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Review of the California Ambient Air Quality Standard For Ozone

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California Environmental Protection Agency

Air Resources Board
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11 Health Effects of Ozone Exposure

11.1 Ozone Dosimetry

11.1.1 Uptake

Ozone (O_3) is a highly reactive gas, with a negligible half-life in liquid or solid media. Therefore, O_3 uptake is limited to anatomic air-liquid interfaces, particularly the mucous membranes of the respiratory tract. Ozone uptake represents an example of reactive absorption, in which it reacts with oxidizable substances on the inner walls of the respiratory tract.

11.1.2 Human Studies

Ozone absorption in the respiratory tract has been studied using multiple approaches, which in general have yielded reasonably consistent results (US EPA 1996). In resting subjects, approximately 40 to 50% of inspired O_3 is taken up in the nose, mouth and throat, while upwards of 90% of the O_3 reaching the lower respiratory tract is removed, principally in the conducting airways, resulting in a total respiratory tract uptake of approximately 90% (range of estimates 76 - 97%) (US EPA 1996; Gerrity 1995; Gerrity et al. 1988; Hu et al. 1992a; Asplund et al. 1996; Hu et al. 1992b; Johansen et al. 1992). One team of investigators reported that oral or oronasal (compared with exclusively nasal) breathing resulted in small, but statistically significant, increases in extrathoracic uptake of O_3 in human subjects (Gerrity et al. 1988). However, Kabel et al. (1994) reported greater uptake efficiency of nasal versus oral breathing, suggesting that exercise sufficient to shift the breathing pattern from nasal to oronasal or oral, could result in an increased dose of O_3 reaching the distal lung. The importance of this observation is related to the nature of the meteorology that favors O_3 formation: i.e., the warm, sunny days that invite outdoor activity.

However, two studies have reported no differences in O_3-induced changes in pulmonary function or respiratory symptoms among volunteers breathing orally, oronasally, or nasally, when O_3 was administered via a facemask (Adams et al. 1989; Hynes et al. 1988). These reports suggest that O_3 is likely to be scrubbed more or less equally by the nose and mouth. Ozone removal efficiency increases directly with increasing concentration and inversely with breathing rate (Gerrity et al. 1988). Continuous O_3 inhalation decreases the efficiency of O_3 absorption in the central airways, facilitating increased delivery of O_3 to the deep lung (Asplund et al. 1996). Presumably this occurs because of the reduction of reactive mucus substrates in the airway. Reduction of tidal volume, a common functional response to O_3 exposure, results in a significant decline in lower respiratory tract uptake of O_3 (Gerrity et al. 1988). Increasing ventilation rates decrease absorption by the upper and lower airways, enhancing O_3 penetration to the deep lung (Hu et al. 1992a; Hu et al. 1992b).

11.1.3 Animal Studies and Animal to Human Extrapolation

Several experimental and theoretical dosimetry studies have estimated the amount or rate of O_3 absorbed by target sites within the respiratory tract. An understanding of the dosimetry of O_3 can assist in extrapolation of animal data to estimate human responses. Mathematical models have incorporated species differences in airway anatomy,

regional airway differences in O_3 dose, and physicochemical interactions within the liquid lining layer of the upper and lower respiratory tracts (Miller et al. 1985; Overton et al. 1987). These models support experimental animal studies that suggest that the primary site of lung damage due to O_3 inhalation is in the centriacinar region. Experimental dosimetry studies with ^{18}O-labeled O_3 show that exercising humans had four- to five-fold greater ^{18}O concentration in their BAL fluid constituents than rats exposed at rest to an identical O_3 concentration (Hatch et al. 1994). While this finding supports the conclusion that experimental rodent species are more resistant than humans to O_3-induced injury at a given dose, differences in exertion level show that ventilatory parameters, such as tidal volume (V_T) and respiratory frequency (f_R) are also important determinants of O_3 dose to target tissues. Nevertheless, theoretical models have predicted greater sensitivity of humans compared to rodents, in that a given exposure concentration of O_3 may result in a local dose to the lower lung of rats roughly half that predicted for humans (Cheek et al. 1994; Overton et al. 1987; Gerrity et al. 1988). This comparative dosimetry is consistent with greater effects of O_3 on lung function in humans than in animals (Costa et al. 1989; Overton et al. 1987). While knowledge of dosimetry has allowed quantitative animal-to-human extrapolation for effective O_3 doses, species sensitivity issues, such as antioxidant status, metabolic rates, and repair/defense mechanisms, are also important determinants of effective O_3 dose and are not as well defined.

11.1.4 Effective Dose Concept

The degree of response to O_3 is related to three factors: 1) the O_3 exposure concentration, 2) V_E, the ventilation rate, and 3) T, the duration of exposure. The consensus of available research is that O_3 concentration is the most important of the three factors (Adams 2003a; Adams et al. 1981; Folinsbee et al. 1978). Adams (2003a) provides an illustration of the significance of O_3 concentration, compared to V_E and V_T. The subjects in this study completed 6.6-hour exposures to 0.08 ppm O_3 (protocol described in Section 11.4.3.2), and 2-hour exposures to 0.30 ppm with alternating 15 min periods of rest and exercise. Although the inhaled O_3 dose in the 2-hour protocol was only 1.44 times greater than that for the 6.6-hour protocol, the decrements in FEV1 averaged -3.51% following the 6.6-hour protocol (0.08 ppm), and –12.36% following the 2-hour protocol (0.30 ppm). Normal, healthy people exposed to O_3 concentrations $\geq$ 0.12 ppm (the federal one-hour standard) typically develop significant, transient reversible decrements in pulmonary function if V_E or T are increased sufficiently.

11.2 Mechanisms of Ozone Toxicity

11.2.1 Introduction

Acute responses reported to occur with controlled exposures to O_3 concentrations within the historical ambient range (up to 0.5 ppm) include alterations in: 1) lung function, 2) airway caliber, 3) bronchomotor responsiveness, 4) symptoms, 5) breathing pattern, and 6) airway inflammation.

Several lines of evidence point to involvement of more than one biological mechanism in mediating responses to O_3 exposure. For example, research has shown that the onset of increased airway resistance (R_{aw}) with O_3 exposure is rapid onset (Beckett et al. 1985) compared with the gradual onset of decrements in forced expiratory endpoints

(Kulle et al. 1985). There is also evidence that while pulmonary function is substantially recovered by 12 to 20 hours after O_3 exposure (Foster et al. 2000; Hiltermann et al. 1995) after O_3 exposure, methacholine responsiveness remains increased. Folinsbee and Hazucha (2000) reported that young adult females exposed for 75 min to 0.35 ppm O_3 still demonstrated reduced pulmonary function and increased airway hyperresponsiveness at 18 hour post exposure, although all endpoints had returned to baseline by 42 hour after O_3 exposure.

Results also indicate that exposure to O_3 typically results in reductions in lung function and in excess symptoms of respiratory irritation. Some studies have reported that lung function decrements and symptoms are correlated (Horstman et al. 1990; Kulle et al. 1985; Adams et al. 1981). However, when Aris et al. (1995), in a considerably larger study (n=66), examined data on an individual level, there were subjects who developed responses of one but not the other category, or whose responses of one type were not proportional to those of the other. On a group level, however, pulmonary function changes and symptoms were weakly correlated. Ostro et al. (1989) used logistic regression models to reanalyze data from four controlled human exposure studies, and concluded that a 10% decline in FEV1 was associated with a 30% increase in the probability of the subject also having a respiratory symptom, and a 15% increase in the probability of the subject having a respiratory symptom of moderate intensity. O_3-induced increases in R_{aw} appear to be poorly correlated with changes in pulmonary function (McDonnell et al. 1983; Aris et al. 1995).

There is also evidence for a relatively weak association between the degree of nonspecific airway responsiveness and individual-specific symptomatic and lung function responses to O_3 (Aris et al. 1995). McDonnell et al. (1987) and Frampton et al. (1997) have reported that bronchial responsiveness was not predictive of pulmonary function changes, although Hackney et al. (1989) reported a significant relationship for the individual subjects in the group who were O_3-responsive, compared to those who were not. Results by Kreit et al. (1989) and Aris et al. (1991) lend support to the hypothesis that nonspecific airway responsiveness is a risk factor for O_3 sensitivity. Aris et al. (1995) suggested that the differences among these studies may be related, at least in part, to differences in the innate O_3-responsiveness of the subjects in the groups studied by the various investigators. Moreover, Aris et al.'s subject group (n=66) was considerably larger than the others cited, resulting in greater statistical power. However, uncertainty remains as to the distribution of O_3-responsiveness in the population as a whole, and the degree to which the subjects studied by these investigators are representative of the general population is unknown. It is clear from these data that in some individuals there is a relationship between baseline bronchial responsiveness and O_3-sensitivity, but this relationship does not appear to be universal at the individual level.

The available literature suggests that airway inflammation peaks several hours after exposure ends, at a time when pulmonary function decrements and symptoms have largely resolved (Foster et al. 2000; Hiltermann et al. 1995; Schelegle et al. 1991). There is also evidence for a relatively weak association between degree of nonspecific airway responsiveness and individual-specific symptomatic and lung function responses to O_3 (Aris et al. 1995). Several studies have reported that changes in pulmonary

function and symptoms are not significantly associated with airway inflammation (Balmes et al. 1996; Schelegle et al. 1991; Frampton et al. 1997; Torres et al. 1997; Jorres et al. 2000; Holz et al. 1999), substantiating that multiple mechanisms are involved in mediating the various responses observed in response to O_3 exposure.

Given the lack of consistent temporal associations and correlations between these various endpoints, it is likely that multiple biological mechanisms mediate the various responses observed consequent to acute O_3 exposure.

Collectively, the available data suggest several mechanisms as mediating the observed responses to acute O_3 exposure. These mechanisms can be broadly categorized as neural or inflammatory. Due to the nature of this document, the descriptions below discuss these mechanisms on a relatively general level, as a detailed review of the evidence for each potential mechanism, such as details of each individual reaction, chemical or cell type involved, is beyond the scope of this review.

11.2.2 Neural Mechanisms

11.2.2.1 Vagal

Parasympathetic innervation of the lungs is via the vagus nerves. These are large neurons that contain several different types of cholinergic nerve fibers whose primary mediator is the neurotransmitter acetylcholine. Stimulation of vagal fibers that innervate airway smooth muscle fibers results in bronchoconstriction and increased airway resistance; activity of the parasympathetic nervous system is the principal determinant of airway tone. Studies by Beckett et al. (1985) and Adams (1986) support the involvement of a vagal reflex mechanism for O_3-induced bronchoconstriction. Studies in dogs by Gertner et al. (1983a,b) also showed that O_3-induced changes in airway resistance were initially mediated through the parasympathetic system, while later phase responses were partially mediated by histamine.

Further, Holtzman et al. (1979) reported that O_3 inhalation leads to hyperresponsiveness to methacholine, suggesting that acute O_3 exposure leads to increased sensitivity of airway smooth muscle to acetylcholine, independent of a vagal reflex mechanism. Observations that circulating epinephrine increases in exercising subjects in a correlated fashion proportional to workload suggests that stimulation of airway smooth muscle beta-adrenoreceptors may be activated to counteract O_3-induced airway smooth muscle contraction (Galbo 1983; Warren and Dalton 1983).

A recent study by Schelegle et al. (2001) used inhaled tetracaine, a local anaesthetic, to block parasympathetic pathways in the lungs of subjects exposed to 0.30 ppm O_3 for 65 min in an attempt to separate the neural pathways involved in O_3-induced changes in pulmonary function, respiratory symptoms, and ventilatory pattern. The results suggest that vagal afferent endings located within the large conducting airways of the tracheobronchial tree are primarily responsible for O_3-induced subjective symptoms. The results also provide evidence that O_3-induced inhibition of maximal inspiratory effort is due to a reflex, and is unrelated to sensations of inspiratory discomfort. In addition, the data suggest that parasympathetic afferent endings located in more distal airways, and possibly the alveoli, mediate the majority of the O_3-induced reduction in inspiratory

capacity and development of a rapid shallow breathing pattern, although further studies are required to confirm this.

11.2.2.2 C-Fibers

C-fibers are one of the sub-types of vagal afferents located in the smooth muscle layer of the airways. These fibers are involved in local regulation of bronchomotor tone and vascular permeability through release of biochemicals such as substance P and other tachykinins. Tachykinins are neuropeptides that function as neurotransmitters. Functionally, they act as neuromodulators that regulate stress responses, pain, and control of vasomotor tone. They also have a nociceptor, or pain/irritation sensory, function. These fibers can be activated through mechanical or chemical stimulation by a variety of anaphylactic mediators, prostaglandins, other autocoids and toxic chemicals, in addition to acetylcholine, the usual parasympathetic mediator. Several investigators have suggested that one mechanism by which O_3 acts may be through stimulation of C-fibers via either an axonal or spinal reflex that inhibits the inspiratory muscles, preventing deep inhalation (Hazucha et al. 1989; Passannante et al. 1998; Schelegle et al. 1993; Coleridge et al. 1993). Reduction in maximal inhalation reduces FVC, and by extension, expiratory flow rates, because expiratory flow rate is a function of the lung's elastic recoil pressure. Elastic recoil pressure is proportional to lung volume. Studies in human and animal subjects (Hazucha et al. 1989; Passannante et al. 1998; Schelegle et al. 1993; Coleridge et al. 1993) point to this mechanism as mediating involuntary inhibition of full inspiration, which leads to reduction in forced vital capacity (FVC), and a concomitant decrease in maximal expiratory flow rates. The study by Passannante et al. (1998) also supports the notion that bronchial C-fibers contribute to O_3-induced symptoms of respiratory discomfort and irritation.

Exercising subjects exposed to O_3 typically demonstrate alterations in breathing pattern, including a decrease in tidal volume (V_T) and a compensatory increase in respiratory frequency (f_R), but no change in ventilation (V_E) (Adams et al. 1981; Folinsbee et al. 1978; McDonnell et al. 1983; Kulle et al. 1985). Reduction in V_T is likely related to the reduction in inspiratory capacity (IC), as described above, and possibly also to breathing discomfort caused by pain on deep inspiration. Studies in dogs (Lee et al. 1979; Schelegle et al. 1993) suggest that the rapid, shallow breathing pattern induced by O_3 inhalation is related to O_3 stimulation of C-fiber afferents through reflex inhibition of inhalation, which leads to a smaller V_T (Lee et al. 1979; Schelegle et al. 1993). Consequently, to maintain adequate V_E, f_R increases to compensate for the reduced V_T.

Biochemical evidence for involvement of bronchial C-fibers comes from studies by Hazbun and colleagues (1993), who reported significant increases in alveolar lavage fluid substance P concentration after O_3 exposure, compared to after filtered air exposure. Substance P is a C-fiber neuropeptide that is released when C-fibers in the airways are stimulated. It is degraded by neutral endopeptidase, an airway lining enzyme that is inhibited by oxidants (Murlas et al. 1990). Hazbun et al.'s (1993) findings support the notion that O_3, as a strong oxidant, diminishes neutral endopeptidase activity (not measured in this study), resulting in an increase in substance P (Murlas et al. 1990; Murlas et al. 1992) release from the afferent endings of bronchial C fibers during excitation, based on analysis of segmental airway washings of healthy subjects who inhaled 0.25 ppm O_3, compared to filtered air.

11.2.2.3 Rapidly Adapting Stretch Receptors

Rapidly adapting pulmonary receptors, also known as “irritant receptors,” are located in the major bronchi. When they are stimulated, they initiate hyperventilation and bronchoconstriction. Evidence suggests that O_3 stimulation of these fibers contributes to symptoms of pulmonary irritation that lead to involuntary inhibition of maximal inhalation (reduced inspiratory capacity), and symptoms of respiratory irritation, such as pain on deep breath and coughing (e.g., Bates et al. 1972; Lee et al. 1979; Schelegle et al. 1993; Coleridge et al. 1993; Coleridge et al. 1976, Coleridge et al. 1978; Hazucha et al. 1989; Hazbun et al. 1993).

11.2.2.4 Summary

Available data suggest that O_3 stimulation of vagal afferents, including C-fibers and rapidly adapting receptors, resulting in vagal reflexes is the principal mechanism for responses measured during and shortly after O_3 exposure. These responses include reduced pulmonary function, increased airway resistance, increased frequency of respiration (f_R), reduced tidal volume (V_T), and increased symptoms of respiratory irritation.

11.2.3 Biochemical and Inflammatory Mechanisms

11.2.3.1 Introduction

Inflammation is a stereotypical biological response to injury and infection. It has several features, the purposes of which are to kill infectious agents, and clear injured cells. Inflamed areas are characterized by vasodilatation of local blood vessels, increased capillary permeability, fluid leakage into the interstitial spaces, release of proteins from damaged cells, migration of macrophages and neutrophils to the damaged area, and swelling of tissue cells. Damaged cells, as well as activated macrophages and neutrophils release a number of mediators that recruit inflammatory cells, and regulate the inflammatory process.

11.2.3.2 Mechanisms of Inflammation – Human Studies

Inflammation begins with tissue damage, and release of proteins and inflammatory mediators from the damaged cells. This is followed by migration of alveolar macrophages to the damaged sites where they begin phagocytizing damaged cells. Activated macrophages also release mediators that recruit neutrophils to the lungs from the blood over the first few hours after the onset of inflammation. If inflammation is severe, neutrophils are also recruited from the bone marrow. Neutrophil recruitment appears to peak at several hours after the beginning of inflammation. As inflammation continues, blood monocytes enter the inflamed tissue, where they enlarge to become macrophages. This phase of inflammation is much slower than the invasion of neutrophils, because the storage pool of monocytes is small, and they require at least 8 hours to develop into macrophages. After several days to weeks, macrophages again dominate the phagocytic cells of the inflamed area due to stimulation of the bone marrow to increase monocyte production. However, it takes three to four days for newly formed immune cells to develop to the stage where they leave the bone marrow. The marrow can produce these cells in tremendous quantities for months or years at rates as high as 20 to 50 times normal if inflammation continues (Guyton and Hall 1996).

O_3 does not penetrate cell membranes, although it appears to initiate a cascade mechanism that begins with reaction with unsaturated fatty acids at the air-tissue barrier interface. There is considerable evidence that O_3 exposure results in airway inflammation of the lung tissues (see Sections 11.4.3.4 and 11.4.3.6.6; Tables 11-5, 11-8, 11-11, and 11-12). Given the chemical reactivity of O_3, it is unlikely that it penetrates far into tissues, or that it can pass unreacted into cells, much less penetrate into lung tissue (Pryor 1992). It is more likely that O_3 reacts with the fluid lining the respiratory tract (epithelial lining fluid, or ELF) or in areas where the ELF is thin or absent with the epithelial cell membranes. Further, it is likely that O_3 primarily reacts with lipids. The fluid lining the respiratory tract ELF and the epithelial cell membranes are constituted of about 90% lipid, with a high concentration of unsaturated fatty acids. Ozonation of lipids results in small, diffusible products that are structurally similar to known lipid-derived signal transduction species. If ozonation occurs in an area with significant water content, such as the lungs, ozonation produces aldehydes, hydroperoxides and small amounts of the Criegee ozonides (Pryor et al. 1995; Frampton et al. 1999; Leikauf et al. 1993). These ozonation products stimulate airway epithelial cells to respond with release of a variety of pro-inflammatory lipid mediators, for example eicosanoids (Schelegle et al. 1989; Leikauf et al. 1995), platelet activating factor (PAF), reactive oxygen species, and cytokines (Pryor et al. 1995).

In vitro studies by Devlin et al. (1994) have shown that epithelial cells exposed to O_3 secrete increased amounts of interleukin-6 (IL-6), IL-8 and fibronectin, all inflammatory mediators. The results also showed that macrophages similarly exposed to O_3 were not stimulated to produce any of these substances. Another study by Devlin et al. (1996), showed that the concentrations of some inflammatory mediators, IL-6 and prostaglandin E_2 (PGE_2), were higher one hour after O_3 exposure, while fibronectin and tissue-plasminogen activator levels were higher 18 hour after O_3 exposure. The number of polymorphonuclear cells and the protein concentrations in the fluid recovered at in the study subjects' bronchoalveolar lavage fluid (BALF) were similar at both times.

Cytokines released by damaged epithelial cells recruit macrophages and neutrophils to the lungs and activate them. Then, these activated cells also secrete various cytokines and chemokines that contribute to the burden of inflammatory mediators that appear in the lung consequent to O_3 exposure. Because it takes several hours for the inflammatory cascade to become activated, available data suggest that the cascade pathway is responsible for responses to O_3 exposure that are not evident until several hours after exposure, when pulmonary function and symptoms responses have largely abated (Foster et al. 2000; Schelegle et al. 1991; Blomberg et al. 1999). Presence of these inflammatory mediators in BALF is indicative of epithelial injury and inflammation (Foster and Stetkiewicz 1996; Aris et al. 1993b; Balmes et al. 1996; Koren et al. 1989b).

