppm), lagged 1 day.

Source: Burnett et al. (1994)

8. Adams et al. (1981) predict lung function changes in humans as a function of the product of C × T × $\dot{V}_E$ for C = 0 to 0.4 ppm O_3, T = 30 to 80 min, and $\dot{V}_E$ = 33 or 66 L/min.
9. Rombout et al. (1989) predict the concentration of protein in BAL fluid of rats as a function of C (0.25 to 4.0 mg/m^3 O_3) and T (0 to 12 h) for daytime and nighttime exposures.
10. Highfill and Costa (1995) compare the fits of quadratic, exponential, and sigmoid-shaped models to published human lung function data and O_3 laboratory animal BAL data.
11. Thurston et al. (1994) predict hospital admissions in Toronto as a function of C (previous day 1-h O_3 maximum) for Toronto hospitals.

9.3.4.2 Prediction and Summary of Individual Responses

It is well known that considerable interindividual differences in the magnitude of response to O_3 exposure exist. The individual lung function and, to a lesser extent, respiratory symptom responses to O_3 have been demonstrated to be reproducible over a period of time, indicating that some individuals are consistently more responsive than others to O_3. The basis for these differences is not known, with the exception that young adults have been observed to be more responsive than older adults (see Figure 9-6).

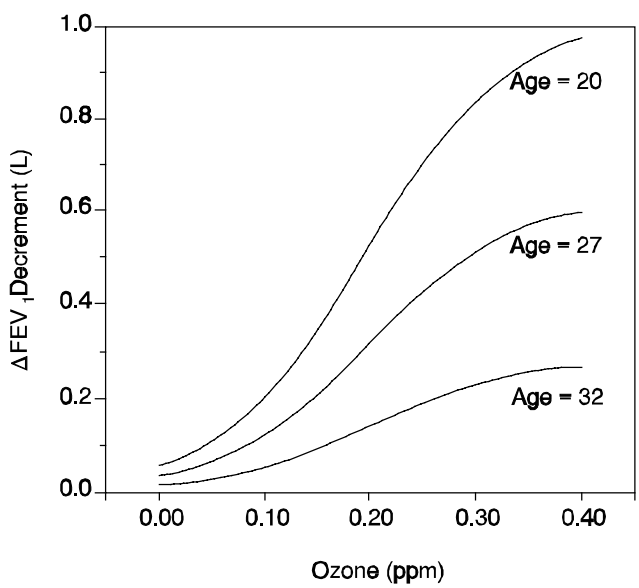

Figure 9-6. Predicted mean decrements in forced expiratory volume in 1 s (ΔFEV_1) following 2-h exposures to ozone while undergoing heavy intermittent exercise for three ages. (Note: To convert ΔFEV_1 to $\%\Delta FEV_1$, multiply by 22.2%.)

Source: McDonnell et al. (1993).

Calculation of group mean responses for a population that includes both more and less responsive individuals is useful for making inferences regarding the probability that a population effect is present or absent for a given exposure. Because the frequency distribution of individual responses to O_3 changes with changing exposure conditions, however, knowledge of the mean and variance of population responses does not provide reliable information on the distribution of individual responses for a given exposure, and, hence, is not particularly useful for estimating risks to members of the population. One method of presenting individual data is illustrated in Figure 9-7 in which histograms are presented for individual responses of subjects participating in four 6.6-h studies of low-level O_3 exposure.

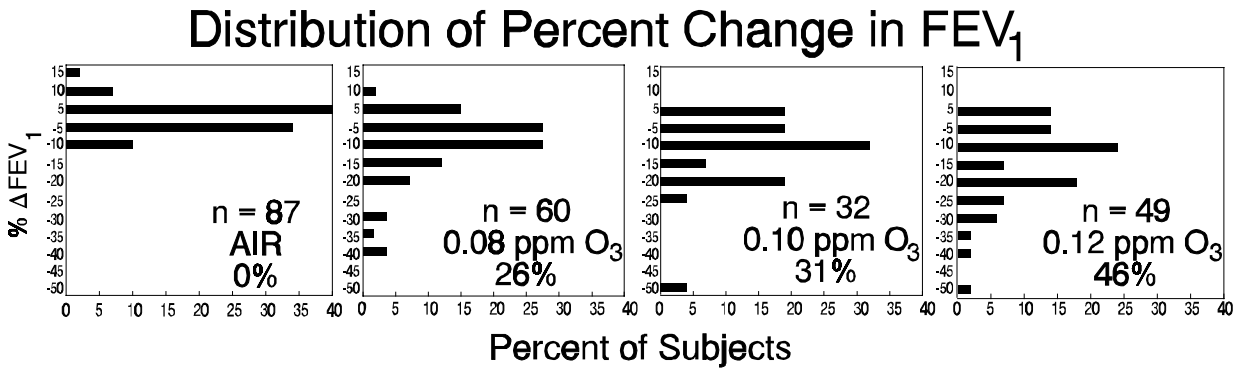

Figure 9-7. ***The distribution of response for 87 subjects exposed to clean air and at least one of 0.08, 0.10, or 0.12 ppm ozone (O_3). The O_3 exposures lasted 6.6 h, during which time the subjects exercised for 50 min of each hour, with a 35-min rest period at the end of the third hour. Decreases in forced expiratory volume in 1 s (FEV_1) are expressed as percent change from baseline. For example, the bar labeled, "−10" indicates the percent of subjects with a decrease in FEV_1 of >5% but ≤10%, and the bar labeled "5" indicates improvement in FEV_1 of >0% but ≤5%. Each panel of the figure indicates the percentage of subjects at each O_3 concentration with a decrease of FEV_1 in excess of 10%.***

Similarly, the histograms of regression slopes of the lung function-O_3 concentration relationship for children participating in a camp study illustrate a large range of variability in response (Figure 9-8).

Another method that allows interpolation between observed data points involves definition of the effect of interest (e.g., a 10% decrement in FEV_1) and modeling of the proportion of individuals who experience such an effect as a function of exposure conditions. Figures 9-9 and 9-10 show the predicted proportion of individuals (humans) experiencing 10% FEV_1 decrements and respiratory symptom responses, respectively, as a function of C (0.0 to 0.4 ppm O_3) for independent studies conducted at either 1 or 2 h of exposure with heavy exercise.

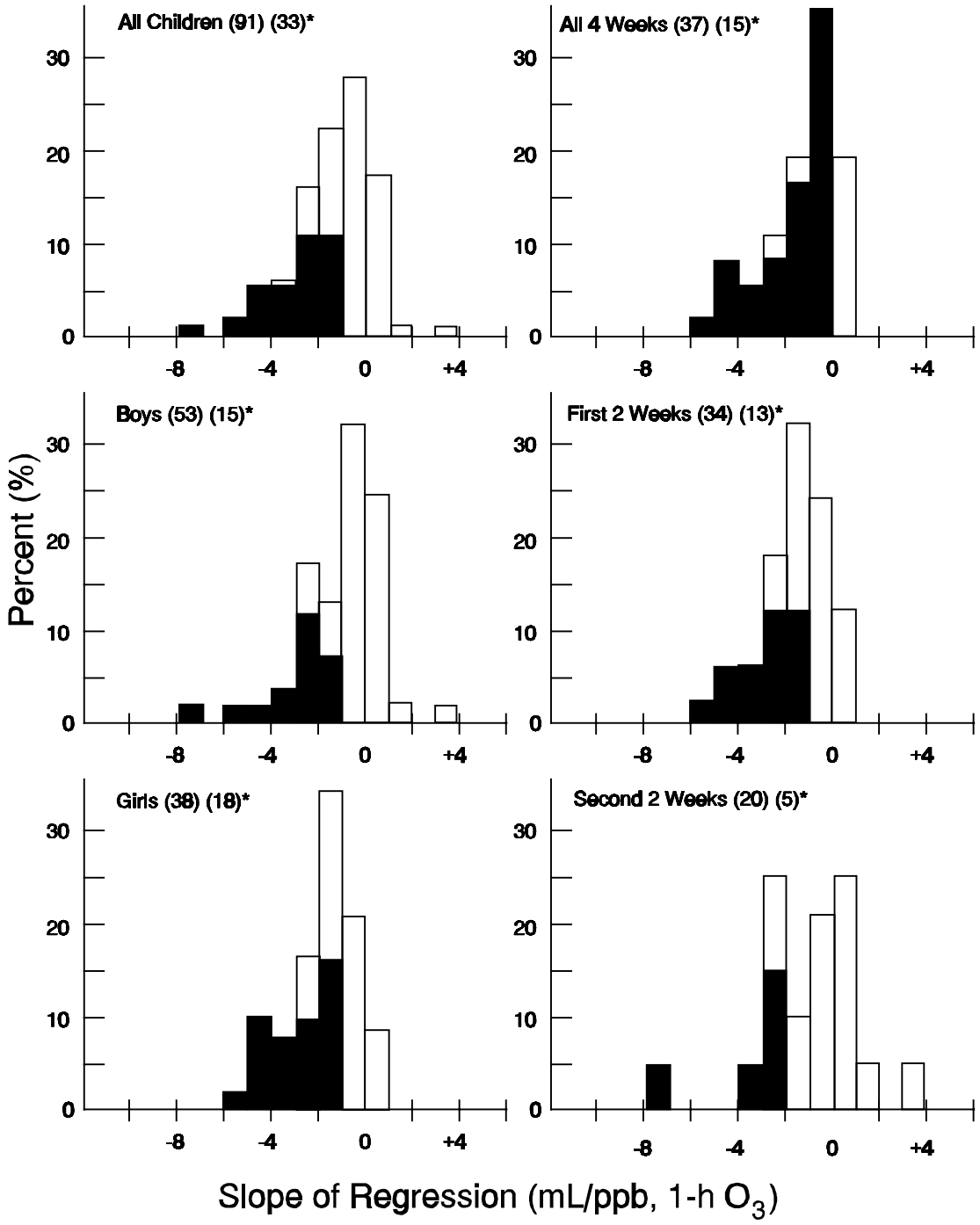

Figure 9-8. ***Histograms of regression slopes for FEV_1 versus 1-h ozone (O_3) concentration in children attending a summer camp in northwestern New Jersey. The numbers of children in each group are indicated in parentheses; an asterisk identifies the number of children with slopes that were significantly different from zero ($p < 0.05$). Shading represents the percent of significant ($p < 0.05$) slopes across the distributions.***

Source: Spektor et al. (1988).

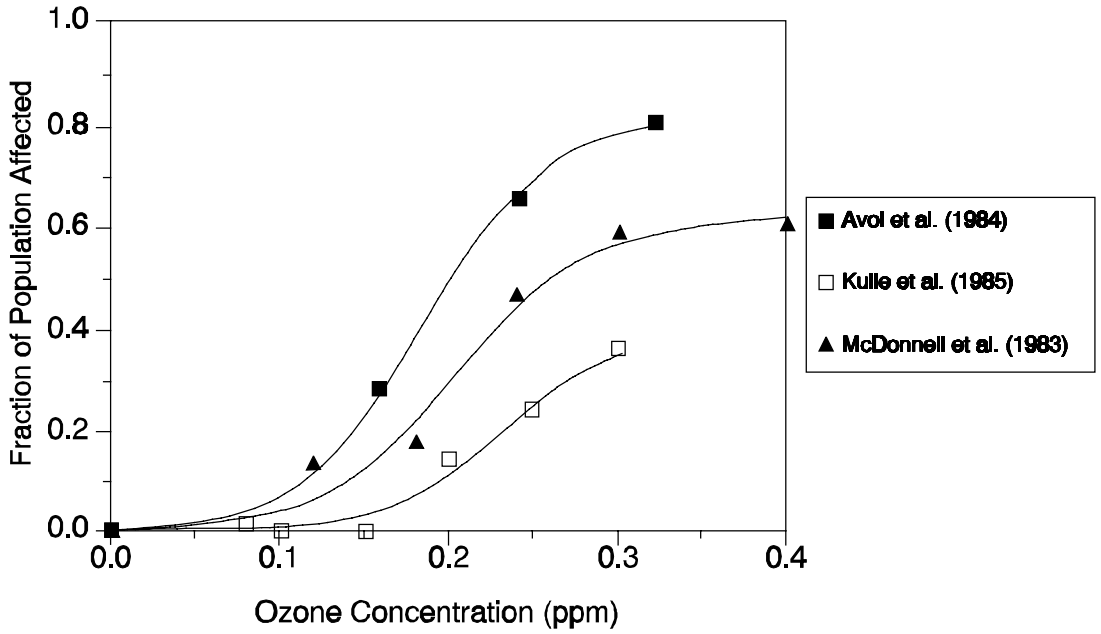

Figure 9-9. Proportion of heavily exercising individuals predicted to experience a 10% decrement in forced expiratory volume in 1 s following a 1- or 2-h exposure to ozone.

Source: U.S. Environmental Protection Agency (1989).

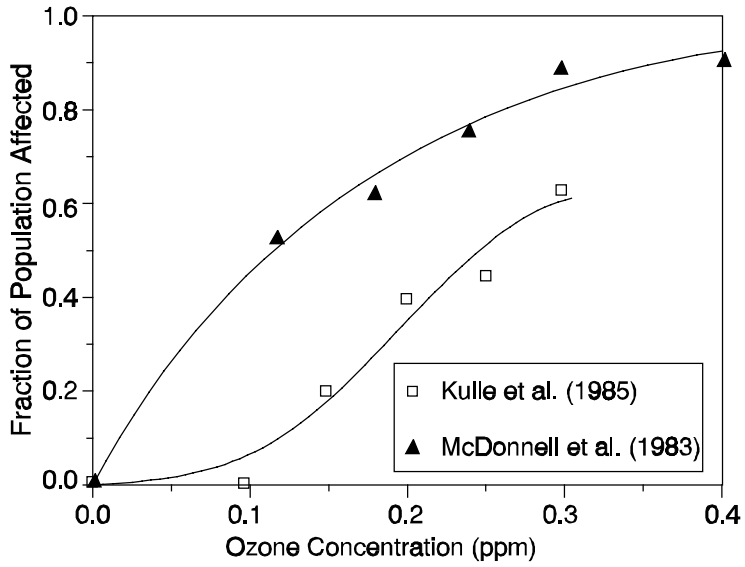

Figure 9-10. Proportion of heavily exercising individuals predicted to experience mild cough following a 2-h ozone exposure.

Source: U.S. Environmental Protection Agency (1989).

Predictions of the proportion of individuals experiencing 5, 10, or 15% FEV_1 decrements as a function of C (0.0 to 0.12 ppm O_3), T (1 to 6.6 h), at a specific age (24 years) for exposures with moderate exercise are shown in Figure 9-11.

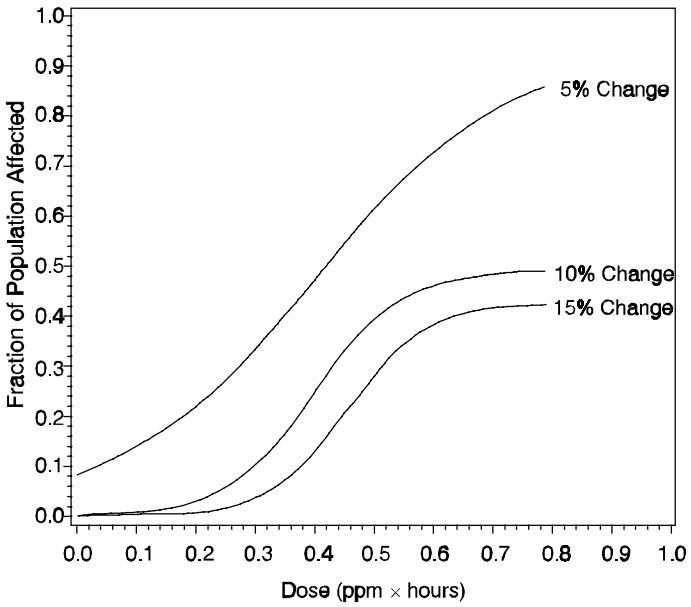

Figure 9-11. Proportion of moderately exercising individuals exposed to ozone for 6.6 h predicted to experience 5, 10, or 15% decrements in forced expiratory volume in 1 s as a function of C × T for age = 24 years.