There is evidence that some of the mediators released through this cascade mechanism contribute to the immediate responses to O_3 exposure, as well as to late-phase responses. For example, prostaglandins and cyclooxygenase products of arachidonic acid appear to play a role in pulmonary function responses to O_3 exposure (Coleridge et al. 1976; Coleridge et al. 1978; Schelegle et al. 1987; Eschenbacher et al. 1989; Ying et al. 1990) in the short-term. Koren et al. (1991) reported a positive correlation between O_3-induced pulmonary function decrements and the level of

prostaglandin E_2 in bronchoalveolar lavage fluid collected within 1 hour of the end of exposure in human subjects who varied greatly in O_3 responsiveness. Hazucha et al. (1996) concluded that the changes in inflammatory markers including PGE_2, thromboxane B_2 (TBX_2), IL-6 and percentage of neutrophils in BALF one-hour following O_3 exposure were not significantly related to the magnitude of responses in forced vital capacity (FVC), forced expiratory volume in one second (FEV1) or specific airway resistance (SR_{AW}) in subjects who were pretreated with placebo or the cyclooxygenase inhibitor ibuprofen.

Collectively, the available data suggest that, while the chemical substances released during the early part of the cascade mechanism contribute to the short- term effects on pulmonary function and symptoms observed with O_3 exposure, the cascade mechanism is primarily related to development of airway inflammation, which occurs over a longer time-frame. The data also indicate that O_3-induced inflammatory responses can amplify oxidative damage to the lung tissues due to O_3 directly, and to its initial reaction products.

11.2.3.3 Mechanisms of Inflammation - Animal Studies

Ozone likely initiates inflammation of pulmonary tissue through the process of lipid peroxidation of unsaturated fatty acids in cellular membranes. Changes in cell membranes may alter cell metabolism and biochemistry leading to injury or death of the cells. Consequences of O_3-induced lung inflammation include disruption of the pulmonary epithelial barrier, resulting in increased transmucosal permeability, and recruitment of inflammatory cells to lung airways (Bhalla 1999). In addition, O_3-induced inflammation and increased permeability can enhance the accumulation of inhaled particles in interstitial lung tissue, where clearance to blood is very slow. Even though rodents appear to be more resistant to the inflammatory effects of O_3 compared to humans, the permeability and inflammatory findings of the rodent data parallel the data from counterpart studies in humans using similar exposure protocol and effect parameters. Recent work supports previously reviewed studies, in that indications of inflammatory and permeability changes in the lungs of experimental animals occur at O_3 concentrations as low as 0.1-0.13 ppm. Inflammatory and permeability effects in rodents occurred with nighttime exposure to 0.13 ppm O_3 for 4 hours (Rombout et al. 1989; Van Bree et al. 1995). In larger mammals, acute O_3 exposures have resulted in inflammatory reactions at 0.2 ppm with 6 hour exposure in dogs (Freed et al. 1999) and 0.4 ppm with 2 hour exposure in monkeys (Plopper et al. 1998). With repeated exposure, inflammatory responses in rabbits occurred following 2-hour daily exposures to O_3 concentrations as low as 0.1 ppm for 7 days (Driscoll et al. 1987). Chronic exposure of rats to an urban profile of O_3 that reached a daily peak concentration of 0.25 ppm resulted in pulmonary inflammation only during the first 1-3 weeks of exposure (Chang et al. 1992). An attenuation of the inflammatory response occurs with repeated exposure to O_3, while other effects of O_3 exposure, including morphological and biochemical effects, may not attenuate (Tepper et al. 1989). A detailed summary of the animal toxicology literature is provided in Appendix A.

11.2.4 Mutagenic and Carcinogenic Potential of Ozone

Ozone has been shown to be genotoxic and mutagenic in some, but not all, *in vitro* and *in vivo* bacterial and animal test systems (Victorin 1996). The extreme reactivity, gaseous nature, and toxicity of O_3 likely present difficulties for these test systems. Lung tumor development studies that employed less-than-lifetime exposures in rats, hamsters and mice at concentrations as high as 0.8 ppm were either negative or ambiguous for carcinogenicity (Witschi et al. 1993; Ichinose and Sagai 1992; Hassett et al. 1985; Last et al. 1987; Witschi et al. 1999). These studies included carcinogenicity experiments with A/J mice, reported to be susceptible to lung tumor formation in response to some carcinogens. In two-year and lifetime carcinogenicity studies conducted by the National Toxicology Program, O_3 was determined to be carcinogenic in female mice, uncertain in male mice, and not carcinogenic in rats (Herbert et al. 1996; Boorman et al. 1994). In mice, there was a trend toward increased incidences of lung neoplasms with increasing O_3 exposure (0.12, 0.5, and 1.0 ppm), but only female mice exposed to 1.0 ppm O_3 exhibited an increased incidence of lung neoplasms over control values. Unique mutations, together with a higher frequency of mutations, were found on the K-ras gene of O_3-induced neoplasms of mice, suggesting O_3 exposure leads to DNA damage on this proto-oncogene (Sills et al. 1995). Co-carcinogenicity studies with pulmonary carcinogens are negative or ambiguous for O_3 acting as a tumor promoter (Boorman et al. 1994; Witschi et al. 1993; Hassett et al. 1985; Ichinose and Sagai 1992; Last et al. 1987). There are no human studies on this subject.

11.2.5 Summary

It is clear that several mechanisms interact to produce the spectrum of responses observed following O_3 exposure. The apparent chain of events seems to include factors that influence O_3 delivery to the tissue (i.e., inhaled concentration, breathing pattern and airway geometry, and exposure duration. O_3 reactions with components in airway surface liquid and epithelial cell membranes, local tissue responses including tissue injury and inflammation, as well as stimulation of bronchial C-fiber afferents and rapidly adapting receptors, and vagal reflex responses also contribute to observed responses. Injured airway epithelial cells release cyclooxygenase metabolites of arachidonic acid, as well as other chemical mediators. This leads to recruitment of inflammatory cells to the lungs, and their activation to release inflammatory mediators.

Responses that develop during and shortly after O_3 exposure seem to be primarily mediated by vagal fibers. Later phase responses seem related primarily to inflammatory mechanisms activated by injury to epithelial cells. The interrelationships among these factors and how each contributes to the pulmonary responses induced by O_3 inhalation are not entirely understood.

11.3 Morphological Effects

11.3.1 Introduction

The tissue response of the respiratory system to exposure to oxidant air pollutants such as O_3 follows a well-characterized pattern of cellular injury, inflammatory and repair events, which is highly dependent upon the inhaled concentration and the length of the exposure. There is a clear acute dose-response relationship for the initial exposure of naïve animals and humans under experimental conditions. The initial cellular injury sets initiates a series of inflammatory and repair processes which follow a relatively uniform time course regardless of the extent of the acute injury, unless it is sufficiently massive as to be fatal. Under experimental conditions, these repair processes lead to the reestablishment of the pre-exposure steady state within a finite period of time. Imposition of additional periods of exposure to injurious concentrations during the repair process alters the cellular events and leads to the establishment of a new steady-state where inflammation is markedly reduced and the cells which repopulate the injury site are resistant to further acute injury by oxidant gases. This is true regardless of how long the exposures are continued. Despite the very large number of long-term exposure studies, the utility of experimental animal studies for estimating the long-term risk to human populations of ambient exposure conditions appears limited. One of the limitations is that concentration multiplied by time does not appear to equal effect. Depending on the measures used to assess effects, the response may actually appear to diminish over time. A second limitation is that ambient conditions are such that the periods when oxidant gas concentrations are elevated to levels, which can produce injury are highly variable. The period below threshold concentrations can vary from as little as 18 hours to as long as many months. Additionally, these periods generally cycle annually.

All animal species studied show generally similar morphological responses to <1960 $\mu g/m^3$ (1 ppm) O_3. The precise characteristics of the structural changes due to O_3 are dependent on the exposure regimen, time of examination, distribution of sensitive cells, and the type of centriacinar region (i.e., junction between the end of the terminal bronchioles and the first few generations of either respiratory bronchioles or alveolar ducts, depending on the species). Primates (e.g., humans, monkeys) have terminal bronchioles leading to respiratory bronchioles, which have gas exchange areas proximal to the alveolar ducts. In small laboratory animals, the terminal bronchioles typically lead directly to alveolar ducts; if respiratory bronchioles are present, they are rudimentary. Although all regions of the respiratory tract are affected, the centriacinar region, which is predicted to receive the highest dose of O_3, is the site of the most pronounced lesion. The lesions extend further into the acinus with increasing concentration or duration of exposure, but alveoli at the periphery of an acinus have not been reported to be affected. In the nasopharyngeal and tracheobronchial regions, the ciliated epithelial cells are the most sensitive to O_3. Cilia are lost or altered and cells are destroyed, depending upon exposure regimens. Damage to the ciliated cells appears most severe in the terminal or respiratory bronchioles (depending on species), where these cells are replaced by nonciliated bronchiolar (Clara) cells, which themselves show morphological changes. The loss of ciliated epithelium is likely to affect mucocillary clearance, and the hyperplasia of nonciliated bronchiolar (Clara) cells, which are rich in

mixed function oxidases, is likely to impact lung xenobiotic metabolism. In the centriacinar region, type 1 cells (the most sensitive), across which gas-exchange occurs, are destroyed and replaced by the thicker type 2 cells, which thickens the interaveolar septa. Inflammatory changes (e.g., increased PMNs and AMs and edema) also occur in this region.

Responses to duration of exposure are not linear. As exposure continues, there is an initial (over about the first week) wave of inflammation with necrosis and sloughing of more sensitive cell phenotypes (ciliated cells and alveolar type 1 cells) followed by proliferation of more resistant cell phenotypes (Clara cells and alveolar type 2 cells), replacing the ciliated and alveolar type 1 cells. Soon these changes return to near-control or control levels, but then over months of exposure, they slowly increase again and typically persist during exposure. Remodeling of distal airways and the centriacinar region results from the cumulative effects of cell necrosis followed by replacement by a different type of epithelium. Walls of centriacinar alveoli thicken with inflammatory cells and collagen. Thus, the changes become more chronic in nature. The remodeling of the centriacinar region and the increased thickness of the interstitium may impact pulmonary function, if O_3 levels are sufficiently high. The hyperplasia of nonciliated bronchiolar and alveolar type 2 cells and macrophages, rich in antioxidant and proteolytic enzymes, respectively, can result in multiple types of biochemical changes. The increase in fibroblasts and collagen can lead to development of interstitial fibrosis, which may persist after long-term exposure ceases.

Because morphological changes are central to several of the classes of effects of O_3 and can be diagnostic of various forms of lung disease, but cannot normally be examined in humans, there is great interest in extrapolating them from animals to humans. Qualitatively, the extrapolation is strong because numerous studies of several species of laboratory animals, ranging from mice to nonhuman primates, have shown similar types of O_3-induced changes, in spite of species anatomical and ventilatory differences. Comparison of published quantitative (morphometric) data from rats and nonhuman primates suggests that the respiratory system of nonhuman primates (monkeys) may respond more to ambient concentrations of O_3 than does that of rats (Plopper et al. 1991; Paige and Plopper 1999). In addition, all small animal and nonhuman primate studies of repeated exposures to O_3 in the range of 196 to 392 $\mu g/m^3$ (0.1 to 0.2 ppm) have demonstrated morphological changes of the type seen in early stages of human lung disease from other pollutants such as cigarette smoke (Tyler et al. 1991).

There are many investigations of the effects of O_3 on lung cellular organization and architecture, and several include correlated measurements of pulmonary function or lung biochemistry. The presentation of this morphology section will begin with short-term exposure effects (<1 week). The subsequent discussion of long-term effects (>1 week) is based on the fact that repair has already been initiated at this time; the chronic studies that included evaluation of various durations of exposures are presented totally in this subsection to illustrate the effects of exposure duration. The impact of differences in interexposure period is also addressed.

As a basis for defining their biological significance, exposure patterns will be characterized by three key parameters: exposure concentration, duration of exposure

(or exposure period), and the length of time between exposures when the concentration is near background (the interexposure interval). Ambient exposures are variable in nature, with daily and seasonal variations in concentration. Under ambient conditions the duration of exposure to elevated O_3 concentrations on a daily basis, is approximately 6 hours. The peak concentrations during this 6-hour period are highly variable by season. There are many days, even during seasons associated with high average ambient levels, when the ambient concentration is very low or near background. The key issue regarding the period of time when concentrations reach background (or the interexposure interval) is whether the background is below the level which will overcome the ability of target cell populations in the respiratory system to detoxify the oxidant species and limit cellular injury.

11.3.2 Morphological Effects Associated with Short-Term Exposure

Effects in experimental animals of exposures generally lasting less than 1 week are summarized in Table 11-1. Few studies of the nasal epithelial response to O_3 exposure have been reported. The ciliated epithelium of the anterior nasal region of monkeys was altered by a 6-day (8 h/day) exposure to 294 µg/m^3 (0.15 ppm) (Harkema et al. 1987b). Johnson et al. (1990) found that cellular proliferation of rat nasal epithelial tissues was increased by 3 days of intermittent exposure to 1,568 µg/m^3 (0.8 ppm) O_3. The cuboidal/transitional epithelium was the most responsive. Lower O_3 concentrations were not effective in altering the nasal epithelium of the rat. Thus, the rat nasal epithelium appears less responsive to O_3 than does that of monkeys. Following up on their earlier observations of nasal and centriacinar region (CAR) effects after a single exposure (Hotchkiss et al. 1989a; Hotchkiss et al. 1991) sought to examine the effect of cumulative O_3 exposure (1,568 µg/m^3, 0.8 ppm; 6 hour/day) on the nasal nonciliated cuboidal epithelium of rats. Both a seven-day exposure (examination 18-hour post exposure) and a three-day exposure (examination 4 days post exposure) caused equivalent epithelial hyperplasia and secretory metaplasia. The three-day exposure induced no effects with examination at 18-hour post-exposure. The investigators concluded that either maximal response occurred by Day 3, with no further damage possible, or that the ongoing repair may have involved the proliferation of a more O_3-resistant epithelium.

Very short exposures (as little as two hours) initiate the acute response to O_3 in primates (Plopper et al. 1998). After two hours exposure to 1 ppm O_3 there was a significant increase in the abundance of necrotic cells corresponding with a significant decrease in the abundance of intact epithelial cells. While polymorphonuclear leukocytes and eosinophils were significantly increased in number following a 2-hour exposure to 1 ppm O_3, macrophages exhibited a significant decrease.

In the ciliated airways of the tracheobronchial region, changes similar to those in the nasal cavity have been reported in all animal species examined after 1 week of exposure to levels as low as 392 µg/m^3 (0.2 ppm) (Boatman and Frank 1974; Boatman et al., 1974; Castleman et al. 1977; Mellick et al. 1977; Schwartz et al. 1976; Wilson et al. 1984; Stephens et al. 1974; Stephens et al. 1974b; Dungworth et al. 1975; Suzuki et al. 1992; VanBree et al. 1989). Cilia are shortened and less dense after O_3 exposure, and damaged ciliated cells are replaced by nonciliated bronchiolar (Clara) cells, which

become hyperplastic. Mucous-secretory cells are relatively resistant, even though some effects occur.

In the centriacinar region also, different species have similar responses to low levels of O_3 (392 µg/m^3, 0.2 ppm; 1 week) (Castleman et al. 1977; Schwartz et al. 1976; Boatman and Frank 1974; Stephens et al. 1974; Freeman et al. 1973; Stephens et al. 1974b; Stephens et al. 1978; Stephens et al. 1973; Mellick et al., 1977; Brummer et al. 1977; Dormans et al. 1999). Type 1 cells are destroyed, exposing the basal lamina, and inflammatory cells especially alveolar macrophages, (AMs) accumulate. Hyperplastic Type 2 and nonciliated bronchiolar (Clara) cells covers the denuded areas and the interalveolar septa thicken, primarily due to thickening of the interstitium. There may be several causes of this thickening, including increases in collagen and reticulin fibers(Last et al. 1979), eosinophilic hyalin material and mononuclear cells (Schwartz et al. 1976), and edema (Castleman et al. 1977). Last et al. (1979) found that after 7 days of exposure to 980 to 3,920 µg/m^3 (0.5 to 2.0 ppm) O_3, there was a concentration-related increase in lung lesions (e.g., collagen and reticulin) and collagen synthesis in rats. These fibrotic changes increased slightly by 14 days of exposure, but then plateaued.

The effects of exposure duration are complex and are likely responsible for the similar patterns of biochemical responses. Ciliated and alveolar type 1 cells became necrotic and were sloughed as soon as 2 to 4 hour into an exposure of rats to around 980 µg/m^3 (0.5 ppm) (Stephens et al. 1974; Stephens et al. 1974b), with the damage no greater after 48 hour of exposure (Stephens et al., 1974a). Repair of the damage, as indicated by increased DNA synthesis by nonciliated bronchiolar (Clara) and alveolar type 2 cells, begins by 18 to 24 in both rodents and monkeys (Evans et al. 1976; Stephens et al., 1974a; Castleman et al. 1980; Castleman et al. 1980). While cell damage, including necrosis, continued throughout short (Castleman et al., 1980) and long (Barr et al. 1990; Schelegle et al. 2003) exposures in rodents and primates, the morphological lesion was fully developed after about 3 days of continuous exposure. After that time, the rate of damage was exceeded by the rate of repair and the animal entered the “reparative-adaptive” period. Adaptation may be due in part to the greater resistance to O_3 of the newly formed nonciliated bronchiolar (Clara) cells and alveolar type 2 cells. For example, Evans et al. (1976) observed that alveolar type 2 cell hyperplasia in rats peaked around Day 2 of an exposure to 686 to 1,960 µg/m^3 (0.35 to 1.0 ppm) O_3 and decreased to near control by Day 4, although exposure continued through Day 8. In the centriacinar region of nonhuman primates, alveolar type 1 cell necrosis was maximal after 4-hour exposure to 1,568 µg/m^3 (0.8 ppm) O_3, but continued at a reduced rate throughout a 50-hour exposure (Castleman et al., 1980). In that study, DNA labeling was maximal at the end of the 50-hour exposure. The same was true for both midlevel bronchi and centriacinar bronchioles and alveolar ducts in adult rats (Schelegle et al. 2003). In the tracheas of monkeys, ciliated cell lesions consequent to exposure to 1,254 µg/m^3 (0.64 ppm) O_3 were more severe after 3 days of exposure; by 7 days of exposure, the epithelium had returned towards normal (Wilson et al. 1984). As another indicator of the complexity of the response, Schwartz et al. (1976) found that there was very little difference in the intensity of lung lesions in the terminal bronchioles or centriacinar regions of rats exposed for 8 hour/day versus 24 hour/day to 392 to 1,568 µg/m^3 (0.2 to 0.8 ppm) for 7 days.

The time course of effects after cessation of exposure has also been explored, with mixed findings. For example, both Hotchkiss et al. (1989a) and Van Bree et al. (2001) found progressive thickening of tracheobronchial walls and CAR alveolar duct septa with increasing time after a short term exposure of rats. Dormans et al. (1990) reported persistent effects on the increases in alveolar macrophages in rats after a short-term exposure. Using transmission electron microscopy morphometry, a wide range of temporal responses were observed in O_3-exposed rats by Pino et al. (1992), depending on the exact endpoint. For example, the volume of the connective tissue was increased in terminal bronchioles 4 hour after an 8-hour exposure ceased, and in alveoli, immediately after the exposure ceased.

A number of factors alter susceptibility. The age of the animals at the beginning of exposure has a significant impact on the pattern of the acute (Shore et al. 2000; Stiles and Tyler 1988; Tyler et al. 1988; Vincent and Adamson 1995). Stephens et al. (1978) investigated age responsiveness in rats (1 to 40 days old) to a 72-hour exposure to 1,666 µg/m^3 (0.85 ppm). When exposure started prior to weaning (20 days of age), there were no effects. As age increased (from 21 days old) at the start of exposure, centriacinar lesions increased progressively, reaching a plateau at 35 days of age. Older rats (444 days old) responded differently than young adult (60-days old) rats to a 3-day exposure to 686 or 1,568 µg/m^3 (0.35 or 0.8 ppm) O_3 (Stiles and Tyler 1988). Younger rats had larger centriacinar lesions than older rats. Older rats had smaller lung volumes at the higher O_3 concentration; lung volumes of young rats were unaffected. Thus, the age susceptibility was dependent on the endpoint examined. Senescent rats showed greater susceptibility to acute centriacinar injury and a greater proliferative response (Vincent and Adamson, 1995).

A number of dietary factors, including vitamin A (Paquette, et al, 1996), Vitamin E (Chow et al. 1981; Plopper et al. 1979) and taurine (Gordon et al. 1998; Schuller-Levis et al. 1995) appear to modulate the impact of O_3 exposure on acute injury in rodents and primates. At low O_3 levels (196 µg/m^3, 0.1 ppm), a 7-day exposure caused more effects in vitamin E-deficient rats (Chow et al. 1981; Plopper et al. 1979). However, Stephens et al. (1983) did not find an influence of vitamin E deficiency at higher exposure levels (1,764 µg/m^3, 0.9 ppm; up to 72 hour). Deficiency in vitamin A levels enhanced the injury and inflammatory responses in mice (Paquette et al. 1996), while elevation in taurine levels served to protect against acute injury and reduced inflammation (Schuller-Levis et al. 1995; Gordon et al. 1998).

Alteration of the lung's ability to respond to acute injury by blocking neutrophils not only reduced inflammation, but also inhibited repair of acute injury (Bassett et al. 2001; Hyde et al. 1999; Vesely et al. 1999b). Modulation of the components thought to be involved in the response to acute exposure also altered the response. Mice with the gene for extracellular superoxide dismutase blocked showed increased cellular injury (Jonsson et al. 2002). Elevation of hemeoxygenase-1 by lipopolysaccharide (LPS) attenuated the response (Li et al. 2000), as did elevation of the anti-inflammatory cytokine IL-1O (Reinhart et al. 1999). Mice with inducible nitric oxide synthase knocked out have reduced injury and inflammatory responses (Fakhrzadeh et al. 2002) to O_3 exposure. Inhibition of receptors for platelet activating factor (PAF) has a similar effect (Longphre et al. 1999).

Alteration of physiological functions also appears to modulate responses to O_3 exposure. Changes in respiratory pattern (increased rapid shallow breathing), whether produced artificially in isolated perfused lungs (Joad et al. 2000; Postlethwait et al. 2000) or by blocking airway c-fiber function with capsaicin (Sterner-Kock et al. 1996; Vesely et al. 1999a), altered the injury pattern, indicating that rapid shallow breathing is protective of the distal lung. Rats with a laboratory-induced emphysema-like condition were more susceptible to a brief (3 or 7 days) exposure to up to 980 $\mu g/m^3$ (0.5 ppm) (Dormans et al. 1989). Exercise can increase parenchymal lesions (Mautz et al. 1988), as can increased body temperature (Wiester et al. 1996b; Huffman et al. 2001).

Table 11-1 Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960, 1176, 1960	0.3, 0.6, 1.0	3 hr	Rat, M, (SD)	Serum levels of CC16 elevated immediately after exposure, and then decreased, and was correlated with lavage protein, cells and LDH. Lavage CC16 decreased with exposure early and returned late	Arsalane et al. 1999
1960, 3920	1.0, 2.0	6 hr	Rat, M, (BN, F-344, 2 strains of SD, WSTR), 65-70days	Based on lavage albumin, strain differences in acute injury, least to most susceptible: BN, SD, F-344, WSTR. Close inverse correlation with level of tissue PMN and eosinophils	Bassett et al. 2000
1960 or 3920	1.0 or 2.0	3 hr or 48 hr	Rat, M, (SD)	Blocking PMN in both serum and tissue necessary to reduce acute injury response based on lavage PMN and albumin (anti serum). Blocking serum PMN but not lung tissue resident PMN does not alter injury (cyclophosphamide)	Bassett et al. 2001
1960	1.0	3 hr	Rat, M, (SD), 6-8wk	Exposure increased lavagable cells and protein, including alkaline phosphatase and fibronectin, peaking at 12hr post exposure. Blocking PMN had no effect. Macrophages and type z cells primary sources of alkaline phosphatase and fibronectin	Bhalla 1999
1960	1.0	3 hr	Rat, M, (SD), 6-8wk	Close correlation between injury, based on elevation of lavage albumin, PMN, and MIP-2, with increases in ICAM-1, but not B-2 integrin or LTB4	Bhalla and Gupta 2000
510, 980, 1960	0.26, 0.5, 1.00	4.7-6.6 hr, endotracheal tube	Cat	Desquamation of ciliated epithelium. Focal swelling or sloughing of Type 1 cells. (LM, EM)	Boatman and Frank 1974
784	0.4	1,3,7,28, or 56 days, continuously	Rat, M, (WSTR)	Cellular necrosis and hyperplasia in centriacinar regions with 3 - 7 days continuous exposure	Van Bree et al. 2001

Table 11-1 (cont.) Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980, 1568	0.5, 0.8	2, 4, 6, 8 or 24 hr/d, 7 days	Rat (SD)	Increased centriacinar inflammatory cells (mostly AMs). At 980 µg/m^3, the magnitude of the increase progressively increased between 2-6 hr/d of exposure; response at 6 hr and 8 hr <24 hr/d. At 1,568 µg/m^3, progressive increase with hr/d of exposure. Intensity of a 24-hr/d exposure to either concentration similar. (EM)	Brummer et al. 1977
392, 686	0.2, 0.35	8 hr/d, 7 days	Monkey (bonnet)	All had lesions. Trachea and bronchi had areas of shortened or less dense cilia. RBs had AM accumulation and cuboidal cell hyperplasia. Alveoli of RBs had AM accumulations and increased number of Type 2 cells. RB walls of the 686-µg/m^3 group were often thickened due to mild edema and cellular infiltration. (LM, EM)	Castleman et al. 1977
1568	0.8	4 to 50 hr	Monkey (rhesus)	Degeneration and necrosis of RB Type 1 cells predominates from 4-12 hr. Labeling index highest at 50 hr; mostly cuboidal bronchiolar cells, but some Type 2 cells. Increase in numbers of AMs. Bronchiolar epithelium hyperplastic after 50 hr and persisted 7 days PE.	Castleman et al. 1980
196-980	0.1-0.5	0.5 hr	Rat, M, (SD)	*In vitro* study of alveolar type 2 monolayers showed concentration dependant increase permeability, adding PMN promoted repair at low concentrations and increased injury at high concentrations.	Cheek et al. 1995
1960 3920	1.0 2.0	6 hr, 18 hr	Rat, M, (F344), 90 d	Extracellular lining fluid volume, protein and albumin increased with concentration but not to the same degree	Cheng et al. 1995
196	0.1	24 hr/d, 7 days	Rat (SD)	Vitamin E-deficient rats had increased centriacinar AMs and bronchiolar epithelial lesions. Rats with diet supplements of 11ppm vitamin E had lesser but similar lesions. Fewer rats supplemented with 110 ppm Vitamin E had lesions and they were less severe. (EM).	Chow et al. 1981 Plopper et al. 1979

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
250, 1000, 1500	0.12, 0.5, 0.75	Continuous for 1-7 d	Rat, M (WSTR) 8 wks	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.	Dormans et al. 1990
294, 980	0.15, 0.5	Continuous for 3 or 7 d	Rat, M (WSTR) 8 wks	Elastase-induced emphysema and saline control rats. LM histopathology and morphometry for alveolar size. 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
392,784	0.2, 0.4	3, 7, 28, 56 d	Rat, M, (Wistar), 7wk	All three species same centriacinar inflammatory response and cell injury up to 7 days.-all changes concentration dependant- mouse bronchiolar hyperplasia at 3 days	Dormans et al. 1999
392, 686, 980, 1568	0.2, 0.35, 0.5, 0.8	8 hr/d, 7 d	Monkey (rhesus and bonnet)	Respiratory bronchiolitis 392 µg/m^3. Increased number of AMs. Bronchiolar epithelium both hyperplastic and hypertrophic. Increase in Type 2 cells. Random foci of short, blunt cilia or absence of cilia. (LM, EM)	Dungworth et al. 1975
980	0.5	8 hr	Rat, Male, (F344, WSTR, SD), 14 mon	Strain differences in injury based on lavage LDH, protein, fibronectin, PGE2 and IL6 levels and increased PMN and macrophages. Wistar>SD or F344. In vitro epithelial response is reflective of lavage	Dye et al. 1999
686, 980, 1372, 1470, 1960	0.35, 0.5, 0.7, 0.75, 1.00	1, 2, 4, 5, 6, or 8 d	Rat (SD)	All tritiated thymidine labeled cells increased and then decreased to near control levels by D 4. Type 2 cells showed largest changes in labeling index. (LM, autoradiography)	Evans et al. 1976
784 1568	0.4 0.8	3 hr	Mice, F 8-16 wks	Mice with inducible nitric oxide synthase knocked out had reduced inflammation and injury, based on lavage protein and cells, and lower macrophage number, peroxynitrite and PGE2 product.	Fakhrzadeh et al. 2002

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
3920	2	3 hr	Rat, F, (SD)	Taurine pretreatement (50% in drinking H_20) reduced mild inflammation and injury shortly (6 hr) post exposure and reduced disruption of tight junctions in epithelium	Gordon et al. 1998
235, 1568, 2940	0.12, 0.8, 1.5	6 hr	Rat, M (F344) 12-18 wks	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 hr PE. At later PE times, there was mild, patchy CAR bronchiolitis and alveolitis. Increase in AMs and PMNs from 18-66 hr PE. Progressive thickening of TB walls and CAR AD septa at 18, 42, and 66 hr PE.	Hotchkiss et al. 1989a
235, 1568, 2940	0.12, 0.8, 1.5	6 hr	Rat, M (F344) 12-18 wks	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 hr PE.	Hotchkiss et al. 1989b
1568	0.8	6 hr/d, 3 or 7 d	Rat	Nasal nonciliated cuboidal epithelium studied. Epithelial hyperplasia and secretory metaplasia in rats exposed for 7 days and examined 18 hr PE and exposed for 3 days and examined 4 days PE, but not in rats exposed for 3 days and examined 18 hr PE.	Hotchkiss et al. 1991
980 - 5880	0.5 -3.0	3 hr	Rat, M, (SD)	Experimental hyperthyroidism (thyroxine injection, 0.1-1.0 mg/kg) increased acute injury based on elevated lavage protein, LDH, albumin, PMN levels. Not completely dependant on high metabolic rate	Huffman et al. 2001
1568	0.0, 0.8	8 hr	Primate, M, (Rhesus), 3yrs	Inhibition of PMN migration with specific anti-CD18 antibody increased the abundance of necrotic cells in distal airways. Promoting migration of PMN into airways by lavage of C5a promoted removal of necrotic cells from injured sites	Hyde et al. 1999

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980	0.5	6 hr	Primate (Rhesus)	*In vitro* exposure of bronchial epithelial cells at air/liquid interface alters apical and basolateral expression of ß-1 integrins without increased mRNA production	Jabbour et al. 1998
1960	1.0	90 min	Rat, (SD)	Using isolated perfused lung, showed that distribution of airway epithelial injury, based on exclusion of cell impermeant dye, is altered by breathing pattern and that injury is high in many sites besides the central acinus	Joad et al. 2000
235, 529, 1568	0.12, 0.27, 0.8	6 hr/d, 7 d	Rat (F344)	Effects in nasal epithelium only at 1,568 µg/m$_3$. At Days 3 and 7, increased DNA replication in nonciliated cuboidal/transitional epithelium. At Day 3 only, increased DNA replication in respiratory and olfactory epithelia. Seven days PE, decreased DNA replication in squamous epithelium. (LM, EM)	Johnson et al. 1990
2940	1.5	48 hr	Mice	Mice without the gene for extracellular superoxide dismutase had increases in inflammation and injury, based on lavage IL6 and histopathology, without differences in lavage protein, cells, or LDH	Jonsson et al. 2002
490,3920	0.25, 2.0	3 hr	Mice, M, (C3H/HeJ), 8-9wk	HNE based protein adduct formation, is produced in lung cells obtained by lavage	Kirichenko et al. 1996

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980 to 2940	0.5 to 1.5	4 hr/d, 1, 2, or 3 wks	Rat, M (SD)	Elevated collagen synthesis rates and histologically discernible fibrosis 980 µg/m^3. At 980 µg/m^3, minimal or no thickening of walls or evidence of fibrosis and increased number of cuboidal cells and AMs by Day 7; at 2 or 3 weeks, sometimes minimal thickening of alveolar duct walls with mildly increased reticulin and collagen. At 1,568 µg/m^3, moderate thickening of alveolar duct walls and associated IAS by fibroblasts, reticulin, and collagen, with narrowing of the ducts and alveoli. Thickening decreased with increased length of exposure. (LM)	Last et al. 1979
1960	1.0	3 hr	Mice, M, (C57Bl/6), 8-9wk	Elevation of heme oxygenase-1 by pretreatment with endotoxin (LPS) reduced inflammation and injury in lung cells obtained by lavage. Co-treatment with a heme oxygenase inhibitor (SnPP) abolished the reduction in inflammation	Li et al. 2000
1960 5880	1.0 3.0	6 hr	Hamsters, M, (Syrian Golden), 4-18 mo	Elevation of 0_3-induced lipid peroxidation products (F2-isoprostanes) in lavage PAF, but not reductions in antioxidants (urate, ascorbate) in lavage fluid and plasma. Exercise during exposure elevated F2-isoprostanes, but not inflammatory indicators, in lavage fluid	Long et al. 2001
3920	2.0	3 hr	Mice, M, (C57-BL/6J), 6-8 wk	Inhibition of platelet activating factor receptors by an antagonist (UK-74505) prior to or after exposure reduced lavage protein, PMN and epithelial cells and epithelial proliferation and ICAM-1 expression,	Longphre et al. 1999

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
686, 1176	0.35, 0.6	4 hr at rest, 3 hr with exercise	Rat, M (SD) 7 wks	Examination 48 hr PE. Lung: LM morphometry of lesions as a percent of parenchyma section area. No statistical evaluation of groups exposed to O_3 at rest or exercise. The lesion percent of parenchyma appears concentration-dependent and increased by exercise. Nasal epithelium: Evaluated percent thymidine labeled cells in respiratory epithelium. No change due to O_3 at rest.	Mautz et al. 1988
980, 1568	0.5, 0.8	8 hr/d, 7 days	Monkey (rhesus)	Lesions similar at both O_3 levels, but less severe at 980 µg/m^3. Patchy areas of epithelium devoid of cilia in trachea and bronchi. Luminal surfaces of RBs and proximal alveoli coated with AMs, a few PMNs and eosinophils, and debris. Nonciliated cuboidal bronchiolar cells were larger, more numerous, and sometimes stratified. Proximal alveolar epithelium thickened by increased numbers of Type 2 cells. Progressive decrease in intensity of lesions from proximal to distal orders of RBs.	Mellick et al. 1977
980	0.5	12 hr	Horse	Exposure increased airway inflammation, based on lavage PMN levels, and elevated reduced and oxidized glutathione and Fe in lavage fluid, but did not alter plasma levels	Mills et al. 1996
5880	3.0	72 hr	Mice, M/F, (B6 &C3), 6-8 wk	Modulation of vitamin A and retinyl palmitate levels in lung and liver altered inflammatory responses in sensitive (B6) and resistant (C3) strains. Vitamin A deficiency elevated lavage PMN, in mice and lavage protein and epithelial loss in both strains	Paquette et al. 1996

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	4, 6, 8, 24 hr	Rat, M (SD) 63 days old	TEM morphometry. Volume of necrotic cells per area basal lamina (Vs) in the TB larger than controls at the end of 4- and 24-hr 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 Vs of necrotic alveolar cells was increased after 4, 6, and 24 hr of exposure. Viable undifferentiated cell Vs in TBs was increased after 6-hr exposure followed by 18 hr PE, 8-hr exposure followed by 16 hr PE, and after a 24-hr exposure. In alveoli, viable Type 1 cell Vs was increased after a 24-hr exposure. Total connective tissue Vs changes only increased in TBs after 8-hr exposure followed by 4 hr PE and in alveoli at the end of 8-hr exposure. The Vs of migratory cells in TB interstitium was only increased 4 hr after a 6-hr exposure. In alveoli, the Vs of capillaries was increased after 8-hr exposure.	Pino et al. 1992
784-1960	0.4-1.0	2 hr	Primate, M, (Rhesus), 3 yr	Local dose, based on excess $^{18}0_2$, varied within airway tree with respiratory bronchiole highest and parenchyma lowest. Degree of epithelial injury and inflammatory cell infiltration closely correlated with local dose and loss of reduced glutathione in a concentration dependant manner	Plopper et al. 1998
490, 980, 1960	0.0, 0.25, 0.5, 1.0	20-90 min	Rat, M, (SD)	Exposure of isolated perfused lungs produced acute epithelial injury, based on permeability of cell-impermeant dye (Ethidium homodimer-1): The greatest injury was in minor daughter side branches of proximal bronchi and the least injury in terminal bronchioles	Postlethwait et al. 2000
1568	0.8	3 hr	Rat, M, (SD)	Elevation of anti-inflammatory cytokine IL-10 prior to exposure reduces lung inflammation based on lavage protein, albumin, PMN and fibronectin content	Reinhart et al. 1999

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	8 hr, 5 d	Rat, M, (SD), 70 d	Severe epithelial necrosis at day 1 with increased inflammation in midlevel bronchi, terminal bronchioles and proximal alveolar ducts, that was reduced by 5th day of exposure	Schelegle et al. 2003
3920	2.0	3 hr	Rat, F, (SD)	Preexposure treatment to elevate taurine levels markedly reduced acute inflammation, based on total cell, PMN and hydroxyproline in lavage, and acute epithelial injury and cell infiltrates in the central acinus	Schuller-Levis et al. 1995
392, 980, 1568	0.2, 0.5, 0.8	8 or 24 hr/d, 7 d	Rat (SD)	Focal areas of missing or damaged cilia in trachea and bronchi. In TBs, Clara cells shorter with increased surface granularity and less smooth endoplasmic reticulum. Ciliated cells of TBs had fewer cilia and focal blebs. Centriacinus had clusters of AMs and PMNs. Type 1 cells swollen and fragmented, and Type 2 cells frequently in pairs or clusters. Proximal IAS was minimally thickened. Lesions in 392-µg/m^3 rats were mild. Only slight differences between 8- and 24-hr/d exposure groups. (LM, EM)	Schwartz et al. 1976
3920	2	3 hr	Rat, M/F, (SD), 2 wk	Younger rats (2 wk) had greater acute injury, based on lavage protein and PGE2 levels than did adults	Shore et al. 2000

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1058, 1725	0.54, 0.88	2, 4, 8, 12, 48 hr	Rat (SD)	Severe loss of cilia from TBs after 2 hr. Necrotic ciliate cells in TB epithelium and free in lumen after 6 to 12 hr of 1,725 µg/m^3; necrosis continued until 24 hr, when little evidence of further damage was seen. Only minimal loss of ciliated cells at 1,058 µg/m^3. Nonciliated cells were "resistant" to injury from O_3 and hypertrophic at 72 hr. At 1,058 µg/m^3 for 2 hr, Type 1 cell "fraying" and vesiculation; damage greater at 1,725 µg/m^3, "basement lamina" denuded, Type 2 cells resistant. AMs accumulated in proximal alveoli. Repair started at 20 hr: Type 2 cells divided and replaced Type 1 cells. Continued exposure resulted in thickened alveolar walls and tissue surrounding TBs. Exposure for 8 to 10 hrs followed by clean air until 48 hr resulted in a proliferative response (at 48 hr) about equal to that observed after continuous exposure. (LM, EM)	Stephens et al. 1974a
980	0.5	2 to 6 hr	Rat, M (SD) young	Centriacinar Type 1 cells were swollen, then sloughed. Type 2 cells were not damaged and spread over the denuded basement membrane. Damage was most severe only in the most central two to three alveoli. Interstitial edema occasionally observed. (LM, EM)	Stephens et al. 1974b
1666	0.85	24 hr/d, 1, 2, 3 d	Rat (SD)	Birth to weaning at 20 days old: Very little indication of response or tissue nodules with dissecting microscope. 12 days old: No lesions. 22 days old: Loss of cilia, hypertrophy of TB cells, tendency towards flattening of luminal epithelial surface. 32 days old: Loss of cilia, and significant hypertrophy of TB epithelial cells. 21 days old and older: Alveolar injury, including sloughing of Type 1 cells resulting in bare basal lamina. 35 days old: Response plateau is reached. (LM, EM)	Stephens et al. 1978

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	8 hr	Rat, (WSTR)	Elimination of lung C-fibers by neonatal capsaicin treatment produced marked increases in the degree and distribution of lung injury and inflammation, including coagulate necrosis in all airway epithelium	Sterner-Kock et al. 1996
1960	1.0	8 hr	Rat, Primate (rhesus), Ferret	Compared to rats, the acute epithelial injury and inflammation in lungs of ferrets is much greater and very comparable to the response in rhesus monkeys	Sterner-Kock et al. 2000
686, 1568	0.35, 0.8	72 hr	Rat (SD)	Typical O_3 effects. Older rats (444-days old) had smaller lung volumes after 1,568 µg/m^3, but young rats (60-days old) were not affected. No impact of age on TB responses or volume fraction of parenchyma. Younger rats had more of an increase in AMs, but older rats had a greater accumulation of mucus. Younger rats had a larger volume fraction of centriacinar lesions. Older rats had a decrease in body weight; young rats did not.	Stiles and Tyler 1988
784	0.4	Continuous up to 14 days	Rat, M (WSTR) 5 wks	SEM morphometry, immunocytochemistry. Number of Clara cells/µm2 increased at 14 days, but not earlier. The length of the Clara cell apical projection was increased after 6 hr, 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
1568, 784	0.8, 0.4	Continuous for 7 days	Rat, M (WSTR) 8 wks	LM: Clara cell numbers/mm of TB basement membrane unchanged. Cell isolation: Although the number of isolated Clara cells 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.	VanBree et al. 1989

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	8 hr	Rat, Male, (WSTR), 6-9 wk	Elimination of lung C-fibers by neonatal capsaicin treatment prevented 0_3-induced rapid shallow breathing which correlated closely with increased airway epithelial injury, but without higher levels of cell proliferation or inflammation	Vesely et al. 1999a
1960	1.0	8 hr	Rat, Male, (WSTR), 6-9 wk	Elimination of PMN using an antiserum did alter 0_3-induced respiratory changes, but elevated 0_3-associated epithelial injury in nasal and distal airways while depressing cell proliferation	Vesely et al. 1999b
1568	0.8	6 hr	Rat, M, (F-344)	Senescent adult rats had slightly greater central acinar (terminal bronchiole and alveolar duct) epithelial injury with a greater cell proliferation (50%) during repair than do young adult rats	Vincent and Adamson 1995
1254	0.64	23 hr/d, 3 or 7 d	Monkey (bonnet)	Effects in tracheal epithelium. Necrosis of ciliated cells, decreased numbers of ciliated cells and loss of cilia. Extracellular space was increased and focal areas of epithelial stratification were seen. Small mucous granule cells were increased and an intermediate cell was described. Regular mucous cells had decreased density and smaller irregularly sized secretory granules, which contained only filamentous or granular material. Most severe lesions occurred at Day 3, and the epithelium had returned towards normal after Day 7. (EM)	Wilson et al. 1984
980	0.5	24 hr	Mice, M, (B6C3F1) adult	Prior exposure to aged and diluted sidestream cigarette smoke (ADSS) before 0_3 produced increased inflammation based on number of lavage cell s, PMNs and lymphocytes and protein. Centriacinar epithelial proliferation was elevated by 0_3 (280% of control) and augmented further by ADSS/0_3 (402%). Alveolar macrophage release of IL-6 with LPS was not different between 0_3 and ADSS/0_3 exposed groups	Yu et al. 2002

Table 11-1 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
196, 980, 1960	0.1, 0.5, 1.0	8 hr/d, 1, 10, 75, 90 d	Rat, M, (SD) adult	Reduction p450 2E1 expression in bronchi and bronchioles, greatest in bronchioles 0, 1, 2, and 7 days after 8 hr exposure	Watt et al. 1998
980	0.5	6-23h/d, 5 d	Rat (F344), adult	Lower ambient temperature (10°C vs. 22°C and 34°C) produced greater inflammation based on lavage protein, lysozyme, alkaline phosphatase and PMN	Wiester et al. 1996b

See Glossary for abbreviations

11.3.3 Morphological Effects From Longer-Term Exposures

When the exposure period is extended beyond 1 week, and the interexposure interval is kept short enough (less than 3 days) to prevent the later stages of the repair cycle to occur, chronic lesions develop (Table 11-2) in experimental animals exposed to O_3. As length of exposure increases, the severity of the lesions does not increase. As exposure continues past 1 week to 1 year, alveolar type 1 cell necrosis and inflammatory responses generally decreased to near control values, and hyperplasia and fibrosis remained elevated. After chronic exposure ends, some indices of fibrosis persist and may even became more severe during postexposure periods in clean air (Last and Reiser 1984; Last et al. 1984; Pickrell et al. 1987).