Source: McDonnell et al. (1995).

As an example of differences between the mean and individual responses, it was stated earlier that exposure for 5.6 h to 0.08 ppm O_3 was the shortest duration for which a 5% mean decrement in FEV_1 was observed. For those same exposure conditions, 41, 17, and 10% of the subjects studied experienced FEV_1 decrements larger than 5, 10, and 15%, respectively.

The clinical significance of individual responses to O_3 exposure depends on the health status of the individual, the magnitude of the changes in pulmonary function, the severity of respiratory symptoms, and the duration of the response. Tables 9-1 and 9-2 categorize individual functional and symptomatic responses to O_3 exposure as normal (or none) and by increasing levels of severity in healthy persons and in persons with impaired respiratory systems, respectively. Pulmonary function responses are represented in these tables by changes in spirometry (e.g., FEV_1), SR_{aw}, and nonspecific bronchial responsiveness. Respiratory symptom responses include cough, pain on deep inspiration, and wheeze. The changes in spirometry that have been focused on most frequently are O_3-induced decrements in FEV_1 because they are easily quantified, have a continuous distribution, and have been used to provide most of the exposure-response relationships described in this section. The combined impact of both functional and symptomatic

Table 9-1. Gradation of Individual Responses to Short-Term Ozone Exposure in Healthy Persons[a]

Functional Response	None	Small	Moderate	Large
FEV_1	Within normal range (±3%)	Decrements of 3% to ≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness[b]	Within normal range	Increases of <100%	Increases of ≤300%	Increases of >300%
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Symptomatic Response	Normal	Mild	Moderate	Severe
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Impact of Responses	Normal	Normal	Mild	Moderate
Interference with normal activity	None	None	A few sensitive individuals choose to limit activity	Many sensitive individuals choose to limit activity

[a]See text for discussion; see Appendix A for abbreviations and acronyms.
[b]An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).

responses to O_3 exposure generally is displayed as an interference with normal activity or a change in medical treatment (see Tables 9-1 and 9-2).

In healthy individuals, the importance attached to individual changes in FEV_1 and nonspecific bronchial responsiveness depends, in part, on the magnitude and persistence of the response, but it is also important to consider the circumstances in which changes in lung function occur with other responses. For example, a 20% decrement in FEV_1 or a 100 to 200% increase in SR_{aw} that is induced as a result of a nonspecific bronchial responsiveness test and one that is almost completely reversible within an hour is associated with little, if any, airway epithelial damage. If, in addition, there are no respiratory symptoms (except chest discomfort), then this response, in itself, would not be considered clinically significant. On the other hand, a smaller decrement in FEV_1 of 15%, accompanied by marked pain on deep inspiration and persistent cough that is reversed in approximately 24 h, may be considered clinically significant in some individuals. In other words, it is important to

Table 9-2. Gradation of Individual Responses to Short-Term Ozone Exposure in Persons with Impaired Respiratory Systems[a]

Functional Response	None	Small	Moderate	Large
FEV_1 change	Decrements of <3%	Decrements of 3 to ≤10%	Decrements of >10% but <20%	Decrements of ≥20%
Nonspecific bronchial responsiveness[b]	Within normal range	Increases of <100%	Increases of ≤300%	Increases of >300%
Airway resistance (SR_{aw})	Within normal range (±20%)	SR_{aw} increased <100%	SR_{aw} increased up to 200% or up to 15 cm H_2O/s	SR_{aw} increased >200% or more than 15 cm H_2O/s
Duration of response	None	<4 hours	>4 hours but ≤24 hours	>24 hours
Symptomatic Response	Normal	Mild	Moderate	Severe
Wheeze	None	With otherwise normal breathing	With shortness of breath	Persistent with shortness of breath
Cough	Infrequent cough	Cough with deep breath	Frequent spontaneous cough	Persistent uncontrollable cough
Chest pain	None	Discomfort just noticeable on exercise or deep breath	Marked discomfort on exercise or deep breath	Severe discomfort on exercise or deep breath
Duration of response	None	<4 hours	>4 hours, but ≤24 hours	>24 hours
Impact of Responses	Normal	Mild	Moderate	Severe
Interference with normal activity	None	Few individuals choose to limit activity	Many individuals choose to limit activity	Most individuals choose to limit activity
Medical treatment	No change	Normal medication as needed	Increased frequency of medication use or additional medication	Physician or emergency room visit

[a]See text for discussion; see Appendix A for abbreviations and acronyms.
[b]An increase in nonspecific bronchial responsiveness of 100% is equivalent to a 50% decrease in PD_{20} or PD_{100} (see Chapter 7, Section 7.2.3).

consider the pattern of responses and not simply to focus on a single marker of the effect of O_3.

The magnitude of individual changes can become more important in persons with impaired respiratory systems (e.g., asthmatics) who already have reduced baseline lung function. Any change in function that causes these individuals to drop below 40 to 50% of predicted would be considered clinically adverse. For example, O_3-induced changes in SR_{aw}, a measure of airway narrowing, are small and of minimal clinical significance in nonasthmatic individuals. Asthmatics, however, often have baseline airway narrowing and experience larger changes in SR_{aw} on exposure to O_3 than do nonasthmatics. Because of these baseline differences, the clinical significance of increases in SR_{aw} depends both on percent change from baseline and on absolute increases in SR_{aw}.

9.4 Effects of Long-Term Ozone Exposures

In both humans and test animals, the response to a single O_3 exposure nominally can be characterized by lung dysfunction, lung cell injury and inflammation, and leakage of plasma proteins into the airspace lumen. However, when such an exposure is repeated for several consecutive days, many of these effects appear to wane, suggesting attenuation or the development of tolerance to the continued intermittent challenge. In spite of this apparent state of attenuation, long-term O_3 exposures have been linked to subtle pulmonary effects, some of which have irreversible components, thereby enhancing concern about chronic effects. The following section will provide an overview attempting to synthesize the current understanding of the phenomenon of attenuation during brief, repeated exposures and the evidence for potential health impairments resulting from protracted exposures to this oxidant.

9.4.1 Repeated Exposures

It is well established that a brief exposure of laboratory rodents to an O_3 concentration, which causes minimal effects, will protect the animals from a subsequent lethal challenge of O_3 a week later. This phenomenon, called tolerance, bears a similarity to the pattern of attenuated nonlethal effects (sometimes referred to as "adaptation") observed in both human volunteers and animals when exposed to episodic levels of O_3 ($\leq$0.5 ppm) for 1 to 7 h/day over a succession of 5 or more days. Generally, over a 5-day exposure period, the effects of Day 1 are accentuated on Day 2 and diminish thereafter. Attenuation of the functional effects include spirometric deficits and associated symptoms as well as irritative alterations of breathing; nonspecific airway responsiveness, however, does not revert to normal levels. Measures of tissue effects that attenuate include inflammation and impaired phagocytic capabilities of alveolar macrophages. However, some evidence from animal studies suggests that tissue alterations persist, although the observed changes may be part of a transition to a chronically affected stage of the lung. Thus, in general, cell-associated indicators of injury or damage within the lung appear to diminish in spite of the continued O_3 exposure.