Morphological effects of O_3 on the upper respiratory tract have been reported by Harkema et al. (1987a,b). Bonnet monkeys were exposed to 294 or 588 µg/m^3 (0.15 or 0.3 ppm) O_3 for 6 or 90 days. Quantitative changes were evident in the nasal transitional respiratory epithelium. At both time periods and at both O_3 levels, a lesion developed consisting of ciliated cell necrosis, shortened cilia, and secretory cell hyperplasia. Ultrastructural changes in goblet cells, along with alteration of stored secretory products, were evident at 90 days. The authors suggested that ambient levels of O_3 could induce significant nasal epithelial lesions that may compromise the upper respiratory tract defense mechanisms.

Within the terminal bronchioles of mice and rats and the respiratory bronchioles of monkeys, ciliated cells lose cilia and become necrotic and are then replaced by nonciliated bronchiolar (Clara) cells, which may also be affected by O_3 (Barry et al. 1988; Chang et al. 1992; Boorman et al. 1980; Eustis et al. 1981; Zitnik et al. 1978). The terminal bronchioles of neonatal and adult rats were examined by Barry et al. (1988), using morphometric procedures. Six weeks of exposure (12 hour/day) of one-day-old and 42-day-old rats to 490 µg/m^3 (0.25 ppm) O_3 caused loss of cilia (20 to 30%), loss of the Clara cell dome, and a decreased (16 to 25%) luminal surface area of Clara cells. Changes in both of these cell types suggest that the normal functions of these cells are likely to have been compromised. No statistically significant interactions between the effects of O_3 and animal age were found.

Barry and Crapo (1985) studied the effects of O_3 on the proximal alveolar region (i.e., centriacinar region) of the rats discussed above (Barry et al. 1988). In the animals exposed to 490 µg/m^3 (0.25 ppm) O_3 from one day of age, the number of Type 1 cells doubled, their mean surface area decreased by 38%, and their mean thickness increased by 24%. The number of alveolar Type 2 epithelial cells increased, and the number of macrophages doubled. Adult animals exposed to 490 µg/m^3 (0.25 ppm) O_3 showed similar patterns of changes and also exhibited a doubling of interstitial macrophages. Adult animals exposed to 235 µg/m^3 (0.12 ppm) O_3 showed smaller, still statistically significant changes in Type 1 cells. In both the terminal bronchioles and proximal alveolar region, no statistically significant age-dependent effects were found. However, as shown by

Stephens et al. (1978), rats were not sensitive to O_3 until they were at least 20 days old. Others who used image analysis to compare newborn to adult mice exposed intermittently for 6 weeks to 588 µg/m^3 (0.3 ppm) O_3 also found that the neonates were more resistant (Sherwin and Richters 1985). Therefore, the neonates in the Barry et al. (Barry and Crapo 1985; Barry et al. 1988) studies may have received effective exposures only 22 of the 42 days of exposure, whereas the 42 day old "adults" received effective exposures each of the 42 days. As stated by the investigators, this raises the possibility that the neonates may have been more sensitive because they developed as severe a lesion after 22 days of exposure as the "adults" did after 42 days of exposure.

Four extensive chronic studies have elucidated the effects of low-level ambient patterns of O_3 (Chang et al. 1992; Tyler et al., 1988, 1991; Catalano et al. 1995b). In the Chang et al. (1992) study, rats were exposed to a background of 118 µg/m^3 (0.06 ppm) O_3 for 13 hour/day, 7 days/week and a slow 9-hour spike (5 days/week) that rose to 490 µg/m^3 (0.25 ppm) O_3. The integrated concentration of the spike was 372 µg/m^3 (0.19 ppm) O_3. Morphometric and morphologic examinations of the terminal bronchioles and proximal alveolar region were made at 1, 3, 13, and 78 weeks of exposure; 6 weeks after the 13-week exposure ceased; and 17 weeks after the 78-week exposure ceased. In the terminal bronchioles, cilia were lost (78 weeks of exposure) and the surface area of Clara cells decreased (1, 3, and 78 weeks of exposure). The rare preciliated cells increased in number and size only after 1 week of exposure. Several weeks post exposure, recovery of the terminal bronchioles occurred. The proximal alveolar region had a biphasic response with duration of exposure. Acute, 1-week responses (i.e., epithelial inflammation, interstitial edema, interstitial cell hypertrophy, and influx of AMs) subsided by 3 weeks of exposure. However, from 13 through 78 weeks of exposure, slower progressive changes occurred, including epithelial hyperplasia (especially of Type 2 cells, but including Type 1 cells), fibroblast proliferation, and thickening of the interstitial matrix (due to increased deposition of basement membrane and collagen fibers). Generally, postexposure recovery occurred, except for interstitial changes. In the interstitium of rats exposed for 78 weeks, there was a recovery of the changes due to increased volume of cells, but the collagen fibers were still increased, especially at alveolar duct bifurcations or in the septum bordering alveolar ducts. Pulmonary function alterations in identical groups of rats (Costa and Tepper 1988; Costa et al. 1988) were consistent with the fibrotic lesions observed.

Tyler et al. (1988; 1991) investigated the impacts of seasonal ambient patterns of exposure to determine whether the classical inhalation toxicology protocols (i.e., exposure every day for months) adequately represented "real world" exposure patterns in the U.S. and some other areas of the world that typically have higher concentrations over the summer months compared to the winter months. Both young monkeys (7 mo old) (Tyler et al. 1988) and rats (Tyler et al. 1991) were examined after "daily" and "seasonal" exposures to 490 µg/m^3 (0.25 ppm) O_3. The daily group was exposed for 8 hour/day for 18 mo, whereas the seasonal group was exposed to O_3 for 8 hour/day every other month (i.e., 9 mo of exposure spread over an 18-mo period). At termination of the monkeys'

exposure, both the daily and seasonal groups had respiratory bronchiolitis, an increased volume of respiratory bronchioles, and alterations of lung growth. The daily group (only) had an increase in inflammatory cells. The seasonal group (only) had an increase in total lung collagen and pulmonary function changes suggestive of a delay in lung maturation. Thus, the seasonal group of monkeys, which received half of the O_3 exposure compared to the daily group, had equivalent responses, and in some cases (e.g., collagen deposition) greater changes, than the daily group. In the rats, there was no significant difference between the daily and seasonal exposure regimens. Both groups had more bronchiole/alveolar duct junctions (as a total number and in the number/unit area). One month post-exposure, this effect was not observed. Use of other seasonal regimens at higher O_3 levels (1,862 µg/m^3, 0.95 ppm) for shorter periods (89 days) resulted in a similar total volume of affected parenchyma following both exposures, even though the episodic exposure was for only 35% of the hours as the daily exposure (Barr et al. 1990). There was an apparent, but not statistically significant, attenuation of O_3-induced respiratory bronchiolar formation. This body of work suggests that seasonal exposure patterns are of more concern than continuous exposures. Another study with nonhuman primates (Bonnet monkeys) found that subchronic exposures could also increase the acellular and cellular matrix of respiratory bronchioles (Harkema et al. 1993).

Catalano et al. (1995a) summarized a comprehensive study of rats exposed to O_3 (235, 980, or 1960 µg/m^3; 0.12, 0.5, or 1.0 ppm) for 6 hour/day, 5 days/week, for 20 mo. Reports by Harkema and Mauderly (1994), Chang et al. (1995), Plopper et al. (1994a), and Pinkerton et al. (1995) provide additional details on the morphometric analyses. Effects were found in the nasal, tracheobronchial, and centriacinar region, with the centriacinar region being the most affected. The lowest concentration did not significantly affect the nasal region. However, the two higher levels caused a moderate mucosal inflammation and other changes, including mucous cell metaplasia in the transitional epithelium (at 1960 µg/m^3, 1 ppm, only). In the centriacinar region, there was an increase in nonciliated cells and the total cell mass, depending on the dose delivered to the site (as estimated from a dosimetry model). Effects were observed at the lowest concentration tested. The epithelium of the alveolar duct was reorganized, as well (bronchial epithelial metaplasia), suggesting bronchiolarization. For this latter effect, males were more responsive than females, possibly due to differences in regional deposited dose, as calculated with dosimetry models. As shown by an additional morphometric approach, 980 and 1960 µg/m^3 (0.5 and 1.0 ppm) O_3 caused increases in the volume of the interstitial matrix of the proximal alveolar region. This thickening resulted from bronchial epithelial metaplasia. The highest concentration caused a mild fibrotic response, with increases in the noncellular and cellular components of the intersitium; increases were observed in collagen, elastin, basement membrane and acellular spaces. The increase in interstitial fibroblasts caused the increase in the cellular interstitium. As would be expected, the terminal bronchioles were less affected. Few changes were found in other endpoints (e.g., lung function or lung biochemistry) examined in these rats. The

investigators' interpretation of the entire study is that rats exposed to the two higher concentrations had some structural hallmarks of chronic airway disease.

Remodeling of the distal airways and centriacinar regions after long-term exposure to O_3 has been observed in numerous studies. Boorman et al. (1980), Moore and Schwartz (1981), Fujinaka et al. (1985), Tyler et al. (1987), Gross and White (1987), Smiler et al. (1988), Wright et al. (1990;1989) and Barr et al. (1990) have presented evidence of bronchiolization of airways that were previously alveolar ducts; that is, bronchial epithelium replaced the Type 1- and 2-cells typical of alveolar ducts. For example, using morphometric techniques, bronchiolization was reported in nonhuman primates exposed to 1,254 µg/m^3 (0.64 ppm) for 1 year (Fujinaka et al. 1985). In addition, the walls of centriacinar alveoli become thickened following long-term exposure in rodents and primates (Schwartz et al. 1976; Boorman et al. 1980; Fujinaka et al. 1985; Barry and Crapo 1985; Chang et al. 1992; Moffatt et al. 1987; Zitnik et al. 1978). Studies of the nature of these thickened centriacinar interalveolar septa and bronchiolar walls revealed increases in inflammatory cells, fibroblasts, and amorphous extracellular matrix (Fujinaka et al. 1985; Barry et al. 1985; Zitnik et al., 1978). Morphological evidence of mild fibrosis (i.e., local increase of collagen) in centriacinar interalveolar septa following exposure to <1,960 µg/m^3 (<1.0 ppm) has been reported by (Last et al. 1979; Boorman et al. 1980; Chang et al. 1992; Pickrell et al. 1987; Freeman et al. 1974; Moore and Schwartz 1981; Jakab and Bassett 1990). In addition to centriacinar remodeling, Pinkerton et al. (1998) reported thickening of tracheal, bronchial, and bronchiolar epithelium after 3 or 20 months exposure to 1960 µg/m^3 (1 ppm). No such responses were observed at either time point for exposures to 235 µg/m^3 (0.12 ppm).

There are several older reports where chronic exposure of rats to 1,960 µg/m^3 (1.0 ppm) O_3 caused emphysema (P'an and Jeiger 1972; Freeman et al. 1974; Stephens et al. 1976). Since the time of these publications, no other similar reports have appeared in the literature. The precise definition of emphysema is critical to evaluating these studies. The American Thoracic Society (ATS, 1995) defines emphysema as "an abnormal permanent enlargement of the airspaces distal to the terminal bronchioles which in humans is accompanied by destruction of their walls and without obvious fibrosis." In analyzing the reports of emphysema according to the ATS criteria above, it must be concluded that there is currently no unequivocal evidence that O_3 causes emphysema (US EPA 1996). In addition, the three reports noted above either (1) did not adequately describe the examination methods or results to permit independent conclusions or (2) did not use appropriate lung fixation methods.

Table 11-2: Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1862	0.95	8 hr/d, 90 d; 7 units of 8 hr/d for 5 d then 9 d air for 89 d total	Rat (SD)	Centriacinar lesion from episodic exposure similar but less severe than in daily exposure group, but more than predicted on C-T basis. (LM, EM)	Barr et al. 1990
1862	0.95	8 hr/d for 90 d	Rat, M (SD) 61 d	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

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
235, 490	0.12 0.25	12 hr/d, 6 wks	Rat (F344)	Effects on the TB region: decreased ciliated surface of Clara cells and number of brush cells per mm^2 of TB basement membrane. Effects on proximal alveolar region: Type 1 cells had increased numbers, decreased surface area, and increased thickness; Type 2 cells and AMs increased in number. Adult animals, in addition, had an increase in interstitial thickness and macrophages. Similar, but smaller changes at 235 µg/m^3. Although there were no statistically significant differences between neonates and adults in either region, others have found that rats prior to weaning are relatively insensitive to O_3. This implies that juveniles (post weaning) may be more responsive (see text). (EM)	Barry and Crapo 1985 Barry et al. 1988
392, 980, 1568	0.20, 0.5, 0.8	8 hr/d, 20, 50, or 90 d	Rat (SD)	Minimal or no effect at 392 µg/m^3. Changes at Day 20 and 50 generally similar to those at Day 90, but were slightly greater. The 980- and 1,568µg/m^3 groups had increased centriacinar AMs at all times. The 90-day 1,568-µg/m^3 group had changes in the TB/alveolar duct junction to TB/respiratory bronchiole/alveolar duct junctions. TBs had loss of or shortening of cilia. Nonciliated cells were flattened. Proximal alveoli of 20- and 90-day 1,568-µg/m^3 groups had thicker blood/air barriers due to increased volume of the interstitium, accompanied by accumulation of collagen and mononuclear cells. (LM, EM)	Boorman et al. 1980
784	0.4	1,3,7,28, or 56 d, continuously	Rat, M, (WSTR)	Centriacinar epithelial hyperplasia, reduced proliferation and increased cellularity after 28 and 56 days exposure. Some hyperplasia and fibrosis present after recovery but collagen content reduced	Van Bree et al. 2001

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
118 base, spike rising to 490	0.06 base, spike rising to 0.25	13 hr/d, 7 d/wk base; ramped spike, 9 hr/d, 5 d/wk; 1, 3, 13, and 78 wks	Rat (SD)	Shift from acute inflammatory phase to more chronic character. Changes in TBs: Decreased surface areas of ciliated and Clara cells, and domes of Clara cells reduced after 78 weeks; recovery PE. Changes in proximal alveolar region. Type 1 cells: increased in number at 1 week and remained changed to about the same degree at 3, 13, and 78 weeks; still altered 6 weeks after the 13-week exposure, but not 17 weeks after end of 78-week exposure. Type 2 cells: biphasic response, increased at 1 and 78 weeks; recovery PE. Interstitial volume: cells increased at 1 week only; matrix increased at 1, 13, and 78 weeks. Changes did not return to normal 17 weeks after the 78-week exposure. Increased collagen observed after 78-week exposure; partial recovery 17 weeks PE, but basement membrane still thickened. AMs: Increased at 1 week only. (LM, EM)	Chang et al. 1992
686	0.35	4.5 hr/d, 5 d/wk for 4 wks, 380 mmHg (5,400 m) or sea level P_B	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

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
392 784	0.2, 0.4	3, 7, 28, 56 d	Rat, M, (WSTR), 7wk	All species had epithelial hyperplasia and fibrosis at 56 days	Dormans et al. 1999
980, 1568	0.5, 0.8	24 hr/d, 8 hr/d, 7, 28, or 90 d	Monkey (bonnet)	Principal lesion was a "low-grade respiratory bronchiolitis" characterized by "intraluminal accumulations of macrophages and hypertrophy and hyperplasia of cuboidal bronchiolar epithelial cells." Conducting airway lesions not apparent by LM, but parallel linear arrays of uniform shortening and reduction of density of cilia were apparent by EM.	Eustis et al. 1981
1254	0.64	9 hr/d, 1 yr	Monkey (bonnet)	Increased volume density and volumes of RBs, which had thicker walls and narrower lumens. Peribronchiolar and perivascular connective tissue was increased by increased inflammatory cells and amorphous extracellular matrix, rather than stainable fibers. In RBs, cuboidal bronchiolar cells increased, and Type 1 cells decreased. (LM, EM)	Fujinaka et al. 1985
1058, 1725	0.54, 0.88	4 hr to 3 wks	Rat (SD)	Lungs heavier and larger. Increased centriacinar AMs. Hyperplasia of distal airway epithelium. Increased connective tissue elements. Collagen-like strand formed bridges across alveolar openings. Fibrosis more pronounced at 1,725 µg/m^3.	Freeman et al. 1974
1372	0.7	20 hr/d for 4 wks	Rat, M (F344) 14 wks	LM histopathology. 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 1987

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980	0.5	20 hr/d, 7 d/wk for 52 wks	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
294, 588	0.15, 0.3	8 hr/d, 6 or 90 d	Monkey (bonnet)	Effects in nasal epithelium. At 6 or 90 days, 294 mg/m^3, ciliated cell necrosis, shortened cilia, and secretory cell hyperplasia. Inflammation at Day 6. Morphometric changes in epithelium, especially in goblet cells, at Day 90. (LM, EM)	Harkema et al. 1987b
294, 588	0.15, 0.3	8 hr/d, 6 or 90 d	Macaca radiata, F, M 2-6 yrs	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
980	0.5	Continuous for 120 days	Mice, F (Swiss) 20-23 g	LM morphometry and histopathology. 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
510	0.26	8 hr/d, 5 d/wk, 1-90 d	Mice, M, 6-8 wk	Exposure produced fewer lavagable macrophages, epithelial cells, and PMN and reduced cell proliferation in mast cell deficient mice than in wildtype controls	Kleeberger et al. 2001

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980, 1568, 2156, 2940	0.5, 0.8, 1.1, 1.5	24 hr/d, 7, 14, 21 d	Rat, M (SD)	Increased collagen synthesis rate at all exposures; correlated with fibrosis (histological) of alveolar duct walls after 14 or 21 days. Effects were concentration dependent; for duration, 7 <14 = 21 days.	Last et al. 1979
784, 1254	0.4, 0.64	8 hr/d, 90 d	Monkey (bonnet) adult	Changes in RBs: (1) thicker walls, narrower lumens; (2) more cuboidal cells, fewer Type 1 cells; (3) thicker interstitium; and (4) more cellular organelles associated with protein synthesis. Many of the changes were not concentration dependent. (LM, EM)	Moffatt et al. 1987
980	0.5	24 hr/d, 3, 50, 90, 180 d	Rat, M (SD)	Inflammatory cell response of centriacinar region peaked at Day 50, but was still elevated at Day 180 and 62-days PE. Total lung volume increased at Day 180 and 62-days PE. Formation of respiratory bronchioles in centriacinar region at Day 180 only partially reversed PE. (LM, EM)	Moore and Schwartz 1981
1882	0.96	8 hr/night, 7 d/wk for 3 or 60 nights	Rat, M (SD) 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. 1988
1568	0.8	8h/d, 90 days	Rat, M, (SD)	P450 2B activity and protein elevated in distal bronchioles after 90-d exposure. Protein localized to Clara cells in all airway levels	Paige et al. 2000

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1117, 2156	0.57, 1.1	19 hr/d, 11 d	Rat	At Day 12, concentration-dependent inflammation and Type 2 cell hyperplasia. Increased elastolytic/collagenolytic activities. At 60-days PE, increase in total collagen and alveolar ductal fibrosis. (EM)	Pickrell et al. 1987
235, 980, 1960	0.12, 0.25, 0.5	6 hr/d, 5 d/wk 20 mo	Rat, M (F344) 6-8 wks	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. At 0.5 and 1.0 ppm increased volume of interstitium and epithelium along alveolar ducts due to epithelial metaplasia. Bronchiolar epithelial hyperplasia. At 1.0 ppm mild fibrotic response (increase in interstitial matrix and cellular interstitium; the latter due to increase in volume in interstitial fibroblasts). More effects in PAR than in terminal bronchioles.	Pinkerton et al. 1995 Chang et al. 1995
1921	0.98	8 hr/d, 7 d/wk for 90 d	Rat, M (SD) 65 d	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 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
235, 980, 1960	0.12, 0.5, 1.0	6 hr/d, 5 d/wk for 20 mo	Rat, M (F344) 6-8 wks	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 volume fraction (Vv) of nonciliated bronchiolar cells. Vv also increased in caudal (long-path) TB of 0.5-ppm exposed group. Mass ($\mu m^3/\mu m^2$) (Vs) of nonciliated cells increased in caudal (long-path) TBs of all exposed rats, but not in short-path TBs. CAR: Increased Vs 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
1960	1.0	8 hr, 5 d	Rat, M, (SD), 70 d	Alternation of acute inflammatory response and cellular injury with 2nd to 4th 5-day exposure periods. Hyperplasia and macrophage accumulations increase with each reexposure and recovery period	(Schelegle et al. 2003)
196	0.1	2 hr/d, 5 d/wk for 1 yr	Rabbit, M (NZW) 3-3.5 kg	LM morphology and morphometry of intrapulmonary conducting airways. 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
588	0.3	7 hr/d, 5 d/wk, 6 wk	Mouse (Swiss Webster)	Newborns had increase in Type 2 cell area, but no effect on cell number or alveolar wall areas or linear intercepts. Adults had increase in all these parameters. (Image analysis)	Sherwin and Richters 1985

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
235, 490, 980	0.12, 0.25, 0.5	20 hr/wk, 7 d/wk for 2 yrs (Examined at 4, 12, 26, 52, 78, and 104 wks)	Rat, M (F344) 42 days	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. 1990 Wright et al. 1989
1254, 1882	0.64, 0.96	8 hr/night, 42 nights	Rat (SD)	Rats 28-days old at start of exposure. At 1,254 µg/m^3, increase in volume of RBs immediately (both O_3 levels) and 42 days post-exposure (high O_3 level). At 1,882 µg/m^3, increase in lung volume and volumes of parenchyma and alveoli immediately after exposure; latter two also increased PE. (LM, EM)	Tyler et al. 1987
490	0.25	8 hr/d, 18 mo "daily" and "seasonal" (1 mo O_3, 1 mo air repeated for 18 mo)	Monkey, M, (Rhesus) Rat, M, (SD)	Monkey: Both groups: respiratory bronchiolitis, increased number of RBs, alterations in lung growth. (LM, EM). Daily group: Increased number of inflammatory cells (mostly macrophages) in lumen and interstitium. Seasonal group: Increase in total lung collagen, chest wall compliance (suggestive of delay in lung maturation), and inspiratory capacity. Rat: Daily and seasonal groups had equivalent increase in number of bronchiole/alveolar duct junctions, as total number and number/unit area.	Tyler et al. 1988

Table 11-2 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1254	0.64	8 hr/d for 12 mo	Macaca faciscularis, M, 6-7 mo	LM morphometry of CAR airway remodeling. Both Vv 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. Vv free cells, mostly AMs, increased only at exposure end. No differences in BW or fixed lung volumes.	Tyler et al. 1991
980	0.5	24 hr/d, 7, 21, or 35 d	Mouse (Swiss Webster)	Most severe damage at TB/alveolar duct junction. TBs had focal hyperplastic nodules of nonciliated cells. Proximal alveoli had accumulations of AMs and thickening of IAS by mononuclear cells at Day 7. At Day 35, changes much less evident, but numbers of Type 2 cells increased.	Zitnik et al. 1978
392 980	0.2 0.5	7 d 8 hr/d	Rat	Focal areas of missing or damaged cilia in trachea and bronchi. TB nonciliated (Clara) cells were shorter and had increased surface granularity and less smooth endoplasmic reticulum. Ciliated cells of TB had fewer cilia and focal blebs. Centriacinus had clusters of AMs and PMNs. Type 1 cells swollen and fragmented and type 2 cells frequently in pairs or clusters. Proximal IAS were minimally thickened. Lesions in 0.2 ppm rats were mild.	Schwartz et al., 1976

See Glossary for abbreviations

11.3.4 Interexposure Interval

Critical to understanding the effects of long term O_3 exposure is the length of time between exposures. Once that interval approaches the length of time for repair to be complete (7 days) the pattern of injury changes. Plopper et al. (1978) compared the response of rats exposed to 0.8 ppm O_3 either 6 or 27 days after an initial 3 day exposure to O_3. Early responses to O_3 included an influx of neutrophils followed by necrosis. The neutrophil influx resolved by the end of the 3 day exposure. Necrosis reached maximum after day 3 and resolved by day 6 (3 days after cessation of exposure). Re-exposure on day 30 (27 days after cessation of exposure) resulted in the same pattern of neutrophil influx and necrosis. This is not necessarily surprising since the normal course of repair should result in an epithelium that was completely repaired more than three weeks after cessation of exposure. Re-exposure on day 9 (6 days after cessation of exposure) resulted in a response similar to that observed in naive animals and in rats re-exposed 27 days after the initial exposure. For two exposure cycles, the acute inflammatory response and subsequent necrosis were the same as the initial exposure.

In an exposure regimen with a longer interexposure interval, Barr et al. (1990) used an episodic exposure pattern of five 8-hour days of exposure to 0.95 ppm O_3 followed by a 9-day recovery period. This pattern was repeated for a total of 90 days. Alternately, rats were exposed daily. While epithelial hypertrophy was not significantly different between daily and episodically exposed rats, the interstitial components were markedly different with a significant increase in interstitial thickness in episodically-exposed compared to daily-exposed rats.

The biologic response is further altered by additional increases in the length of time between exposures. Hyde et al. (1999) assessed total lung collagen and bronchiolar hyperplasia in Rhesus monkeys exposed to 0.25 ppm O_3. Monkeys were exposed 8 hours per day for either 18 continuous months or for alternating one-month periods. There was no discernable difference in the degree of bronchiolar hyperplasia in either exposure group, yet monkeys exposed on alternate months had considerably greater total lung collagen compared to monkeys exposed for 18 continuous months. This suggests that while the acute response (e.g., necrosis, inflammation) appears to be equivalent for subsequent exposures, the late responses involving repair may be altered.

It is apparent from the above data that an episodic exposure with an extended interexposure interval and multiple sampling points provides a better understanding of the impact of variable exposure conditions on pathogenesis. Using an exposure scenario similar to that of Barr et al. (1990), but with more frequent sampling, Schelegle et al. (2003) sampled rats at the beginning and end of each 5-day exposure period to 0.5 ppm O_3, 8 hour/d, and at the end of each 9-day recovery period through day 29. On the 5th day of the first exposure period, the epithelium appeared hyperplastic, yet the epithelium appeared similar to control by 9 days after the first exposure period. At the onset of the second set of

5-day exposures, inflammation, necrosis and hyperplasia were attenuated compared to that observed in the first exposure. Nine days after the second 5-day exposure period, bronchiolarization of the central acinus persisted. By the end of the fourth 5-day exposure, the acute inflammatory response was attenuated, macrophage/ monocyte counts were increased, epithelial hyperplasia was elevated, remodeling of the centriacinar region has increased and cell proliferation was decreased. The remodeling and macrophage infiltration were even further elevated by the end of the 9-day recovery period.

The response of the respiratory system to oxidant air pollutants such as O_3 is highly dependent on inhaled concentration and time of exposure. In ambient conditions, the synthesis of tropospheric O_3 is cyclic in nature, with O_3 concentrations usually highest in the mid-afternoon and dropping to lowest in the pre-dawn hours. Additionally, tropospheric O_3 concentrations exhibit daily and even seasonal variations. However, most experimental studies employ exposure protocols with near-continuous exposures. The episodic nature of ambient exposure conditions in humans suggests that reliable assessments of risk must include a clear understanding of the impact of cyclic exposure conditions on biological time response profiles. The biological response of the respiratory system in naive animals to the initial O_3 exposure follows a stereotypic cellular injury and inflammatory cycle. The imposition of additional oxidant stress by repeated exposure impacts the response variably, depending on the time during injury or repair when re-exposure occurs. The length of the interval between exposures appears to be more critical in determining the long-term impact of repeated exposures than the total duration of the exposure episode. Near-continuous exposure for a significant period of time (measured in months) fundamentally alters both the pattern of toxic cellular injury and the nature of the inflammatory response. Not only is the periodicity of the exposure important, but the duration of interexposure intervals also appears to effect biological response. The episodic nature of ambient exposure conditions appears to represent a greater health risk than would be expected based on extrapolation from experimental conditions relying on near-continuous exposure scenarios.

11.4 Controlled Exposure Studies

11.4.1 Characteristics of controlled exposure studies

11.4.1.1 Human volunteers

Experimental exposures of human volunteers to air pollutants under controlled laboratory conditions can provide useful pathophysiological information directly relevant to standard-setting. Controlled human exposure studies all follow a similar general structure. Volunteers are usually exposed to one or more carefully measured pollutants through a mouthpiece (oral breathing only), a face-mask (oronasal breathing), or in a chamber (oronasal breathing). A study subject may also utilize oral breathing if he or she wears a nose-clip. Nasal breathing may also be effectuated by keeping one's mouth closed in a chamber or face-mask experiment. Water-soluble pollutants, such as sulfur dioxide, tend to be scrubbed from incoming air in the nasal passages, so the mode of breathing may

make a marked difference in the health effects observed. However, for O_3, the mode of breathing, per se, does not appear to have a marked effect on lung function or symptoms reported by subjects (Hynes et al. 1988; Adams et al. 1989). Study subjects may breathe quietly at rest, or they may be required to hyperventilate or exercise in order to increase the rate of pollutant delivery, the total dose received, or both.

Typically the data collected in human controlled exposure studies include, at a minimum, responses to questions about respiratory and some nonrespiratory symptoms and measurements of pulmonary function. Measurements of these outcomes are typically taken before and after exposure to the pollutant(s) of interest, then compared with those obtained before and after exposures to clean, filtered air (FA). For multi-hour exposures, both symptoms and lung function measurements may also be taken at several points during the exposure, as well. In these study designs, each subject can serve as his or her own control, increasing the statistical power.

Assessment of other health outcomes may require more invasive procedures, such as bronchoalveolar lavage (BAL) or nasal lavage (NL). Such methods allow the researcher to examine specimens from the lung or nose for evidence of injury and inflammation. Lavage involves the instillation of sterile saline solution into the airways of the lung (BAL) or nose (NL). This fluid is then retrieved and analyzed microscopically (for, e.g., inflammatory cells) or chemically (for, e.g., proteins indicative of cell damage or biochemical messengers that attract inflammatory cells). Evidence of systemic effects may be obtained via the analysis of blood samples taken before and after the exposure, or through the use of an electrocardiograph (for measuring heart rate and rhythm).

Exercise on a treadmill or stationary cycle is often incorporated in the exposure protocol in order to increase ventilation rates (and hence the dose of the pollutant delivered to the respiratory tract), to simulate the effect of active outdoor work or recreation. Indeed, multi-hour O_3 exposure protocols utilized by the U.S. EPA were intended to simulate the exertion of outdoor manual (Folinsbee 1989). It should be noted, however, that exercise may itself modify the outcomes of interest in studies of individuals with asthma. Many asthmatics experience coughing and chest tightness during or shortly after exercise, accompanied by measurable decreases in lung function. Such exercise-induced bronchospasm (EIB) is usually self-limited and reversible within an hour, but may require inhalation of bronchodilator medication to alleviate symptoms. In analyzing the results of chamber studies of asthmatics, one can control for the effects of EIB by comparing lung function after pollutant exposure to that found after filtered air exposure on another day. However, the magnitude of EIB in a given individual may vary from day to day, which may obscure somewhat any pollutant-associated effects, even with such statistical control.

11.4.1.2 Typical outcomes assessed in human controlled exposure studies

One of the primary categories of outcomes assessed in human controlled exposure studies is lung function, which requires the subject to take a maximally

deep breath and exhale forcefully into a spirometer mouthpiece. Typical lung function metrics in controlled human exposure studies include: (i) the total volume of air that can be exhaled after a deep inspiration (forced vital capacity or FVC); (ii) the amount of air that can be exhaled during the first second of a forced expiratory maneuver (forced expiratory volume in one second or FEV1); and (iii) the amount of air exhaled over the middle half of a forced expiration (forced mid-expiratory flow or FEF25-75%). FEV1 is considered the most reproducible measure of acute changes in large airway caliber. FEF25-75% is an indirect measure of the caliber of smaller airways at lower lung volumes. As noted above, measurements of these functions are typically taken before and after exposure to the pollutant(s) of interest, then compared with those obtained before and after exposures to FA.

Numerous studies of controlled O_3 exposures have also involved examination of airway hyperreactivity (AHR) or bronchial hyperresponsiveness. (The terms hyperreactivity and hyperresponsiveness are often used interchangeably.) AHR refers to an exaggerated tendency of the airways to constrict in response to such substances as airborne irritants, cold, dry air, and pharmacological agents (usually methacholine or histamine). Assessment of AHR involves serial challenges with methacholine or another bronchoconstrictor, followed by spirometry. The subject inhales increasing concentrations of bronchoconstrictor until the FEV1 or Sr_{aw} measured after each inhalation decreases (FEV1) or increases (Sr_{aw}) by a pre-specified percentage from baseline. For example, if the experimental protocol targets a 20% decline in FEV1, the results are expressed as the provocative concentration necessary to elicit this response (i.e., PC_{20}). The less bronchoconstrictor required to provoke the targeted decline, the greater the AHR.

Although it has been recognized for decades that acute and chronic O_3 exposure in animals injures the airway epithelium and causes inflammation, this effect was first demonstrated in a controlled exposure study of humans using a relatively high dose in 1986 (Seltzer et al. 1986). Inflammation represents an orchestrated sequence of biological responses to injury or infection, involving (among other things) the release of pro-inflammatory mediators (such as IL-8), which attract white blood cells known as polymorphonuclear cells [PMNs] to the site of injury. Inflammatory changes also include increased local vascular permeability, and influx of inflammatory cells, including PMNs and other types of lymphocytes. A number of biochemical markers of injury or inflammation have been linked with O_3 exposure (e.g., Devlin et al. 1991; Seltzer et al. 1986) and will be discussed in the context of specific studies. One function of inflammation is to kill foreign pathogens with highly reactive oxidants generated by various white blood cells. However, these chemicals can also attack and injure host tissue as well. Thus, the inflammatory process may amplify O_3-associated toxicity.

Investigators have also measured O_3-related effects on host defenses. Alveolar macrophages (AMs) are mobile phagocytic cells responsible for maintaining the sterility of the deep lung. Under normal circumstances, AMs represent about 95% of the immune cells retrieved in BAL fluid. A variety of tests of AM function

(e.g., mobility, phagocytosis, and ability to generate reactive oxygen species) can be conducted on such cells obtained from human subjects' BAL to assess functional changes due to O_3 exposure.

Because many epidemiological studies over the past 15 years have identified adverse cardiovascular outcomes related to acute exposures to air pollution, investigators have also begun to assess measures of cardiovascular function in controlled exposure studies. For instance, subjects' electrocardiograph may be monitored to measure heart rate and rhythm during and after exposure. Another outcome that has been examined is heart rate variability, a measure of the heart's ability to respond to stress, which can be derived from electrocardiographic data.

11.4.1.3 Strengths and limitations of controlled exposure studies

The principal advantage of controlled human exposure studies over epidemiological studies is that exposures to the pollutant(s) of interest can be precisely measured, and therefore exposure-response relationships can be determined with some degree of accuracy. Under limited circumstances, investigators may also be able to identify a threshold of exposure for certain outcomes, which may provide useful information for the standard-setting process. Controlled studies in a clinical setting can also include invasive, labor-intensive procedures, such as bronchoalveolar lavage (described above), allowing for the examination of outcomes that would not be feasible in epidemiological investigations. While exposure conditions can also be controlled in animal experiments, the obvious advantage of human chamber studies in relation to the latter is that no cross-species extrapolation is required.

In contrast to animal studies, pathological examination of pollutant-induced tissue damage (e.g., in lengthwise dissections of airways) is more limited in living humans by both ethical and practical considerations, although small biopsies may be obtained during the BAL procedure. Other limitations of controlled human exposure studies include the following: 1) only short-term responses to acute exposures can be evaluated (minutes to repeated exposures on several days (Balmes et al. 1997; Folinsbee et al. 1994); 2) the small number of subjects usually participating in these experiments limits the statistical power to detect effects; 3) simplified exposure conditions may fail to capture effects that might occur in response to complex, real-world exposures; 4) self-selection of volunteers for such experiments may limit the applicability of the results, as such individuals may not be representative of the general population; 5) eligibility criteria for such studies may also limit the generalizability of the results (e.g., the systematic exclusion of people with a current or recent respiratory infection); and 6) there have historically been fewer studies conducted on potentially susceptible subpopulations that ambient air quality standards are intended to protect, such as asthmatics. While some O_3 chamber studies have focused on potentially susceptible subgroups, selection biases are operative in these studies, as well. For instance, the disease severity of most asthmatics volunteering for controlled exposure studies tends to be relatively mild (with intermittent or sometimes more persistent symptoms). Moreover, those who are experiencing a flare or

exacerbation of their condition would likely be excluded from the exposure protocol, at least until they recovered from the acute illness. Thus, chamber study volunteers do not necessarily reflect the entire spectrum of this disease in the population. It should be noted, however, that these limitations of chamber studies generally tend to result in underestimates of pollutant effects. Therefore, identifying responses related to specific exposure conditions constitutes an important dimension of the standard-setting process. In contrast, given the potential shortcomings of this genre of research, null or negative findings may in some cases reflect the constraints of study design as much as or more than biological realities. However, these studies do provide the strongest and most quantifiable concentration-response data on the health effects of O_3, in and of itself.

11.4.1.4 O_3 Analyzer Calibration Methods and Health Data Interpretation

Ambient O_3 monitoring in early studies of the responses of human subjects to O_3 exposure was performed with instruments that were calibrated by one of several iodometric methods, while most studies conducted after the late 1970's used the ultraviolet absorption calibration standard. DeMore et al. (1976) compared the standard iodometric methods of several public agencies (ARB, USEPA, and the Los Angeles Air Pollution Control District, predecessor of the South Coast Air Quality Management District) with each other, and also with the ultraviolet absorption method. The iodometric method typically overestimated the O_3 concentration, with the difference as much as 25 to 30% compared to the ultraviolet absorption method, although the differences varied according to the specific iodometric method used. The authors concluded that the ultraviolet absorption (UV) calibration method was the most accurate, and the UV calibration standard was officially adopted by ARB in 1975. Consequently, the O_3 concentrations used in early human studies are most likely lower than reported, although how much lower is unknown.

Since the original papers typically do not give information as to which of the iodometric methods was used, it is impossible to estimate the actual O_3 concentrations to which the subjects were exposed. We have made no attempt to apply a correction factor to iodometrically based O_3 concentrations in this literature review. Instead, studies based on iodometric calibration methods are indicated by (KI) following the stated O_3 concentration, and the results are considered qualitatively rather than quantitatively in the health analysis. Studies not notated (KI) used the UV calibration method.

11.4.2 Short-term Exposure to Ozone

11.4.2.1 Responses of Human Subjects

11.4.2.1.1 Introduction

The primary site of O_3-induced health effects is the lung. Reported effects include reductions in various measures of pulmonary function and decreased inspiratory capacity. A rapid, shallow breathing pattern is particularly evident during exercise, and increases in airway hyperresponsiveness to non-specific

bronchoconstrictor agents (i.e. methacholine, histamine and cold air), symptoms of cough and pain on deep inspiration, and cellular and biochemical markers of lung inflammation have also been reported. The decrease in inspiratory capacity results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC). Some individual subjects show evidence of bronchoconstriction, which contributes to the decrease in forced expiratory volume in 1 sec (FEV1). In many of the studies reporting effects with exposure to O_3 at concentrations less than 0.3 ppm, the decrements in FEV1 are of similar size to those in FVC, and are due to decreased inspiratory capacity, and to a lesser extent, increases in central and peripheral airway resistance (R_{aw}).