A number of mechanisms have been shown to be involved in the evolution of this "adapted" state. These mechanisms range from the replacement of sensitive cells in the alveolar lining (epithelium) by more resistant cells (with or without a thickened fluid barrier on the lumenal surface) to the enhancement of antioxidant metabolism providing cell resistance and more biochemical defenses at the lung surface. However, controlled human

studies show that after a 1-week period without O_3 exposure, subjects regain their spirometric responsiveness to O_3 challenge, although this abrupt transition between unresponsiveness and responsiveness appears less distinct in field-related studies. For example, studies of Southern Californians suggest that they are significantly less responsive to the spirometric effects of an acute episodic-like controlled challenge with O_3 when studied for a period after the "high" O_3 season than after the relatively "low" O_3 season. Likewise, there is some evidence that O_3-exposed urban populations are also somewhat more resistant to the oxidant than populations that receive minimal exposure. This would appear to be in conflict with hospital admissions data suggesting the aggravation of respiratory diseases, like asthma, within such populations. It remains to be shown whether these latter data reflect the responsiveness of a sensitive subpopulation, perhaps less adapted or having less reserve function.

9.4.2 Prolonged Exposures

Most long-term exposure studies in animals have evaluated structural and functional changes. In the few investigations of the immune system or antibacterial host defenses, prolonged exposures of animals either caused no effects or did not increase the magnitude of effects observed after acute exposures. Thus, the following discussion centers on the larger body of knowledge on other endpoints.

Epidemiologic studies attempting to associate chronic lung effects in humans with long-term O_3 exposure provide only suggestive evidence that such a linkage exists. Most studies have been cross-sectional in design and have been compromised by incomplete control of confounding variables and inadequate exposure information. Other studies have attempted to follow variably exposed groups prospectively. Studies of such design have been conducted in communities of the Southwest Air Basin as well as in Canada where comparisons could be drawn between lung function changes over several years in populations from high- or low-oxidant pollution. The findings suggest small, but consistent decrements in lung function among inhabitants of the more highly polluted communities. However, associations between O_3 and other copollutants and, in some cases, problems with study population loss undermine the confidence in the study conclusions. Likewise, recent associations found between O_3 and the incidence and severity of asthma over a decade of study, although derived from well-designed studies, also tend to be weakened by the colinearity of O_3 with other air pollutants. Nevertheless, in all of the studies assessing lung function, the pattern of dysfunction associated with the long-term exposure has been consistent with the functional and structural abnormalities seen in laboratory animal studies.

The advantage of laboratory animal studies is the ability to examine closely the distribution and intensity of the O_3-induced morphologic changes that have been identified throughout the respiratory tract (see Chapter 6, Section 6.2.4). Indeed, cells of the nose, like the distal lung, clearly are affected by O_3. Perhaps of greater health concern are the lesions that occur in the small airways and in the centriacinar regions of the lung where the alveoli meet the distal airways (Figure 9-12). Altered function of the distal airways, the proximal conduits of air to the gas-exchange regions, can result in reduced communication of fresh air with the alveoli and air-trapping. In fact, chronic O_3 lesions as found in animal studies are reminiscent of the earliest lesions found in respiratory bronchiolitis, some of which may progress to fibrotic lung disease (Kuhn et al., 1989; King, 1993).

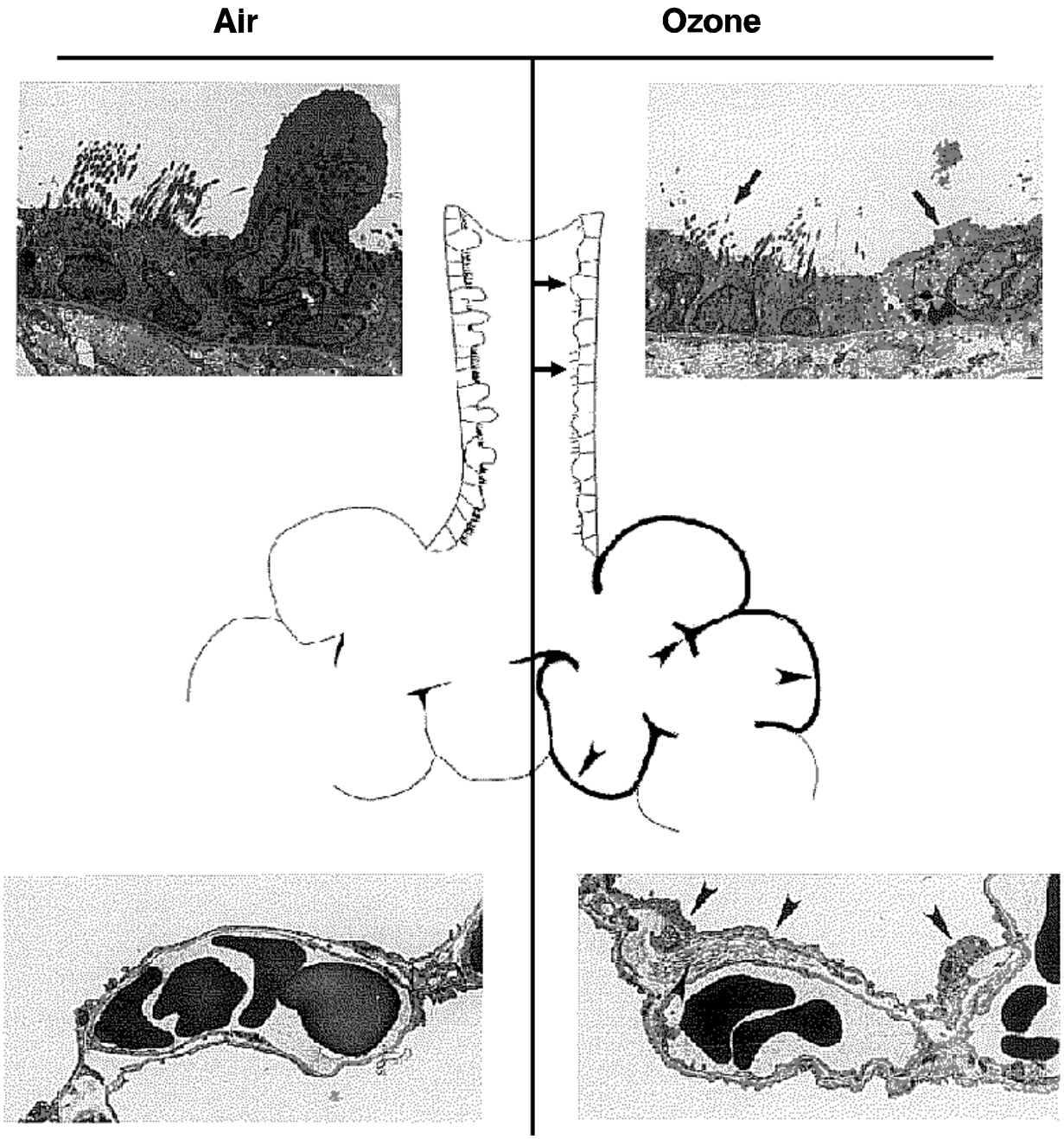

Figure 9-12. A summary of the morphologic lesions found in the terminal bronchioles and centriacinar region (CAR) of the lung following exposure of laboratory rats to filtered air or a simulated ambient pattern of ozone for up to 78 weeks. In the terminal bronchiole, the sizes of the dome of Clara cells became smaller with ozone exposure, and the number of cilia is reduced (arrows). In the CAR, the epithelium becomes thicker, and accumulation of collagen fibers occurs (arrow heads).

Source: Chang et al. (1992).