The degree of response to O_3 is related to three factors: 1) the O_3 exposure concentration, 2) V_E, the ventilation rate, and 3) T, the duration of exposure (see Sections 11.1.4 and 11.4.15.8). The consensus of available research is that O_3 concentration is the most important of the three factors. Normal, healthy people exposed to O_3 concentrations $\geq$ 0.12 ppm (the federal one-hour standard) develop significant, transient reversible decrements in pulmonary function if V_E or T are increased sufficiently. Host factors are also important in determining individual responses to O_3 exposures: there is a large degree of variability (up to about ten-fold) among individuals in the magnitude of acute responses to a given inhaled dose of O_3. These responses appear to be characteristic of a given individual, as they are reproducible over periods of many months (see Section 11.4.2.1.4).

11.4.2.1.2 Responses With One- to Three-Hour O_3 Exposures

Several early studies examined the acute effects of single O_3 exposures to less than 1.0 ppm O_3 in resting humans (Young et al. 1964; Bates et al. 1972; Silverman et al. 1976; Folinsbee et al., 1978; Horvath et al. 1979; Kagawa and Tsuru 1979). The lowest concentration at which significant reductions of pulmonary function were observed was 0.5 ppm (KI) (Folinsbee et al., 1978; Horvath et al., 1979). Effects on R_{aw} in resting subjects exposed to O_3 concentrations below 1.0 ppm are inconsistent.

Bates et al. (1972) and Hazucha et al. (1973) were the first to investigate the effect on pulmonary function responses of increasing ventilation via exercise so as to increase the inhaled dose of O_3. The important findings from these two studies were that increasing V_E (i) increased the magnitude of the observed pulmonary function response at any given O_3 concentration, and (ii) reduced the O_3 concentration at which significant pulmonary function responses could be observed. Subsequent investigations expanded on these two studies, and demonstrated that in healthy young adults performing moderate to heavy exercise, intermittently or continuously, for durations of one to three hours, inhalation of O_3 concentrations in the range of 0.12 to 0.18 ppm elicited statistically significant decrements in pulmonary function, and an increase in respiratory symptoms (Silverman et al. 1976; Folinsbee et al. 1975; Hackney et al. 1975; Adams et al. 1981; McDonnell et al. 1983; Kulle et al. 1985; Linn et al. 1986; Seal et al. 1993; Adams and Schelegle 1983; Folinsbee et al. 1984; Gong

et al. 1986; Avol et al. 1984; Adams WC 1986; Hazucha 1987; McKittrick and Adams 1995).

11.4.2.1.3 The Ozone Concentration-Response Relationship: One- to Three-Hour Exposures

Numerous studies have reported that as the inhaled dose of O_3 increases observed effects also increase in magnitude. As discussed below, inhaled O_3 dose is a function of the O_3 concentration, V_E, and T (duration of exposure). Consequently, there are a large number of possible scenarios under which adverse responses could be expected. Several investigators have reported on the time course of response development, and others have used secondary data to develop statistical models to predict effects under various exposure conditions.

Early studies at relatively high O_3 concentrations using intermittent light exercise protocols of one to three hrs duration clearly demonstrated the potentiating effect of exercise on responses to acute O_3 exposure (Silverman et al. 1976; Bates et al. 1972; Hackney et al. 1975; Hazucha et al. 1973). These studies led to a series of studies (Adams et al. 1981; Folinsbee et al. 1978; McDonnell et al. 1983; Kulle et al. 1985; Linn et al. 1986) that sought to define more precisely the O_3 exposure-response relationship.

Studies employing protocols with different intensities of intermittent exercise for two-hour (Folinsbee et al., 1978; McDonnell et al. 1983; Kulle et al. 1985; Linn et al. 1986; Seal et al. 1993) or continuous exercise (Adams et al. 1981) for about one hour with O_3 concentrations ranging up to 0.5 ppm confirmed that significant pulmonary and symptom responses occurred at 0.3 ppm when subjects exercised at moderately heavy workloads. Multiple regression analysis revealed that O_3 concentration explained the majority of variance in the pulmonary function responses, followed by V_E. The Adams et al. study (1981) varied the length of exposure, and observed that exposure duration was the poorest predictor of response, although only a limited number of V_E and exposure duration combinations were investigated.

Subsequently several investigators (McDonnell et al. 1983; Kulle et al. 1985; Linn et al. 1986; Seal et al. 1993) sought to determine the lowest O_3 concentration at which group mean decrements in pulmonary function occurred in subjects performing moderate intermittent exercise for 2 to 2.5 hours. McDonnell et al. (1983) and Seal et al. (1993) reported small, statistically significant decrements in various measures of pulmonary function beginning at 0.12 ppm O_3, and increases in symptoms beginning at 0.18 ppm O_3, while Kulle et al. (1985) and Linn et al. (1986) reported that the lowest O_3 concentrations at which significant effects could be detected were 0.15 and 0.16 ppm, respectively. The studies by McDonnell et al. (1983), Kulle et al. (1985) and Linn et al. (1986) utilized strenuous exercise (67-70 L/min). The investigation by Seal et al. (1993), however, used an exercise level of 25 L/min/m^2 BSA, which was considerably lower than those used in the other three studies. Similar findings of significant pulmonary function decrements in heavily exercising subjects were confirmed by other investigators (e.g., Adams and Schelegle 1983; Avol et al. 1984; Folinsbee

et al. 1984; Gong et al. 1986), who studied subjects exposed to O_3 concentrations of 0.2 ppm or less with one-hour continuous exercise protocols.

McKittrick and Adams (1995) addressed the important question of whether protocols that used different exercise patterns (continuous vs. intermittent exercise) with the same total inhaled dose of O_3 would result in similar effects. The results indicated that when the total inhaled dose of O_3 was equivalent at a given O_3 concentration, there was no difference between pulmonary function responses induced by continuous versus intermittent exercise protocols of two hour or less, although symptoms were somewhat less with the intermittent exercise protocol.

Collectively, this literature indicates that one to three hour exposures to O_3 concentrations as low as 0.12 ppm with moderate to heavy exercise can induce decrements in pulmonary function and increases in respiratory symptoms. Figure 11-1, derived from Seal et al. (1993), provides an illustration of the relationship between changes in FEV1, Sr_{aw} and cough and O_3 concentration (two-hour exposures with intermittent exercise).

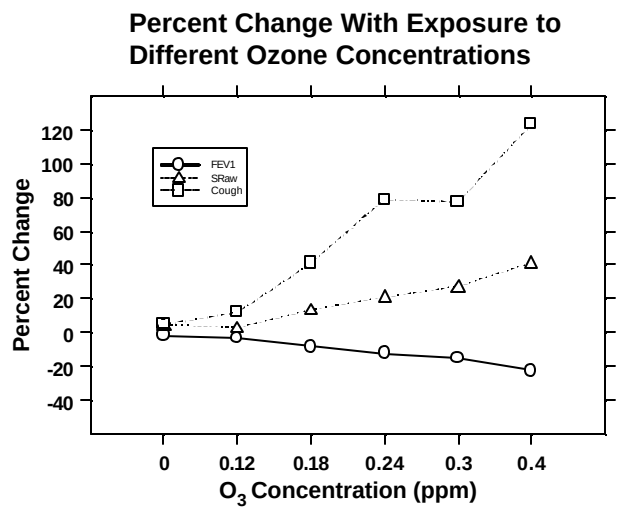

Figure 11-1 Percent change in FEV1, Sr_{aw} and cough with two-hour intermittent exercise exposures to FA, 0.12, 0.18, 0.24, 0.3, and 0.4 ppm O_3. Derived from Seal et al., 1993.

11.4.2.1.4 Inter-subject Variability, Individual Sensitivity and the Association between Responses

In 1972 Bates et al. reported that there was a large degree of inter-subject variability in response to O_3. These observations were subsequently confirmed by numerous other investigators (e.g., Adams et al. 1981; Folinsbee et al. 1978;

Folinsbee et al. 1978; McDonnell et al. 1985b, McDonnell et al. 1983; McDonnell et al. 1993; Kulle et al. 1985; Aris et al. 1995; Holz et al. 1999). It remains unclear what factors account for the variability in responsiveness among individuals.

Folinsbee et al. (1991) combined the data from four studies performed at the US EPA Health Effects Research Laboratory, all which used the same protocol. He then reported on the distribution of change in FEV1 in response to exposure to FA and 0.08, 0.10, and 0.12 ppm O_3 (Figure 11-2).

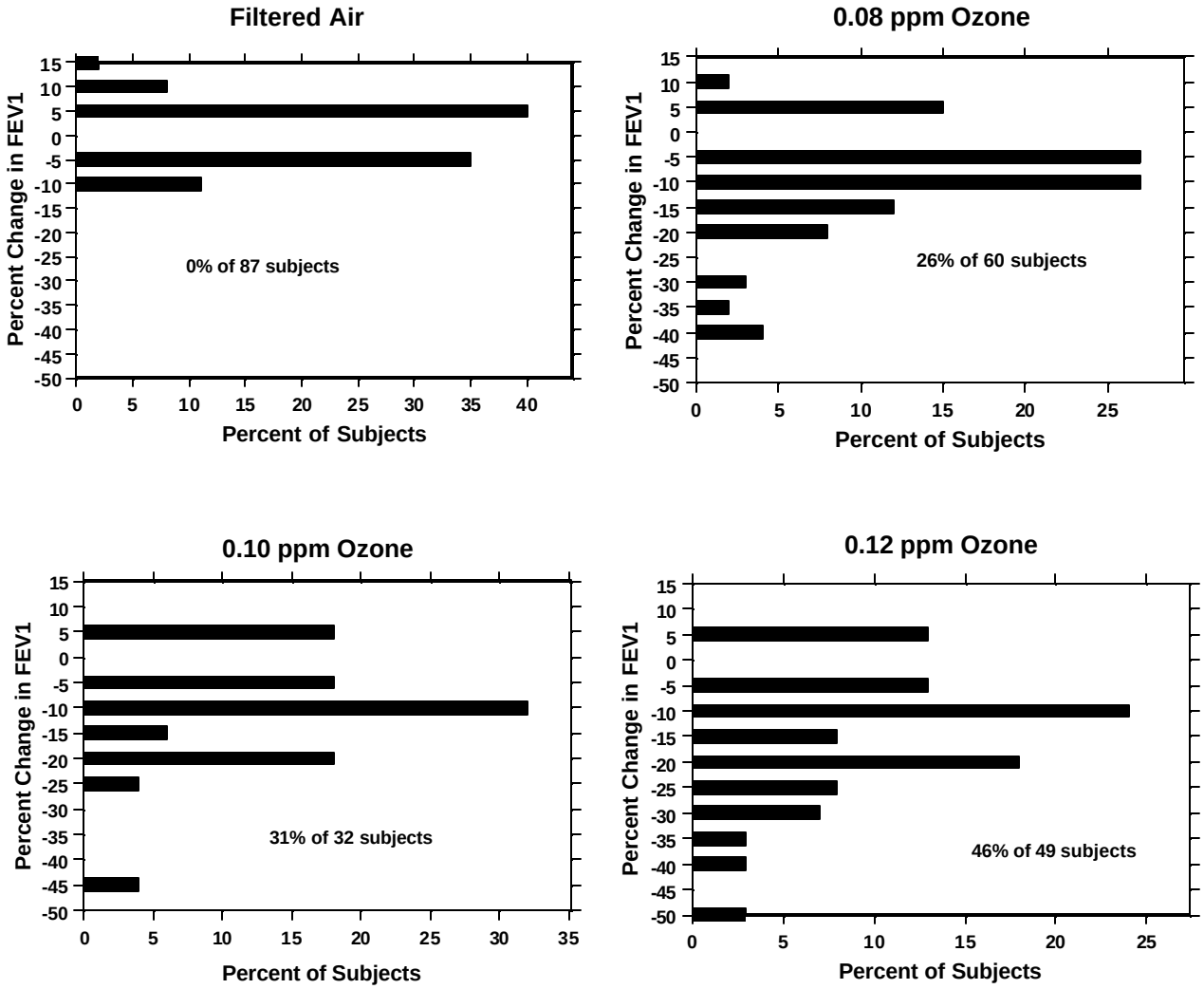

Figure 11-2 Distribution of response for 87 subjects exposed to FA and at least one of 0.08, 0.10, or 0.12 ppm O_3. All exposures were for 6.6-hour, with exercise for 50 min of each hour. Decrease in FEV1 is presented as the change from baseline. Each panel also includes the number of subjects, and the percentage of that number who had a decrease in FEV1 in excess of 10%. Derived from Folinsbee et al., 1991.

The basis of inter-individual variability in responsiveness to O_3 was investigated by McDonnell et al. (1993). The investigators considered a wide range of personal characteristics, including age, height, baseline pulmonary function, presence of allergies, and past smoking history, that might predict individual differences in pulmonary function responses to O_3 exposure in 290 healthy white male subjects between 18 and 32 years of age. The only factor that contributed significantly to inter-subject responsiveness was age (younger subjects were

more responsive). The analysis found that O_3 concentration accounted for only 31% of the variance in responses, demonstrating the importance of some as yet undefined factor(s).

McDonnell and Smith (1994) further investigated differences in individual sensitivity and range of responses in FEV1 among males. The investigators developed a statistical model based on other studies (Folinsbee et al. 1988; Horstman et al. 1990; McDonnell et al. 1991) that included O_3 concentration, exposure duration and age as predictors. The studies had all used the same 6.6-hour protocol, and similar V_E. The results indicated that inclusion of age improved the model fit, but that much of the variability remained unexplained.

Frampton et al. (1997) and Torres et al. (1997) investigated the comparative responses of smokers and nonsmokers to O_3 exposure, and applied multiple logistic regression analysis to ascertain predictors of responsiveness to O_3. The results confirmed previous findings that nonspecific airway responsiveness, gender, and allergies do not predict O_3 responsiveness. There was no age effect, contrary to other reports (e.g., McDonnell et al. 1995; McDonnell et al. 1999), but current smoking was predictive of reduced responsiveness to O_3 exposure. Ozone-induced airway inflammation was independent of smoking status.

Several studies have reported on the within-subject variability of responses to single O_3 exposures that are separated in time. McDonnell et al. (1985b) examined the reproducibility of individual responses to O_3 exposure in healthy human subjects, to determine whether the observed inter-subject variability was related to real differences in O_3 responsiveness among individual subjects, or whether it was related to day-to-day within-subject variability. The subjects participated in two exposures that were separated by 21 to 385 days (mean = 88). The authors concluded that the large range of inter-subject variability in the magnitude of responsiveness reported within groups of individuals exposed to O_3 was due primarily to large differences in the intrinsic responsiveness of the individual subjects, although the factors contributing to inter-subject variability are undefined. The reproducibility of responses varied among endpoints, although the correlations between the two measurements were statistically significant for FVC (R=0.92), FEV1 (R=0.91), FEF25-75% (R=0.83), cough (R=0.77), shortness of breath R=0.60), and Sr_{aw} (R=0.54). Gliner et al. (1983) reported similar decrements in FEV1 following two exposures to 0.42 or 0.5 ppm O_3 that were separated by 31 to 149 days. The intraclass correlation coefficient, R, among O_3 exposures that were separated by one to four weeks ranged between 0.929 and 0.992 for FVC, FEV1 and FEF25-75% in a group of older men and women who completed two-hour intermittent exercise exposures while inhaling 0.45 ppm O_3 (Bedi et al. 1988). Jorres et al. (2000) and Adams (2000a) also noted the reproducibility of pulmonary function and symptom responses to acute O_3 exposures at concentrations more relevant to current ambient O_3 levels.

11.4.2.1.5 Relationship Between Endpoints: Pulmonary Function, Airway Reactivity, Inflammation and Symptom Responses

Several studies have investigated the relationships among the various endpoints that show changes with acute O_3 exposure (pulmonary function responses, symptoms, airway inflammation, and airway reactivity). This subject is discussed in Section 11.2.1).

Table 11-3 Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone – Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.25, 0.50, 0.75 (KI)	2 hr at rest	Healthy adults, (8 M, 5 F), 21-22 yrs	Decreased FVC with 0.50 and 0.75 ppm exposure compared to FA. Small (NS) decrease of 4% in mean VO_{2max} following 0.75 ppm, compared to FA.	Horvath et al. 1979
0.37, 0.5, 0.75 (KI)	2 hr at rest	Healthy adults, (20 M, 8 F), 19-29 yrs	Decreased FEV1, V_{25} and V_{50} with 0.75 ppm exposure, compared to FA.	Silverman et al. 1976
0.50 (KI)	2 hr at rest	Healthy adults, (40 M), 18-28 yrs	Decrease in forced expiratory volume and flows.	Folinsbee et al. 1978
0.08, 0.10, 0.12, 0.14, 0.16	2 hr IE (4x15 min at V_E = 68 L/min)	Healthy adults, (24 M), 18-33 yrs	No significant changes in pulmonary function.	Linn et al. 1986
0.10, 0.15, 0.20, 0.25	2 hr IE (4x14min at V_E=70 L/min)	Healthy nonsmokers, (20 M), 25.3 ±4.1 (SD) yrs	FVC, FEV1, FEF25-75%, SG_{aw}, and TLC all decreased with increasing O_3 concentration and 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	1 hr competitive simulation at mean V_E=87L/min	Highly trained competitive cyclists, (10 M), 19-29 yrs	Decreased FVC and FEV1 with exposure to 0.18 and 0.24 ppm O_3, compared to FA exposure. Decrease in exercise time for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm.	Schelegle and Adams 1986
0.12, 0.18, 0.24, 0.30, 0.40	2.5 hr IE (4x15 min TM exercise at V_E=65 L/min); 1 exposure/subject	Healthy adults, (20 – 29 M/group), 18-30 yrs	Significant decreases in FVC, FEV1 and FEF25-75% at 0.12 ppm O_3; decrease in V_T and increase in f_R and SR_{aw} at 0.24 ppm O_3.	McDonnell et al. 1983

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12, 0.18, 0.24, 0.30, 0.40	2.5 hr IE (4x15 min TM exercise at V_E=35 L/min/m^2 BSA); on two occasions separated by 3 weeks to 14 mo, 1 concentration per subject	Healthy adults (5-8 M/group), 18-30 yrs	Pulmonary function and V_E were not significantly different, regardless of the separation of the two exposures. The results indicated that responses to O_3 concentrations of 0.18 ppm or higher are reproducible for up to 10 mo.	McDonnell et al. 1985b
0.12, 0.18, 0.24, 0.30, 0.40	2.5 hr IE (4x15 min TM exercise at V_E=35 L/min/m^2 BSA), one concentration per subject	Healthy adults, (290 M), 18-32 yrs	Strongest predictors of FEV1 decrements were O_3 concentration and age. Older subjects were less responsive.	McDonnell et al. 1993
0.12, 0.18, 0.24, 0.30, 0.40	2.5 hr IE (4x15 min TM exercise at V_E=25 L/min/m^2 BSA); 1 concentration per subject	Healthy adults, (93 WM; 92 BM; 94 WF;92 BF), 18-35 yrs	FEV1 decreased at all O_3 concentrations, compared to FA. SR_{aw} increase in SR_{aw} at 0.18 ppm or higher compared to FA. No consistent differences between gender or racial groups.	Seal et al. 1993
0.12, 0.20	1 hr CE (mean V_E=89 L/min)	Highly trained competitive cyclists, (15 M, 2 F), 19-30 yrs	Compared to FA exposure, V_{Emax}, V_{O2max}, maximal work load, ride time, FVC and FEV1 decreased with 0.20 ppm exposure during maximal exercise conditions, but no significant change with exposure to 0.12 ppm.	Gong et al. 1986

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.16, 0.24, 0.32	1 hr CE (mean V_E=57 L/min)	Competitive bicyclists, (42 M, 8 F), 26.4 ± 6.9 (SD) yrs	Small decrements in FEV1 at all O_3 concentrations. Decrement larger following exposure to 0.24 ppm than following 0.16 ppm.	Avol et al. 1984
0.125 0.250	3 hr, IE (15 min ex/15 min rest), (V_E=26.2±6.4 l/min)	Healthy (N=11 F, 10 M; mean age=28.5±5 yr) and asthmatic (N=10 F, 5 M; 30.3±7.5 yrs) adults,	Both groups had similar, small, but statistically significant decrements in FVC and FEV1 after exposure to 0.25 ppm O_3. Symptoms increased slightly after exposure to 0.25 ppm O_3. (Primary focus of the study was inflammatory changes. See Table 11-5).	Holz et al. 1999
0.20	4 hr IE (50 min exercise and 10 min rest/hr), (V_E=40 L/min)	Healthy nonsmokers, (11 M and 3 F for FA exposure; 9 M and 3 F for O_3 exposure), 19-41 yrs	Compared to FA, O_3 exposure led to decreased FVC, FEV1, V_T, and SR_{aw}, and increased f_R. Total cell count and LDH increased in bronchial lavage fluid and inflammatory cell influx occurred with O_3 exposure, compared to FA exposure.	Aris et al. 1993b
0.20	4 hr, IE, (50 min ex/10 in rest per hr), (V_E=25 L/min/m^2BSA)	Healthy nonsmokers, (42 M, 24 F), 18-50 yrs, mean=27.0±4.5	O_3 exposure induced statistically significant decrements in FEV1 and FVC, and a statistically significant increase in SR_{aw}. Results indicated that an individual may have large declines in lung function with few symptoms, or vice versa, pointing to different mechanisms underlying the two categories of responses to O_3 exposure.	Aris et al. 1995