The O_3-induced inflammation, cell damage, and altered permeability of epithelial cells lining the respiratory tract allow exudation of fluid, cells, and cellular debris from plasma into lung tissue. The magnitude of intraluminal exudation associated with injury correlates with the initial epithelial necrosis and release of inflammatory mediators.
As shown in Figure 9-13, the temporal pattern of effects during and after a chronic exposure is complex. During the early days of exposure, the end-airway lumenal and interstitial inflammation peaks, and, thereafter, appears to subside at a lower plateau of activity sometimes referred to as a "smoldering" lesion. Several cytokines remain elevated beyond the apparent adaptation phase of the response and may be linked conceptually to the development of chronic lesions in the distal lung. To date, however, a clear association of these BAL-derived mediators and cells with long-term toxicity has not been demonstrated. Some evidence of molecular changes within the matrix of the lung may also link to the chronic effects, but these too remain poorly defined. When exposures to O_3 continue for weeks or months, the diminished O_3-induced exudative response in the distal bronchoalveolar areas is supplanted by hyperplastic epithelia in the alveoli and end-airways. Damaged cells in centriacinar alveoli are replaced by metabolically active progenitor cells that are more resistant to oxidant challenge. Junctional areas between conducting and gas-exchange regions, where the O_3 changes are typically most intense, also undergo epithelial hyperplasia, giving the appearance that airway cells are extending into the mouth of the alveolus, hence the term "bronchiolization". The functional result of this concentration-dependent process is the effective elongation of distal bronchioles, which functionally may alter air distribution within the lung during breathing. These hyperplastic cells also are believed to be more resistant to O_3. When exposure to O_3 ceases, most, but not all, of the hyperplasia appears to reverse with time.

In contrast, within the underlying interstitium (tissue between blood and air spaces) of the affected centriacinar region, proliferating fibroblasts appear to evolve excess noncellular fibrous matrices, which may be only partially reversible and may, in fact, progress after removal from O_3 exposure. This would suggest that O_3 can initiate focal interstitial fibrosis of the lung at the regions where O_3 causes epithelial cell damage as a prelude to chronic degenerative lung disease. The crucial question, then, is whether this latter irreversible process, which clearly occurs at relatively high O_3 concentrations, occurs at ambient levels to which humans are typically exposed, in many cases, over a lifetime. Unfortunately, comparable morphologic data from humans residing in O_3-polluted areas are lacking.

Studies of prolonged O_3 exposures in monkeys and rats reveal generally similar morphologic responses, although it appears that the monkey exhibits somewhat more tissue injury than does the rat under roughly similar exposure conditions. Interspecies comparisons of dosimetric data indicate that the monkey, with its similarity to the human in distal airway structure, provides data that may best reflect the potential effects of O_3 in humans exercising out of doors. As such, monkeys exposed to O_3 at 0.15 ppm for 8 h each day for 6 to 90 days exhibit significant distal airway remodeling. Rats show similar but more modest changes at 0.25 ppm O_3 after exposures of longer duration, up to 18 mo and beyond (near-lifetime). The chronic distal lung and airway alterations appear consistent with incipient peribronchiolar fibrogenesis within the interstitium. Attempts to correlate functional deficits have been variable, perhaps due in part to the degree and distribution of the lesions and the general insensitivity of most measures of the distal lung function. The interstitial changes may progress, however. Moreover, one recent primate study revealed evidence that

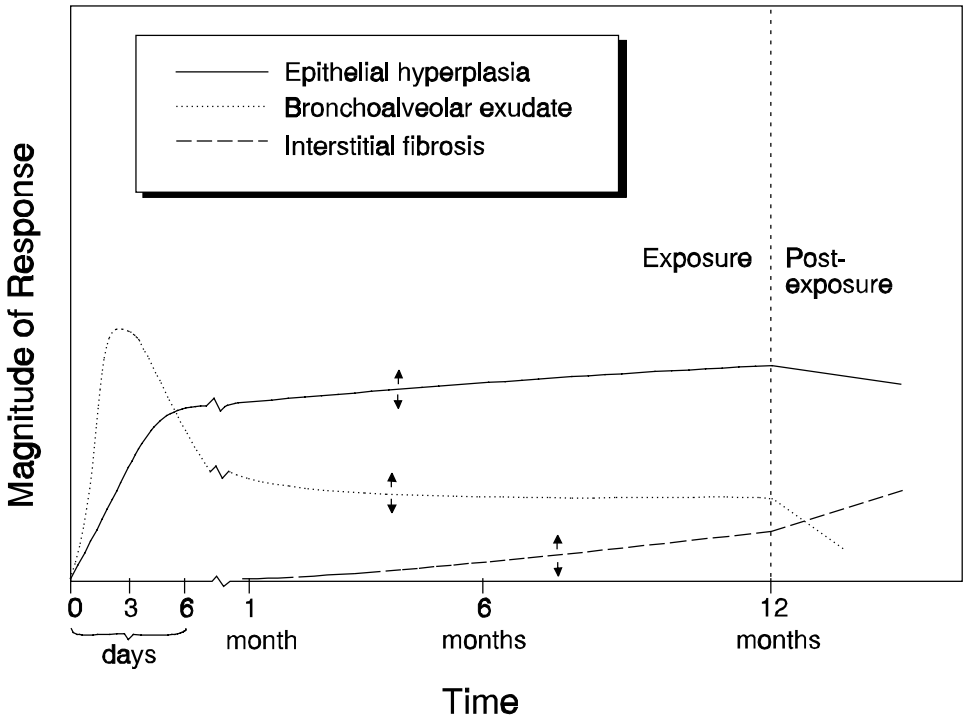

Figure 9-13. Schematic comparison of the duration-response profiles for epithelial hyperplasia, bronchoalveolar exudation, and interstitial fibrosis in the centriacinar region of lung exposed to a constant low concentration of ozone.

Source: Dungworth (1989).

intermittent challenge with a pattern of O_3 exposure more reflective of seasonal episodes, with extended periods of clean air in between extended periods of O_3, actually leads to greater injury. The reasons for this are unclear, but may relate to the known loss of tolerance that occurs in both humans and animal test species with removal of the oxidant burden.

In conclusion, the collective toxicologic data on chronic exposure to O_3 garnered in animal exposure and human population studies have some ambiguities. What is clear is that the distribution of the O_3 lesions is roughly similar across species, is, in part, concentration dependent (and perhaps time or exposure-pattern dependent), and, under certain conditions, has irreversible structural attributes. What is unclear is whether ambient exposure scenarios encountered by humans result in similar lesions and whether there are resultant functional or impaired health outcomes, particularly because the human exposure scenario may involve much longer exposures than can be studied in the laboratory. The epidemiologic lung function data generally parallel those of the animal studies, but they lack the confidence of O_3 exposure history and are frequently confounded by personal or copollutant variables.

9.4.3 Genotoxicity and Carcinogenicity of Ozone

Numerous in vitro exposure studies suggest that O_3 has either weak or no potential to cause mutagenic, cytogenetic, or cellular transformation effects. Most of these experiments utilized high concentrations of O_3 (>5.0 ppm). Because of the exposure systems used, there are unknowns about the formation of artifacts and the dose of O_3. Therefore, these studies are not very useful in health assessment. Cytogenetic effects have been observed in some, but not all, laboratory animal and human studies of short-term O_3 exposure. However, well-designed human clinical cytogenetic studies were negative.

Until recently, in vivo exposure studies of carcinogenicity, with and without co-exposure to known carcinogens, were either negative or ambiguous. A well-designed cancer bioassay study has recently been completed by the National Toxicology Program (NTP) using male and female Fischer 344/N rats and $B6C3F_1$ mice. Animals were exposed for 2 years to 0.12, 0.5, and 1.0 ppm O_3 (6 h/day, 5 days/week). A similar lifetime exposure was conducted, but 0.12 ppm was not used. The NTP evaluated the weight-of-evidence for this study; they found "no evidence" of carcinogenicity in rats but reported "equivocal evidence" of carcinogenicity in O_3-exposed male mice and "some evidence" of carcinogenic activity in O_3-exposed female mice. The increases in adenomas and carcinomas were observed only in the lungs. There was no concentration response. In the male mice, the incidence of neoplasms in the 2-year study was not elevated significantly by O_3 and was within the range of historical controls. The lifetime exposure resulted in an increased incidence of carcinomas that was not statistically significant. When the female mouse data from the two exposure regimens (at 1.0 ppm) were combined, there was a statistically significant increase (almost double) in neoplasms. In a companion study, male rats were treated with a tobacco carcinogen and exposed for 2 years to 0.5 ppm O_3. Ozone did not affect the response and, therefore, had no tumor promoting activity.

In summary, only chronic exposure to a high concentration of O_3 (1.0 ppm) has been shown to evoke a limited degree of carcinogenic activity in the females of one strain of mice. Rats were not affected. Furthermore, there was no concentration response, and there is inadequate information from other research to provide mechanistic support for the finding in mice. Thus, the potential for animal carcinogenicity is uncertain.

9.5 Effects of Combined Pollutant Exposures

In ambient air, people are exposed to mixtures of pollutants, making it important to understand interactions. Epidemiological studies, which inherently evaluate O_3 as part of complex mixtures, are discussed in other subsections dealing with classes of effects. In the laboratory it becomes possible to sort out the role of O_3 in simple mixtures. Complex mixtures are typically not investigated in the laboratory because, even if only six pollutants were involved, the experimental design required to unequivocally sort out which pollutant or pollutant interactions were responsible for the responses or portions of the responses could require as many as 719 additional separate experiments, even if the concentrations of the six pollutants remained the same.

The summary will focus only on binary mixtures because these are by far the predominant type of experiments. Responses to a binary pollutant mixture may represent the sum of the independent responses to the two chemicals (i.e., an additive response). If there is some interaction between either the two responses or the two pollutants, the resultant response

could be larger than additive (synergism) or smaller than additive (antagonism). Interaction between pollutants could result in the production of a more or less toxic byproduct. Alternatively, the response to one pollutant could magnify the response to the other pollutant or could interfere with or block the action of the other pollutant. Binary mixture studies fall into two categories, simultaneous and sequential exposures. In the simultaneous exposures, both the responses and the pollutants can interact. In the sequential exposures, it is primarily the responses that would interact.

In general, controlled human studies of O_3 mixed with other pollutants show no more than an additive response with symptoms or spirometry as an endpoint. This applies to O_3 in combination with nitrogen dioxide (NO_2), SO_2, sulfuric acid (H_2SO_4), nitric acid (HNO_3), or carbon monoxide (CO). Indeed, at the levels of copollutants used in human exposure studies, the responses can be attributed primarily to O_3. In one study, exposure to O_3 increased airway responsiveness to SO_2 in asthmatics. Similarly, other pollutants that may increase airway responsiveness could augment the effect of O_3 on airway responsiveness.

The relatively large number of animal studies of O_3 in mixture with NO_2 and H_2SO_4 shows that additivity, synergism, and antagonism can result, depending on the exposure regimen and the endpoint studied. The numerous observations of synergism are of concern, but the interpretation of most of these studies relative to the real world is confounded by unrealistic exposure designs. For example, ambient concentrations of O_3 often were combined with levels of copollutants substantially higher than ambient, creating the possibility that mechanisms of toxicity unlikely in the real world contributed to the experimental outcome. Nevertheless, the data support a hypothesis that coexposure to pollutants, each at innocuous or low-effect levels, may result in effects of significance.

9.6 Conclusions

This section summarizes the primary conclusions derived from an integration of the known effects of O_3 provided by animal toxicological, human clinical, and epidemiological studies.

1. What are the effects of short-term (<8-h) exposures to ozone?

Recent epidemiology studies addressing the effects of short-term ambient exposure to O_3 in the population have yielded significant associations with a wide range of health outcomes, including lung function decrements, aggravation of preexisting respiratory disease, increases in daily hospital admissions and emergency department visits for respiratory causes, and increased mortality. Results from lung function epidemiology studies are generally consistent with the experimental studies in laboratory animals and humans.

Short-term O_3 exposure of laboratory animals and humans causes changes in pulmonary function, including tachypnea (rapid, shallow breathing), decreased lung volumes and flows, and increased airway responsiveness to nonspecific stimuli. Increased airway resistance occurs in both humans and laboratory animals, but typically at higher exposure levels than other functional endpoints. In addition, adult human subjects experience O_3 induced symptoms of airway irritation such as cough or pain on deep inspiration. The changes in pulmonary function and respiratory symptoms occur as a function of exposure concentration, duration, and level of exercise. Adult human subjects with mild asthma have qualitatively similar responses in lung volume and airway responsiveness to

bronchoconstrictor drugs as nonasthmatics. Respiratory symptoms are also similar, but wheezing is a prevalent symptom in O_3-exposed asthmatics in addition to the other demonstrated symptoms of airway irritation. Airway resistance, however, increases relatively more in asthmatics from an already higher baseline. Recovery from the effects of O_3 on pulmonary function and symptoms is usually complete within 24 h of the end of exposure, although other responses may persist somewhat longer.

- An association between daily mortality and O_3 concentration for areas with high O_3 levels (e.g., Los Angeles) has been suggested, although the magnitude of such an effect is unclear.
- Increased O_3 levels are associated with increased hospital admissions and emergency department visits for respiratory causes. Analyses from data in the Northeastern United States suggest that O_3 air pollution is associated with a substantial portion (on the order of 10 to 20%) of all summertime respiratory hospital visits and admissions.
- Pulmonary function in children at summer camps in southern Ontario, Canada, in the northeastern United States, and in Southern California is associated with O_3 concentration. Meta-analysis indicates that a 0.50-mL decrease in FEV_1 is associated with a 1 ppb increase in O_3 concentration. For preadolescent children exposed to 120 ppb (0.12 ppm) ambient O_3, this amounts to an average decrement of 2.4 to 3.0% in FEV_1. Similar responses are reported for children and adolescents exposed to O_3 in ambient air or O_3 in purified air for 1 to 2 h while exercising.
- Pulmonary function decrements are generally observed in healthy subjects (8 to 45 years of age) after 1 to 3 h of exposure as a function of the level of exercise performed and the O_3 concentration inhaled during the exposure. Group mean data from numerous controlled human exposure and field studies indicate that, in general, statistically significant pulmonary function decrements beyond the range of normal measurement variability (e.g., 3 to 5% for FEV_1) occur
  (1) at >0.50 ppm O_3 when at rest,
  (2) at >0.37 ppm O_3 with light exercise (slow walking),
  (3) at >0.30 ppm O_3 with moderate exercise (brisk walking),
  (4) at >0.18 ppm O_3 with heavy exercise (easy jogging), and
  (5) at >0.16 ppm O_3 with very heavy exercise (running).
  Smaller group mean changes (e.g., <5%) in FEV_1 have been observed at lower O_3 concentrations than those listed above. For example, FEV_1 decrements have been shown to occur with very heavy exercise in healthy adults at 0.15 to 0.16 ppm O_3, and such effects may occur in healthy young adults at levels as low as 0.12 ppm. Also, pulmonary function decrements have been observed in children and adolescents at concentrations of 0.12 and 0.14 ppm O_3 with heavy exercise. Some individuals within a study may experience FEV_1 decrements in excess of 15% under these exposure conditions, even when the group mean decrement is less than 5%.
- For exposures of healthy subjects performing moderate exercise during longer duration exposures (6 to 8 h), 5% group mean decrements in FEV_1 were observed at
  (1) 0.08 ppm O_3 after 5.6 h,
  (2) 0.10 ppm O_3 after 4.6 h, and

(3) 0.12 ppm O_3 after 3 h.

For these same subjects, 10% group mean FEV_1 decrements were observed at 0.12 ppm O_3 after 5.6 and 6.6 h. As in the shorter duration studies, some individuals experience changes larger than those represented by the group mean changes.