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.20, 0.30	30 to 80 min CE cycle ergometry (V_E=33 or 66 L/min)	Aerobically fit, (8 M), 22-46 yrs	Effective dose of O_3 significantly related to pulmonary function decrements and ventilatory pattern changes. O_3 concentration accounted for the majority of pulmonary function variance.	Adams et al. 1981
0.20, 0.35	1 hr CE or competitive simulation (mean V_E=77.5 L/min)	Well-trained distance runners, (10 M), 19-31 yrs	FVC, FEV1 and FEF25-75% decreased with both O_3 concentrations compared to FA. V_T decreased and f_R increased with 50-min continuous exercise. Three subjects unable to complete continuous and competitive protocols at 0.35 ppm O_3.	Adams and Schelegle 1983
0.21	1 hr CE (75% V_{O2max})	Well-trained cyclists, (6 M, 1 F), 18-27 yrs	Decrease in FVC, FEV1, FEF25-75%, and MVV with exposure to 0.21 ppm O_3, compared to FA exposure.	Folinsbee et al. 1984
0.25	1 hr CE (mean V_E=63 L/min)	Healthy nonsmokers, active nonathletes (19 M, 7 F) divided into four groups. Mean ages for the four groups: 20.7±1.97, 20.5±3.27, 22.0±2.45, 20.3±0.76 yrs	FVC, FEV1 and MVV significantly decreased following exposure to 0.25 ppm O_3, compared to FA.	Folinsbee and Horvath 1986

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.25 0.37 0.50	2 hr, IE (15 ex/15 rest), (V_E twice resting)	Healthy adults, (N=13), 22-41 yrs, 5 with history of smoking, two still active smokers, each exposed to one or more of the three O_3 concentrations Six were exposed to 0.25 ppm O_3, five to 0.37 ppm O_3, and seven to 0.5 ppm O_3	Magnitude of changes in forced spirometry measurements and symptoms was related to the O_3 concentration, although changes were statistically significant only in the group exposed to 0.50 ppm O_3.	Hackney et al. 1975
0.30 0.35	1 hr, CE cycle ergometer, V_E=70 l/min for males; 50 l/min for females) 1 hr, CE cycle ergometer, V_E=60 l/min	Healthy, aerobically trained adults (20 M, 20 F), 18-30 yrs Healthy, nonsmokers, (40 M), young adults	O_3 inhalation induced statistically significant decrements in FVC and FEV1, and increases in SR_{aw}, f_R and symptoms.	Adams WC 1986
0.30	1 hr CE cycle ergometer (V_E=60 L/min) and 2 hr IE cycle ergometer (V_E=45-47 L/min)	Moderately fit, healthy, (12 M), 24.3±4 yrs	Equivalent decrease in FEV1 for all O_3 exposure protocols.	McKittrick and Adams 1995

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.35	1 hr CE cycle ergometry (mean V_E=60 L/min)	Moderately fit, healthy, (14 M), 18-34 yrs	FVC and FEV1 significantly decreased by O_3 exposure, compared to FA exposure. Decrements in FVC and FEV1 with O_3 exposure were attenuated significantly with indomethacin pre-treatment, compared to no drug or placebo. The O_3-induced increase in SR_{aw} was not affected by indomethacin.	Schelegle et al. 1987
0.37, 0.50, 0.75 (KI)	2 hr IE cycle ergometer (V_E=2.5 times resting)	Healthy, (20 M, 8 F), 19-29 yrs	Decrements in FVC and FEV1 after exposure to all three O_3 concentrations compared to FA. $V_{25\%VC}$ decreased with 0.37 and 0.75 ppm O_3 exposure, and $V_{50\%VC}$ decreased with all three O_3 exposures, compared to FA. The increase in f_R was correlated with the dose of inhaled O_3, and was accompanied by a compensatory decrease in V_T, resulting in no difference in V_E.	Silverman et al. 1976 Folinsbee et al. 1975
0.37 0.75 (KI)	2 hr, IE, cycle ergometer (V_E=2 times resting)	Smokers (average of 20 cigarettes/day) and nonsmokers, (N=6/group), mean age=23.6±0.7 yrs	Magnitude of decrements in forced expiratory lung functions increased with increasing O_3 concentration. Both smokers and nonsmokers had statistically significant decrements in FVC, FEV1 and FEF25-75% after both one and two hrs of exposure to 0.37 or 0.75 ppm O_3. Symptoms of respiratory discomfort were reported with exposure to both O_3 concentrations.	Hazucha et al. 1973
0.40	2 hr IE treadmill exercise (V_E=50-75 L/min)	Healthy, (8 M), 18-27 yrs	Decreases in FVC, FEV1, VI and TLC, and increases in SR_{aw} and f_R with O_3 exposure compared to FA. Pretreatment with atropine abolished the O_3-induced increase in SR_{aw} and attenuated the FEV1 and FEF25-75% response.	Beckett et al. 1985

Table 11-3 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	1 hr CE treadmill exercise (V_E=20L/min/m^2 BSA)	Healthy, nonsmokers (20 M), 18-35 yrs	V_T decreased by 25% and O_3 uptake efficiency in the lower respiratory tract declined by 9% during O_3 exposure.	Gerrity et al. 1994
0.40	1 hr CE, treadmill (V_E=20 L/min/m^2 BSA)	Healthy nonsmokers, (22 M), 18-35 yrs	Significant decreases in FVC, FEV1, FVC/FEV1, and FEF25-75%. The half-width of an expired aerosol bolus was significantly increased, suggesting an O_3-induced change in small airway function.	Keefe et al. 1991
0.50	2 hr IE, alternating 15 min rest and treadmill exercise (V_E=40 L/min)	Healthy, (18 M), 20-30 yrs	Decrease in FVC, V_T, and maximal transpulmonary pressure, increase in SR_{aw} and f_R with O_3 exposure, compared to FA exposure. Lidocaine inhalation partially reversed the decrease in FVC.	Hazucha et al. 1989
0.75 (KI)	2 hr IE, alternating 15 min rest and cycle ergometry (50 W)	4 light smokers, 9 nonsmokers (13 M) 19-30 yrs	Decrease in FVC, FEV1, ERV, IC, and FEF25-75% after 1 hr of O_3 exposure. Decreases in VO_{2max}, V_{max}, V_{Emax}, maximal workload and heart rate following O_3 exposure, compared with FA.	Folinsbee et al. 1977
0.75 (KI)	2 hr, resting 2 hr, IE, (15 ex/15 rest), (V_E=twice resting)	Healthy adults, (N=10), 22-35 yrs All 10 subjects studies at rest, three studied with the exercise protocol	Effects were greatest in the exercising subjects, although some of the resting subjects experienced significant reductions in measures of lung function, and increases in symptoms.	Bates et al. 1972

Table 11-4: Airway Responsiveness Following Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.10 0.25 0.40	1 hr, IE (15 min ex/15 min rest) (V_E=27 L/min)	Stable mild asthmatics with FEV1>70% predicted, and methacholine responsiveness	No significant changes in FEV1 or FVC with exposure to FA, 0.10 or 0.25 ppm. 12 subjects exposed to 0.40 ppm O_3 had significant reduction in FEV1. O_3 exposure had no effect on responses to an exercise challenge test performed one hr after the O_3 exposures.	Weymer et al. 1994
0.12	1 hr, rest	Mild, stable asthmatics, (8F, 7 M), 19-45 yrs	No significant difference in % change in FEV1 or in bronchial responsiveness to exercise challenge.	Fernandes et al. 1994
0.12 0.20	1 hr at V_E=89 L/min, followed by 3-4 min at V_E=150 L/min	Elite cyclists, (15 M, 2 F)	Greater than 20% increase in histamine responsiveness in one subject at 0.12 ppm O_3, and in 9 subjects at 0.20 ppm O_3.	Gong et al. 1986
0.12	Rest	Atopic asthmatics (5 F, 10 M)	No effect of O_3 on airway response to grass allergen.	Ball et al. 1996
0.12 O_3/0.1 SO_2 0.12 O_3/0.12 O_3 Air/0.1 SO_2	45 min in first atmosphere, followed by 15 min in second atmosphere, IE (V_E=~30 l/min)	Asthmatics, (8 M, 5 F) 12-18 yrs	Greater declines in FEV1 and $V_{max50\%}$, along with greater increase in respiratory resistance after O_3/SO_2 condition than after O_3/O_3 or air/SO_2 conditions.	Koenig et al. 1990
Air/antigen 0.12/antigen	1 hr, rest	Asthmatics, (4 M, 3 F), 21-64 yrs	Increased bronchoconstrictor response to inhaled ragweed or grass after O_3 exposure compared to air.	Molfino et al. 1991
FA / antigen 0.12/antigen	1 hr, rest	Mild, allergic asthma, (6 F, 9 M), 18-49 yrs	No effect of O_3 on airway response to grass or ragweed allergen.	Hanania et al. 1998

Table 11-4 (cont.): Airway Responsiveness Following Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12	45 min, IE (15 min ex at V_E=3 times resting)	Physician diagnosed asthma; SO_2-induced airway hyperreactivity. (12 F, 5 M), 19-38 yrs	Dietary supplementation for 4 weeks before, and during the experimental period (approximately an additional 2 weeks) with 400 IU vitamin E + 500 mg vitamin C reduced airway responses to a challenge with 0.10 or 0.25 ppm SO_2 post-O_3 exposure. Supplementation with vitamins C and E had no effect on pulmonary function responses to O_3 exposure.	Trenga et al. 2001
0.125 0.250 0.125 (on 4 consecutive days)	3 hr, IE (10 min rest/15 min ex), V_E=30 L/min	Mild bronchial asthma: (5 F, 6 M), 20-53 yrs Allergic rhinitis: (6 F, 16 M), 19-48 yrs	Mean early-phase FEV1 response and number of subjects with =20% reduction significantly greater after 0.25 ppm or 4 x 0.125 ppm O_3 exposures. Most of the =15% late-phase responses occurred after exposure to 4 x 0.125 ppm O_3, along with significant inflammatory effects, as indicated by increased sputum eosinophils (allergic rhinitis and asthmatic groups) and in increased sputum lymphocytes, mast cell tryptase, histamine and LDH (asthmatics only).	Holz et al. 2002
0.20 0.40 0.40	2 hr, IE, (V_E=2 times resting) 2 hr/d for 3 days, IE (V_E=2 times resting)	Healthy nonsmokers, (12 M, 7 F), 21-32 yrs	Increased sensitivity to inhaled histamine aerosol challenge after exposure to O_3 at 0.40 ppm, but not after exposure to 0.20 ppm. Increased sensitivity attenuated over the 3 day exposure period.	Dimeo et al. 1981
0.25	3 hr, IE, (15 min ex/10 min rest/5 min without O_3 during each 30 min of exposure), (V_E=30 L/min)	Nonsmokers: mild atopic asthmatics, (11 F, 13 M); mean age = 26 yrs Allergic rhinitics (6 M, 6 F); mean age = 25 yrs Healthy controls (5 M, 5 F); mean age 23 yrs	Asthmatic and allergic subjects had increased bronchial responsiveness to allergen after O_3 exposure, compared to FA exposure. .	Jorres et al. 1996

Table 11-4 (cont.): Airway Responsiveness Following Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	3 hr/d for 5 consecutive days, (V_E= 4 to 5 times resting)	Healthy nonsmokers, (13 M, 11 F), 19-46 yrs	Enhanced response to methacholine after the first 3 days but attenuated to baseline by day 5.	Kulle et al. 1982
0.40	2 hr, IE, (15 min rest/15 min ex),` (V_E=53-55 L/min)	Asthmatics (5 F, 4 M) Healthy (5 F, 4 M) 18-34 yrs	Decreased $PC_{100SRaw}$ from 33 mg/mL to 8.5 mg/mL in healthy subjects, and from 0.52 mg/mL to 0.19 mg/mL for asthmatic subjects after exposure. Asthmatics also had a decrease in $PC_{100SRaw}$ from 0.48 mg/mL to 0.27 mg/mL after exposure to air.	Kreit et al. 1989
0.40	3 hr/d for 5 consecutive days, IE (15 min rest/15 min ex), (V_E=32 L/min)	Mild asthmatics requiring only occasional bronchodilator therapy, (2 F, 8 M), 19-48 yrs	Significant FEV1 and symptoms responses on first two O_3 days, then attenuation. Attenuation reversed partially by four and seven days after the fifth consecutive O_3 exposure. Bronchial reactivity to methacholine was greatest after the first O_3 exposure, but remained elevated following the remaining O_3 exposures.	Gong et al. 1997a
0.40	2 hr IE, (15 min rest/15 min ex), (V_E=20 L/min/m^2 BSA)	Stable, mild asthmatics. No medications within 8 hr of exposures, (5 F, 1 M), 18-27 yrs	Increased airway responsiveness to methacholine 16 hr post-O_3 exposure. Proteinase inhibitor (rALP) treatment prior to O_3 exposure did not alter responses, compared to pre-treatment with placebo.	Hiltermann et al. 1998
0.40	2 hr, IE (40 min ex/hr at 50 W)	Healthy, nonatopic adults, (9 F, 6 M) 31.1 ±2.1 yrs	Decreased FEV1 and FVC, and increased bronchial reactivity to methacholine 4 hr post-exposure. Inhaled budesonide before exposure did not alter responses	Nightingale et al. 2000
0.40	2 hr, IE (15 min rest/15 min ex), (V_E=20 L/min/m^2 BSA)	Stable, mild asthmatics. No medications within 8 hr of exposure, (6 F, 1 M), 19-26 yrs	Increased bronchial responsiveness to methacholine 16 hr after exposure. Inhaled apocynin treatment significantly reduced O_3-induced airway responsiveness.	Peters et al. 2001

Table 11-4 (cont.): Airway Responsiveness Following Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.60	2 hr at rest	Healthy nonsmokers, (5 M, 3 F), 22-30 yrs	300% increase in histamine induced change in R_{aw} at 5 min after O_3 exposure. At 24 hr and 1 week post-O_3 exposure, the increases were 84% and 50%, respectively. Two subjects had an increased response to histamine 1 week after O_3 exposure.	Golden et al. 1978
0.60	2 hr, IE (15 min rest/15 min ex), (V_E=2 times resting)	Atopic (N=9) and nonatopic (N=7) adult nonsmokers (11 M, 5 F), none with asthma, 21-35 yrs	All had increased response to methacholine or histamine challenge after O_3 exposure. On average, atopics had larger responses than nonatopics. Increased responsiveness resolved after 24 hr. Atropine pretreatment blocked O_3-induced increase in airway responsiveness.	Holtzman et al. 1979

Table 11-5: Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
Ambient	variable, outdoor jogging	Healthy nonsmokers, recreational joggers, (18 M, 1 F), 23-38 yrs All completed initial BAL, 15 underwent BAL during the subsequent winter, and 6 completed BAL the following summer	BALF levels of LDH, IL-8, and PGE_2 increased during the summer smog season.	Kinney et al. 1996
0.20	2 hr face mask exposure, IE (15 min ex/15 min rest), (V_E=30 l/min)	Healthy nonatopic, nonsmokers (10 M, 2 F), mean age 28 yrs	Trend for increased PMN and ciliated epithelial cells in BALF 6 hr post-O_3 exposure. Decreased substance P immunoreactivity in submucosa inversely related to changes in PMN and decrease in FEV1.	Krishna et al. 1997
0.30	1 hr (mouthpiece), CE (V_E=60 L/min)	Healthy nonsmokers (5 M)	Significantly increased PMN's in BALF at 1, 6 and 24 hr post O_3 exposure, with the peak increase at 6 hr.	Schelegle et al. 1991
0.40	2 hr IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	BALF at 18 hr post-O_3 exposure showed a significant increase in PMNs, protein, albumin, IgG, PGE_2, plasminogen activator, elastase, complement C3a, and fibronectin.	Koren et al. 1989a
0.40	2 hr IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	Macrophages removed 18 hr after O_3 exposure showed 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	2 hr IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	BALF obtained 18 hr after O_3 exposure contained increased amounts of coagulation factors, tissue factor, and factor VII. Macrophages in the BALF had elevated tissue factor mRNA.	McGee et al. 1990

Table 11-5 (cont.): Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	2 hr IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (10 M), 18-35 yrs	BALF obtained 1 hr after O_3 exposure showed a significant increase in PMNs, protein PGE2, TBX2, IL-6, LDH, a-1-antitrypsin and tissue factor, compared to after exposure to FA. Phagocytosis of yeast by alveolar macrophages was decreased by O_3 exposure.	Koren et al. 1991
0.40	2 hr/d for 5 d, then 2 hr either 10 or 20 d later, IE (15 min ex/15 min rest), (V_E=40 L/min)	Healthy, nonsmokers (16 M), 18-35 yrs	BAL performed immediately after the fifth consecutive exposure, and then 10 or 20 d later. Most markers of inflammation (PMNs, IL-6, IL-8, protein, a-1 antitrypsin, PGE_2, fibronectin) showed complete attenuation with 5 consecutive days of O_3 exposure, while markers of cell damage (LDH, elastase) did not. Reversal of attenuation was not complete for some markers, even after 20 d of nonexposure.	Devlin et al. 1997
0.40	2 hr IE (15 min ex/15 min rest), (V_E=60 L/min)	Healthy nonsmokers (10 M), 20-32 yrs	Subjects were pretreated with 800 mg ibuprofen or placebo 90 min before exposure. Subjects given ibuprofen had smaller reductions in FEV1 after O_3 exposure. BALF obtained 1 hr after O_3 exposure contained similar levels of PMNs, protein, fibronectin, LDH, a-1 antitrypsin, LTB_4 and C3a in both ibuprofen and placebo groups. However, subjects given ibuprofen had decreased levels of IL-6, TBX_2 and PGE_2.	Hazucha et al. 1996
0.40	2 hr IE (15 min ex/15 min rest), (V_E=66 l/min)	Healthy nonsmokers (8 M), 18-35 yrs	Comparison of BALF at 1and 18 hr post-O_3 exposure showed that at 1 hr PMNs, total protein, LDH, a-1 antitrypsin, fibronectin, PGE_2, TBX_2, C3a, tissue factor and clotting factor VII were increased. IL-6 and PGE_2 were higher at 1 hr post-O_3 exposure than after 18 hr. There were no time differences for PMN and protein.	Devlin et al. 1996 Comparison with Koren et al. 1989b

Table 11-5 (cont.): Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	2 hr IE (15 min ex/15 min rest), (V_E=55 L/min)	Healthy nonsmokers, (N=11) , 18-35 yrs.	Mean FEV1 decrement with O_3 exposure was approximately 10%. BAL was performed at 0, 2, or 4 hr post-exposure. PMN number tended to be highest at 4 hr post-O_3 exposure, while LTC_4 was increased at all sampling times. There was no change in PGE_2 or in thromboxane.	Coffey et al. 1996
0.40	2 hr mouthpiece exposure, IE (15 min ex/15 min rest), (V_E=40 l/min)	Healthy nonsmokers, (5 M, 5 F), mean age = 30 yrs	Sputum induction at 4 hr post-O_3 exposure showed a 3-fold increase in neutrophils and a decrease in macrophages. IL-6, IL-8 and myeloperoxidase increased after O_3 exposure. There was a possible relationship of IL-8 and PMN levels.	Fahy et al. 1995
0.40 0.60	2 hr, IE (15 min ex/15 min rest), (83 watts for women, 100 watts for men)	Healthy nonsmokers (7 M, 3 F), 23-41 yrs	BALF obtained 3 hr after O_3 exposure had significant increases in PMNs, PGE_2, TBX_2, and PGF_{2a} at both O_3 concentrations.	Seltzer et al. 1986