- An increase in the incidence of cough has been reported at O_3 concentrations as low as 0.12 ppm in healthy adults during 1 to 3 h of exposure with very heavy exercise. Other respiratory symptoms, such as pain on deep inspiration, shortness of breath, and lower respiratory scores (a combination of several symptoms), have been observed at 0.16 to 0.18 ppm O_3 with heavy and very heavy exercise. Respiratory symptoms also have been observed following exposure to 0.08, 0.10, and 0.12 ppm O_3 for 6.6 h with moderate levels of exercise.
- Increases in nonspecific airway responsiveness in healthy adults have been observed after 1 to 3 h of exposure to 0.40 ppm, but not 0.20 ppm, O_3 at rest and have been observed at concentrations as low as 0.18 ppm, but not to 0.12 ppm, O_3 during exposure with very heavy exercise. Increases in nonspecific airway responsiveness during 6.6-h exposures with moderate levels of exercise have been observed at 0.08, 0.10, and 0.12 ppm O_3.

Short-term O_3 exposure of laboratory animals and humans disrupts the barrier function of the lung epithelium, permitting materials in the airspaces to enter lung tissue, allowing cells and serum proteins to enter the airspaces (inflammation), and setting off a cascade of responses.

- Increased levels of PMNs and protein in lung lavage fluid have been observed following exposure of healthy adults to 0.20, 0.30, and 0.40 ppm with very heavy exercise and have not been studied at lower concentrations for 1- to 3-h exposures. Increases in lung lavage protein and PMNs also have been observed at 0.08 and 0.10 ppm O_3 during 6.6-h exposures with moderate exercise; lower concentrations have not been tested.

Short-term O_3 exposure of laboratory animals and humans impairs alveolar macrophage clearance of viable and nonviable particles from the lungs and decreases the effectiveness of host defenses against bacterial lung infections in animals and perhaps humans. The ability of alveolar macrophages to engulf microorganisms is decreased in humans exposed to 0.08 and 0.10 ppm O_3 for 6.6 h with moderate exercise.

2. What are the effects of repeated, short-term exposures to ozone?

During repeated short-term exposures, some of the O_3-induced responses are partially or completely attenuated. Over a 5-day exposure, pulmonary function changes are typically greatest on the second day, but return to control levels by the fifth day of exposure. Most of the inflammatory markers (e.g., PMN influx) also attenuate by the fifth day of exposure, but markers of cell damage (e.g., lactate dehydrogenase enzyme activity) do not attenuate and continue to increase. Attenuation of lung function decrements is reversed following 7 to 10 days without O_3. Some inflammatory markers are also reversed during this time period, but others still show attenuation even after 20 days without O_3. The mechanisms and impacts involved in attenuation are not known, although animal studies show that the underlying cell damage continues throughout the attenuation process. In addition, attenuation may alter the normal distribution of O_3 within the lung, allowing more O_3 to reach sensitive

regions, possibly affecting normal lung defenses (e.g., PMN influx in response to inhaled microorganisms).

3. What are the effects of long-term exposures to ozone?

Available data indicate that exposure to O_3 for months and years causes structural changes in several regions of the respiratory tract, but effects may be of the greatest importance in the centriacinar regions (where the alveoli and conducting airways meet); this region typically is affected in most chronic airway diseases of the human lung. This information on O_3 effects in the distal lung is extrapolated from animal toxicological studies because, to date, comparable data are not available from humans. The apparent lack of reversal of effects during periods of clean air exposure raises concern that seasonal exposures may have a cumulative impact over many years. The role of adaptive processes in this response is unknown but may be critically dependent on the temporal frequency or profile of exposure. Furthermore, the interspecies diversity in apparent sensitivity to the chronic effects of O_3 is notable, with the rat representing the lower limit of response, and the monkey the upper limit. Epidemiological studies attempting to associate chronic health effects in humans with long-term O_3 exposure provide only suggestive evidence that such a linkage exists.

Long-term exposure in the females of one strain of mice to high O_3 levels (1 ppm) caused a small, but statistically significant increase in lung tumors. There was no concentration-response relationship, and rats were not affected. Genotoxicity data are either negative or weak. Given the nature of the database, potential carcinogenicity in animals is uncertain. Ozone did not show tumor-promoting activity in a chronic rat study (at 0.5 ppm O_3).

4. What are the effects of binary pollutant mixtures containing ozone?

Combined data from laboratory animal and controlled human exposure studies of O_3 support the hypothesis that coexposure to pollutants, each at low-effect levels, may result in effects of significance. The data from human studies of O_3 in combination with NO_2, SO_2, H_2SO_4, HNO_3, or CO show no more than an additive response on lung spirometry or respiratory symptoms. The larger number of laboratory animal studies with O_3 in mixture with NO_2 and H_2SO_4 show that effects can be additive, synergistic, or even antagonistic, depending on the exposure regimen and the endpoint studied. This issue of exposure to copollutants remains poorly understood, especially with regard to potential chronic effects.

5. What population groups are at risk as a result of exposure to ozone?

Identification of population groups that may show increased sensitivity to O_3 is based on their (1) biological responses to O_3, (2) preexisting lung disease (e.g., asthma), (3) activity patterns, (4) personal exposure history, and (5) personal factors (e.g., age, nutritional status).

The predominant information on the health effects of O_3 noted above comes from clinical and field studies on healthy, nonsmoking, exercising subjects, 8 to 45 years of age. These studies demonstrate that, among this group, there is a large variation in sensitivity and responsiveness to O_3, with at least a 10-fold difference between the most and least responsive individuals. Individual sensitivity to O_3 also may vary throughout the year, related to seasonal variations in ambient O_3 exposure. The specific factors that contribute to this large intersubject variability, however, remain undefined. Although differences may be due to the

dosimetry of O_3 in the respiratory tract, available data show little difference on O_3 deposition in the lungs for inhalation through the nose or mouth.

Daily life studies reporting an exacerbation of asthma and decrease in peak expiratory flow rates, particularly in asthmatic children, appear to support the controlled studies; however, those studies may be confounded by temperature, particle or aeroallergen exposure, and asthma severity of the subjects or their medication use. In addition, field studies of summertime daily hospital admissions for respiratory causes show a consistent relationship between asthma and ambient levels of O_3 in various locations in the Northeastern United States, even after controlling for independent contributing factors. Controlled studies on mild asthmatics suggest that they have similar lung volume responses but greater airway resistance changes to O_3 than nonasthmatics. Furthermore, limited data from studies of moderate asthmatics suggest that this group may have greater lung volume responses than nonasthmatics.

Other population groups with preexisting limitations in pulmonary function and exercise capacity (e.g., chronic obstructive pulmonary disease, chronic bronchitis, ischemic heart disease) would be of primary concern in evaluating the health effects of O_3. Unfortunately, not enough is known about the responses of these individuals to make definitive conclusions regarding their relative responsiveness to O_3. Indeed, functional effects in these individuals with reduced lung function may have greater clinical significance than comparable changes in healthy individuals.

Currently available data on personal factors or personal exposure history known or suspected of influencing responses to O_3 follow.

- Human studies have identified a decrease in pulmonary function responsiveness to O_3 with increasing age, although symptom rates remain similar. Toxicological studies are not easily interpreted but suggest that young animals are not more responsive than adults.
- Available toxicological and human data have not conclusively demonstrated that males and females respond differently to O_3. If gender differences exist for lung function responsiveness to O_3, they are not based on differences in baseline pulmonary function.
- Data are not adequate to determine whether any ethnic or racial group has a different distribution of responsiveness to O_3. In particular, the responses of nonwhite asthmatics have not been investigated.
- Information derived from O_3 exposure of smokers is limited. The general trend is that smokers are less responsive than nonsmokers. This reduced responsiveness may wane after smoking cessation.
- Although nutritional status (e.g., vitamin E deficiency) makes laboratory rats more susceptible to O_3-induced effects, it is not clear if vitamin E supplementation has an effect in human populations. Such supplementation has no or minimal effects in animals. The role of such antioxidant vitamins in O_3 responsiveness, especially their deficiency, has not been well studied.

Based on information presented in this document, the population groups that have demonstrated increased responsiveness to ambient concentrations of O_3 consist of exercising, healthy and asthmatic individuals, including children, adolescents, and adults.

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Appendix A

Abbreviations and Acronyms

ADOM	Acid Deposition and Oxidant Model
AGL	Above ground level
AIRS	Aerometric Information Retrieval System
AM	Alveolar macrophage
AQCD	Air Quality Criteria Document
AQCR	Air Quality Control Region
AUSPEX	Atmospheric Utility Signatures, Predictions, and Experiments
C	Carbon
C	Concentration
CA	Chromotropic acid
CAA	Clean Air Act
CAAA	Clean Air Act Amendments of 1990
CAL-RAMS	Coast and Lake Regional Atmospheric Modeling System
CAR	Centriacinar region
CASAC	Clean Air Scientific Advisory Committee
CBM	Carbon-bond mechanism
CCM	Community Climate Model
CFC	Chlorofluorocarbon
CH_3OH	Methanol
CH_4	Methane
CI	Chemical ionization
CIT	California Institute of Technology
CL	Chemiluminescence
CMB	Chemical mass balance
CNG	Compressed natural gas
CO	Carbon monoxide
CO_2	Carbon dioxide
CTWM	Complex Terrain Wind Model
DIAL	Differential absorption lidar

DNPH	2,4-Dinitrophenylhydrazine
DOAS	Differential optical absorption spectrometry
DWM	Diagnostic Wind Model
ECD	Electron capture detection
EKMA	Empirical Kinetic Modeling Approach
EMS	Emissions Modeling System
EPA	U.S. Environmental Protection Agency
EPEM	Event Probability Exposure Model
EPRI	Electric Power Research Institute
EPS	Emissions Preprocessor System
ERAQS	Eastern Regional Air Quality Study
ETBE	Ethyl-tertiary-butyl ether
EtOH	Ethanol
FDDA	Four-dimensional data assimilation
$FeSO_4$	Ferrous sulfate
FEV_1	Forced expiratory volume in 1 s
FVC	Forced vital capacity
FID	Flame ionization detection
FTIR	Fourier transform infrared absorption spectroscopy
GC	Gas chromatography
GMEP	Geocoded Model of Emissions and Projections
GPT	Gas-phase titration
H^+	Hydrogen ion
HC	Hydrocarbon
HCFC	Hydrochlorofluorocarbon
HCHO	Formaldehyde
HNO_2	Nitrous acid
HNO_3	Nitric acid
HO_2	Hydroperoxyl
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography
H_2SO_4	Sulfuric acid
IC	Ion chromatography
ID	Identification (number)
I/O	Indoor/outdoor

IR	Infrared radiation
IR	Incremental reactivity
LMOS	Lake Michigan Oxidant Study
LPG	Liquified petroleum gas
MBTH	3-Methyl-2-benzothiazolone hydrazone
MCCP	Mountain Cloud Chemistry Program
MM4/MM5	Mesoscale Model, versions 4 and 5
MOBILE	U.S. Environmental Protection Agency emissions model for mobile sources
MODELS 3	Modeling framework that consolidates all of the U.S. Environmental Protection Agency's three-dimensional photochemical air quality models
MPAN	Peroxymethacryloyl nitrate
MSA	Metropolitan Statistical Area
MSCET	Month and state current emissions trends
MTBE	Methyl-tertiary-butyl ether
NA	Not available
NAAQS	National Ambient Air Quality Standards
NADP	National Atmospheric Deposition Program
NAMS	National Air Monitoring Station
NAPAP	National Acid Precipitation Assessment Program
NAPBN	Western National Air Pollution Background Network
NAS	National Academy of Sciences
NBKI	Neutral buffered potassium iodide
NBS	National Bureau of Standards; now National Institute of Standards and Technology
NCAR	National Center for Atmospheric Research
NCLAN	National Crop Loss Assessment Network
NDDN	National Dry Deposition Network
NEM	National Air Quality Standards Exposure Model
NF	National forest
NH_3	Ammonia
NH_4HSO_4	Ammonium bisulfate
NH_4OH	Ammonium hydroxide
$(NH_4)_2SO_4$	Ammonium sulfate

NIST	National Institute of Standards and Technology
NM	National monument
NMHC	Nonmethane hydrocarbon
NMOC	Nonmethane organic compound
NO	Nitric oxide
NO_2	Nitrogen dioxide
N_2O	Nitrous oxide
NO_3^-	Nitrate
NO_x	Nitrogen oxides
NP	National park
NPN	*n*-propyl nitrate
NTP	National Toxicology Program
O_3	Ozone
OAQPS	Office of Air Quality Planning and Standards
Obs.	Observations
OH	Hydroxyl
OHBA	Hydroxybenzoic acid
PAMS	Photochemical Aerometric Monitoring System
PAN	Peroxyacetyl nitrate
PANs	Peroxyacyl nitrates
PAR	Proximal alveolar region
PBL	Planetary boundary layer
PBzN	Peroxybenzoyl nitrate
PDFID	Cryogenic preconcentration-direct flame ionization detection
PF/TPLIF	Photofragmentation two-photon laser-induced fluorescence
pH	Hydrogen ion concentration
PL	Liquid-phase vapor pressure
PLANR	Practice for Low-cost Application in Nonattainment Regions
PMN	Polymorphonuclear leukocyte (also called neutrophil)
ppmC	Parts per million carbon
PPN	Peroxypropionyl nitrate
PSD	Passive sampling device
PVOC	Polar volatile organic compound
Q_E	Latent heat flux
Q_H	Heat flux
r	Linear regression correlation coefficient

R^2	Multiple correlation coefficient
RADM	Regional Acid Deposition Model
RAPS	Regional Air Pollution Study
REHEX	Regional Human Exposure Model
RMSD	Root-mean-square difference
ROG	Reactive organic gas
ROM	Regional Oxidant Model
ROMNET	Regional Ozone Modeling for Northeast Transport program
RT	Respiratory tract
SAB	Science Advisory Board
SAI	Systems Applications International
SAPRC	Statewide Air Pollution Research Center, University of California, Riverside
SARMAP	San Joaquin Valley Air Quality Study (SJVAQS)/Atmospheric Utility Signatures, Predictions, and Experiments (AUSPEX) Regional Model Adaptation Project
SAROAD	Storage and Retrieval of Aerometric Data (U.S. Environmental Protection Agency centralized database; superseded by Aerometric Information Retrieval System [AIRS])
SCAQS	South Coast Air Quality Study (California)
SIP	State Implementation Plan
SLAMS	State and Local Air Monitoring Station
SJVAQS	San Joaquin Valley Air Quality Study
SO_2	Sulfur dioxide
SO_4^{2-}	Sulfate
SOS	Southern Oxidant Study
SRM	Standard reference material
SRP	Standard reference photometer
STEM-II	Sulfur Transport Eulerian Model (version II)
SUM06	Seasonal sum of all hourly average concentrations 0.06 ppm
SUM07	Seasonal sum of all hourly average concentrations 0.07 ppm
SUM08	Seasonal sum of all hourly average concentrations 0.08 ppm
SURE	Sulfate Regional Experiment Program
T	Temperature
TAMS	Toxic Air Monitoring Study (U.S. Environmental Protection Agency)
TDLAS	Tunable-diode laser absorption spectroscopy

TEA	Triethanolamine
Tg	Teragram
TGTP	The Global Thinking Project
TNMHC	Total nonmethane hydrocarbons
TPLIF	Two-photon laser-induced fluorescence
TTFMS	Two-tone frequency-modulated spectroscopy
UAM	Urban Airshed Model
UV	Ultraviolet
UV-B	Ultraviolet radiation of wavelengths 280 to 320 nm
VMT	Vehicle miles traveled
VOC	Volatile organic compound
$\dot{V}_E$	Minute ventilation; expired volume per minute
WFM	White Face Mountain
WMO/UNEP	World Meteorological Organization/United Nations Environment Program
W126	Cumulative integrated exposure index with a sigmoidal weighting function