Table 11-6: Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
Nasal Lavage Studies				
0.0, 0.4	2 hr, IE, 15 min ex/15 min rest; (V_E=20 L/min/m^2 BSA)	Placebo group: healthy nonsmokers, (15 M, 1 F), mean age 27 yrs Antioxidant supplement group: healthy nonsmokers, (13 M, 2 F), mean age 27 yrs	Decrements in FVC and FEV1 were significantly smaller in the supplementation group. No difference in inflammatory responses (BAL) between the two groups, either in recovery of cells or in concentrations and types of inflammatory cytokines.	Samet et al. 2001
0.0, 0.22,	4 hr, IE (20 min exercise/10 min rest); (V_E=40-46 L/min)	Healthy nonsmokers, O_3 responders and nonresponders, 18-40 yrs	Glutathione peroxidase (GPx) activity and extracellular GPx protein level were significantly depleted in ELF for at least 18 hr post-O_3 exposure. There was a significant inverse relationship between extracellular GPx and the increase in PMN 18 hr after O_3 exposure, indicating that low GPx is a predictor of susceptibility to O_3-induced airway inflammation.	Avissar et al. 2000
0.0, 0.40	2 hr, IE (20 min ex/10 min rest), Workload = 50 W	Healthy nonsmokers, (6 M, 9 F), mean age 31 yrs	Corticosteroid pretreatment had no effect on post-O_3 exposure decrement in pulmonary function, PMN response, or sputum cell count under either placebo or treatment conditions. Methacholine PC_{20FEV1} was equally decreased under both conditions at 4 hr after exposure. No changes in exhaled NO or CO.	Nightingale et al. 2000
0.12 0.24	90 min, IE (15 min ex/15 min rest), (V_E=20 L/min)	Asthmatics (5 M, 5 F) Nonasthmatics (4 M, 4 F)18-41 yrs	Nasal lavage performed immediately and 24 hr after exposures. Asthmatics exposed to 0.24 ppm O_3 had an increased number of PMNs at both measurement times. There were no changes in nonasthmatic subjects. Neither subject group had changes in lung or nasal function.	McBride et al. 1994

Table 11-6 (cont.): Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.21	1 hr CE cycle ergometry (mean V_E=80 L/min)	Highly fit endurance cyclists, (14 M, 1F), 16-34 yrs	Pretreatment with albuterol had no significant effect on metabolic, ventilatory or pulmonary functions, airway reactivity, or exercise performance compared to pretreatment with placebo. O_3 inhalation induced a decrease in V_{Emax}.	Gong et al. 1988
0.25	1 hr CE cycle ergometer (V_E=30 L/min/m^2 BSA)	Healthy nonsmokers, (5 M, 2 F), 22-30 yrs	12.4% decrease in FEV1, significant elevation of substance P and 8-epi-PGF2a in segmental airway washing, but not bronchoalveolar lavage fluid	Hazbun et al. 1993
0.30	1 hr CE cycle ergometer (mean V_E=60 L/min)	Healthy, (5 M), age not given	Decrease in FVC and FEV1, increase in SR_{aw} 1 hr post-O_3 exposure. Increased percentage PMNs at 1, 6 and 24 hr post-O_3 exposure in bronchial fraction of BALF, compared to FA exposure. PMNs peaked at 6 hr post-O_3 in the bronchial fraction of BALF. Percent PMNs elevated at 6 and 24 hr post-O_3 in pooled samples.	Schelegle et al. 1991
0.40	2 hr, IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy males (N=11), 18-35 yrs	NL performed immediately before, immediately after and 22 hr after exposures. Increased number of PMNs at both post-O_3 exposure times. Increased tryptase immediately after O_3 exposure and increased albumin at 22 hr after O_3 exposure.	Graham and Koren 1990 Koren et al. 1990
0.40	2 hr at rest	Nonsmoking, mild asthmatics, (N=10), 18-35 yrs	Nonsignificant increase in response to allergen after O_3 exposure. PMN and eosinophils increased after O_3+allergen challenge. O_3 alone increased nasal inflammation. An eleventh subject dropped out before completing the protocols.	Peden et al. 1995
0.40	2 hr IE treadmill exercise (V_E=50-75 L/min)	Healthy, (8 M), 18-27 yrs	Decreases in FVC, FEV1, VI and TLC, and increases in SR_{aw} and f_R with O_3 exposure compared to FA. Pretreatment with atropine abolished the O_3-induced increase in SR_{aw} and attenuated the FEV1 and FEF25-75% response.	Beckett et al. 1985

Table 11-6 (cont.): Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	2 hr IE, alternating 15 min rest and treadmill exercise periods (V_E=35 L/min/m^2)	Healthy nonsmokers (11 M), 18-35 yrs	No correlation between pulmonary function and inflammatory endpoints measured in BAL fluid obtained 18 hr after exposure. At 18 hr post-O_3 exposure there was an increase in the percentage of PMNs, total protein, albumin, IgG and neutrophil elastase, and a decrease in the percentage of macrophages, compared to following FA exposure.	Koren et al. 1989b
0.40	2 hr IE, alternating 15 min rest and treadmill exercise periods (V_E=35 L/min/m^2)	Healthy nonsmokers, (10 M), 18-35 yrs	PMN, PGE_2 and IL-6 higher in BAL fluid obtained 1 hr post-O_3 exposure compared to in BAL obtained at 18 hr post-O_3 exposure. Fibronectin and urokinase-type plasminogen activator higher at 18 hr post-O_3 exposure than at 1 hr post-exposure.	Koren et al. 1991
0.40	2 hr IE, alternating 15 min rest and cycle ergometry (V_E=30 L/min/m^2 BSA)	Healthy nonsmokers, (13 M), 18-31 yrs	Indomethacin pretreatment attenuated O_3-induced decrements in FVC and FEV1, compared to O_3 exposure alone. Indomethacin pretreatment did not significantly affect airway hyperresponsiveness.	Ying et al. 1990
0.40 0.60	2 hr IE, cycle ergometry (100 W for males; 83 W for females)	Healthy nonsmokers, (7 M, 3 F), 23-41 yrs	Increased airway responsiveness to methacholine, and in mean percentage of neutrophils, PGF2a, TXB_2 and PGE_2 concentrations in BALF 3 hr after both O_3 exposures, compared to after FA exposure.	Seltzer et al. 1986
0.50	2 hr IE, alternating 15 min rest and treadmill exercise (V_E=40 L/min)	Healthy, (18 M), 20-30 yrs	Decrease in FVC, V_T, and maximal transpulmonary pressure, increase in SR_{aw} and f_R with O_3 exposure, compared to FA exposure. Lidocaine inhalation partially reversed the decrease in FVC.	Hazucha et al. 1989

11.4.3 Responses to Multi-Hour Ozone Exposures: Concentration-Response Relationships

11.4.3.1 Introduction

Historically, most controlled studies of the effects of O_3 have used exposure protocols of one to two hours in duration, primarily based on a diurnal O_3 concentration profile typical of the Los Angeles air basin, and the notion that peak concentration, rather than cumulative dose, was the most important factor mediating O_3 toxicity. Analysis of air quality data from other parts of the US, however, has shown another widespread pattern characterized by a six-to-eight hour period with a relatively constant O_3 concentration near but below that of the existing federal one-hour ambient standard (0.12 ppm) (US EPA 1996). Rombout et al. (1986) advocated an averaging time of seven to ten hours. They pointed out that in the 1970's, when ambient air quality standards were first being developed, it was believed, based on observations in Los Angeles, that O_3 formation was an urban issue due to urban areas being the site of the majority of NO_X and hydrocarbon emissions. At that time it was regarded as surprising when significant O_3 concentrations were measured in rural areas, and in areas regarded as pristine. Subsequent investigations of O_3 concentrations in suburban and rural areas identified another pattern of O_3 concentrations. This second pattern was characterized as a broad peak lasting as long as eight to twelve hours, in contrast to the sharp one- to two-hour peak identified earlier in the Los Angeles, CA area. The broad peak was often only slightly below the one-hour standard concentration.

Recognition of this second pattern of ambient O_3 concentrations led to several studies that investigated the effects of multi-hour exposure to an O_3 concentration lower than the one-hour ambient air quality standard. The protocols for these studies, except for that of Kerr et al. (1975), were designed to simulate a day of heavy outdoor work, recreation or play. The results are particularly relevant for California, because the comparatively mild climate results in a long O_3 season. Moreover, there is a large contingent of California residents who either earn their living by working outdoors, or who engage in multi-hour exercise or active play on a year-round basis, in contrast to many other parts of the US.

11.4.3.2 Healthy Subjects

The first study to report on human responses to multi-hour O_3 exposures was by Kerr et al. (1975), based on the hypothesis that the effects of O_3 exposure would be enhanced with prolonged exposure. The study included 10 smokers and 10 nonsmokers, ranging in age from 21 to 60 years of age (four subjects over 40 yrs). The subjects were exposed to FA for 8 hours on one day, and to 0.50 ppm O_3 (KI) for 6 hours on another day. The exposures were primarily at rest, but did include two 15 min periods of light exercise. Smokers had no significant changes in pulmonary function, while the nonsmokers had significant reductions in FVC and SG_{aw} after the O_3 exposure. Seven nonsmokers reported cough and nine reported chest discomfort. In comparison, among smokers, only one reported

cough and four reported chest discomfort. While the O_3 concentration used is substantially higher than has occurred in California in recent times, the findings illustrate that prolonged exposure, even at rest, can lead to adverse effects. Further, the effective dose of O_3 inhaled by these subjects is in the range used in more recent studies that addressed the effects of exposure to a lower concentration of O_3 over a simulated day of active work or play.

Beginning in 1988 scientists from the US EPA laboratory and elsewhere have investigated responses to multi-hour exposures to O_3 concentrations closer to those typically observed in the ambient environment, 0.04 ppm to 0.16 ppm, for 6.6 or 7.6 hours. The subjects have typically been active, healthy males between 18 and 35 years of age, although a few studies have included females and/or asthmatics. The protocol for these studies required the subjects to perform light or moderate exercise for 50 min. of each hour, with a 35-min. lunch/rest period following the third hour of exposure. Exercise ventilation rates ranged from about 25 L/min in the studies of asthmatics, to about 45 L/min in the other studies. These studies have largely focused on pulmonary function, symptoms and nonspecific airway responsiveness, although several have included bronchoscopy and BALF analysis.

The first of this group of studies was performed by Folinsbee et al. (1988), who compared the responses to FA and 0.12 ppm O_3 (the federal one-hour ambient air quality standard for O_3) of 10 healthy nonsmokers who completed the 6.6 hour protocol described above. Mean forced vital capacity (FVC) and FEV1 decreased in a roughly linear fashion throughout the exposure, and were decreased by 8.3% and 13%, respectively, by the end of the exposure period. The decrement in FEV1 with O_3 exposure became statistically significant at 4.6 hour. The subject group exhibited a wide range of responsiveness to the O_3 exposure (+3% to –48% change in FEV1). Three subjects had decrements in FEV1 in excess of 25%, while the three least sensitive had changes of less than 5%. Methacholine responsiveness was increased following O_3 exposure compared to FA exposure. All subjects reported pain on deep inspiration by the end of the O_3 exposure. There was no apparent relationship between the O_3-induced change in methacholine reactivity and changes in FVC and FEV1, suggesting that these effects were probably mediated by different mechanisms.

Horstman et al. (1990) expanded the database on multi-hour O_3 exposures by exposing healthy young males to filtered air, 0.08, 0.10, and 0.12 ppm O_3 using the 6.6-hour protocol. Each subject served as his own control. The three primary variables investigated were FEV1, pain on deep inspiration and the provocative dose of methacholine inducing a 100% increase in SR_{aw} (PD100). There were significant changes in all three primary variables following all three O_3 exposures. The decrease in FEV1 became significant after the 5th hour of the exposure to 0.08 ppm O_3, the 4th hour. of the exposure to 0.10 ppm and the 3rd hour. of the exposure to 0.12 ppm O_3. The range of subject responses and the mean decrements were somewhat smaller than in Folinsbee et al. (Folinsbee et al. 1988), but the overall findings were similar. Several subjects did not respond to any concentration of O_3, while others were responsive to all the O_3

concentrations used. Subjects returned to the laboratory the next day for pulmonary function measurements. At 20 to 25 hours post-O_3 exposure, FEV1 had returned to within 1% of baseline in these subjects. There was an inverse relationship between PD100 and O_3 concentration, indicating that O_3 exposure increased nonspecific airway reactivity in a dose-dependent manner. Larsen et al. (1991) used the data from Horstman et al. (1990) to develop a "dose-response" relationship for percent change in FEV1 as a function of O_3 concentration and exposure duration. The analysis suggested that FEV1 responses were approximately linear with exposure duration, and that O_3 concentration played a slightly more significant role in predicting the effect size.

McDonnell et al. (1991) continued this series of investigations in subjects exposed to FA and 0.08 ppm and 0.10 ppm O_3. The changes in FEV1 were similar to those reported by Folinsbee et al. (1988) and Horstman et al. (1990). The most important observation from this study comes from the results of a three-parameter logistic model that was applied to fit the dose-response relationship. The model results suggest that the O_3-pulmonary function response relationship may have a sigmoid rather than a linear shape, suggesting a response plateau. That is, for a given O_3 concentration and ventilation level (i.e., dose rate), the FEV1 response tended to reach a limit that would not increase further with continued exposure (which would resemble a plateau in the dose-response curve). The results further substantiated the wide range of inter-subject responses. For example, decrements in FEV1 following the exposure to 0.08 ppm O_3 ranged from +4.3% to –37.9%. Moreover, changes in spirometry did not begin until 2 to 3 hours into the O_3 exposure.

The fourth study in the US EPA series of multi-hour studies (Folinsbee et al. 1994) reported on the responses of 17 young males exposed to 0.12 ppm O_3 for 6.6-hour on 5 consecutive days. The study was designed to evaluate whether the attenuation responses observed in studies performed at higher O_3 concentrations for 1-2 hours (see Section 11.4.9) also occurred with multi-hour exposures to lower levels of O_3. The day-1 results were similar to those of the three studies described above, with the day-1 and day-2 changes in FEV1 averaging -11.9% and -6.23%, respectively. On day-3 through day-5 the changes in FEV1 were not different from the FA condition. Individual decrements in FEV1 ranged from +0.3% to –34.3%, and +2.9% to –33% on day-1 and day-2, respectively. Although most of the subjects had little or no change in FEV1 on day-3 through day-5, there were five subjects who had decrements on FEV1 between 5% and 10%. Reports of lower respiratory symptoms were increased only on day-1. Interestingly, responsiveness to methacholine was increased following all five O_3 exposures compared to the FA exposure, but it was not correlated with either symptoms or with the change in FEV1. Fifteen of the subjects were also exposed to 0.16 ppm O_3 for 4 hour while exercising for 50-min per hour at the same workload as used in the 6.6-hour exposures described above. The group mean decrements in FVC and FEV1 were -9.53% and -16.6%, respectively. Analysis of nasal lavage failed to show evidence of nasal inflammation. The larger decrements in measures of pulmonary function following the 4 hour exposure to 0.16 ppm O_3 compared to the 6.6 hour exposure

to 0.12 ppm O_3 highlights the greater significance of the O_3 concentration compared to exposure duration in inducing pulmonary function decrements. The results also confirm previous findings that pulmonary function responses and non-specific airway responsiveness to O_3 exposure have different biological mechanisms (see Section 11.2).

Folinsbee et al. (1991) combined the FEV1 response data from the studies conducted at the EPA Health Effects Research Laboratory (Folinsbee et al. 1988; Horstman et al. 1990; McDonnell et al. 1991), and examined the distribution of responses among the subjects at 0.0, 0.08, 0.10 and 0.12 ppm O_3. The results of the analysis indicated that 46% of the subjects studied had decrements in FEV1 greater than 10% after a 6.6-hour exposure to 0.12 ppm O_3. The percentages of subjects having greater than 10% reduction in FEV1 were 0%, 26% and 31% for FA, 0.08 and 0.10 ppm O_3 exposures, respectively. Furthermore, the analysis showed that FEV1 decrements as large as 30% to 50% occurred in some individual subjects undergoing 6.6-hour exposure to O_3 concentrations less than 0.12 ppm, confirming the previously observed wide range of individual response to O_3 exposure, as well as adverse effects at levels typical of widespread ambient exposures (see Figure 11-2 above).

The 6.6-hour protocol developed by EPA has also been used to examine the responses of middle-aged adults (30-45 yrs) exposed to FA, and 0.08 ppm O_3 on two consecutive days (Horvath et al. 1991). Although the findings were qualitatively similar to those of Folinsbee et al. (1988), Horstman et al. (1990) and McDonnell et al. (1991), the magnitudes of the pulmonary function and symptom responses were smaller. The mean decrement in FEV1 following the day-1 O_3 exposure was 2.2%, only 3 of the 11 subjects having decrements greater than 2%. Changes in FEV1 during and after the day-2 O_3 exposure were not different from baseline. This difference in results compared to earlier studies is likely related to the older age of subjects in the study by Horvath et al. (1991), and the reduction in responsiveness to O_3 with aging (Drechsler-Parks et al. 1987a). Seven subjects reported at least one symptom (cough, chest tightness and/or pain on deep inspiration) by the end of the day-1 O_3 exposure, 2 subjects reported symptoms on day-2, while none did after the FA exposure. The study reported by Horvath et al. (1991) differed from earlier investigations using the same protocol in that the subjects performed an additional FEV1 test at the end of the mid-exposure lunch period. After the lunch period, FEV1 was improved compared to at the end of the third hour of exposure, and was similar to the response at the same time point of the FA exposure. Although the other studies mentioned above did not measure pulmonary function at the end of the lunch period, Folinsbee et al. (1988) and Horstman et al. (1990) did report that the decline in FEV1 was attenuated between the third and fourth hour measurements. This suggests that there may have been some recovery in lung function during the lunch period when the rate of O_3 inhalation was lower.

To explore the relative influences of O_3 concentration, ventilation rate, and exposure duration on response magnitude, Hazucha et al. (1992) designed a protocol utilizing 8-hour exposures with two different O_3 concentration profiles.

The study compared responses to exposure to a constant O_3 concentration of 0.12 ppm, and to a variable concentration profile (linear increase from 0 to 0.24 ppm over four hours, followed by linear decrease from 0.24 to 0 ppm over 4 hours). The results are illustrated in Figure 11-3, below. The total inhaled effective dose of O_3 was equivalent for the two exposures (difference $<$ 1%). Exposure to the constant O_3 concentration induced a group mean decrement in FEV1 of approximately 5% by the fifth hour of exposure, which did not change over the remainder of the exposure, indicating a response plateau, consistent with Horstman et al. (1990). In contrast, with the variable concentration protocol, the response over the first three hours was minimal, followed by a mean decrease in FEV1 over hours 4 through 6 that peaked at approximately 10%. There appears to be a lag in development of the maximal response, since the maximal O_3 concentration occurred at hour 4, yet the maximal FEV1 response occurred at hour 6. FEV1 improved during the last two hours of the exposure, and by the end of the exposure the FEV1 decrement was nearly identical to that following the constant concentration exposure. Adams (2003a) also compared responses of healthy young adults to two O_3 concentration profiles: (1) a constant O_3 concentration of 0.08 ppm, and (2) a triangular O_3 profile where the O_3 concentration increased from 0.03 ppm to 0.15 ppm over four hours, and then decreased to 0.03 over the next 2.6 hours (mean O_3 concentration = 0.08 ppm). The total inhaled dose of O_3 was equivalent for both protocols. The group mean decrement in FEV1 was smaller, at least partly due to the lower O_3 concentration, compared to Hazucha et al. (1992), the maximal decrement occurred at the time of the peak O_3 concentration with the triangular profile, but after six hours in the constant concentration exposure. The results of both of these studies illustrate that the FEV1 response is dependent on the dose rate as well as the cumulative dose of O_3 inhaled, at least when the O_3 concentration is variable. Furthermore, findings from both Hazucha et al. (1992) and Adams (2003) confirm that O_3 concentration is the most important factor in determining responses to O_3 exposure, compared to either exposure duration or ventilation.

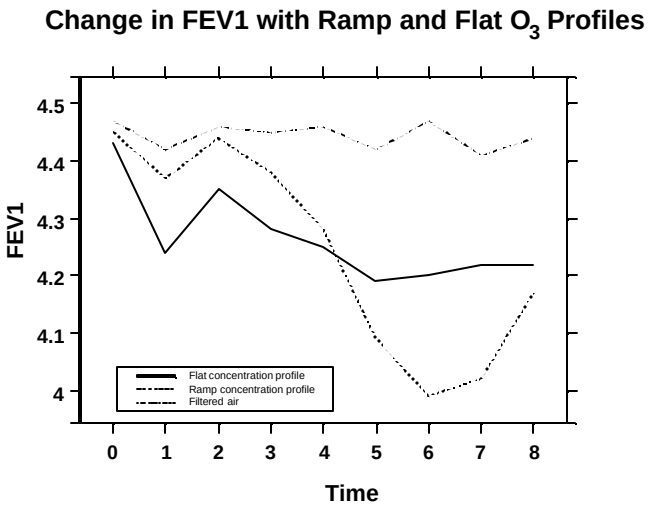

Figure 11-3 Comparison of the change in FEV1 with ramp and flat O_3 concentration profiles. Derived from Hazucha, 1993.

Adams (2000b) investigated whether different equivalent ventilation rates (EVR) between subjects altered responses to 0.12 ppm O_3 using the USEPA 6.6 hour protocol. EVR is a means of normalizing ventilation by scaling it to body surface area (V_E/BSA). Healthy young adult males and females completed nine O_3 exposures involving varying O_3 concentrations, exposure durations, and several EVRs, as well as one FA exposure. The results showed that because of the EVR methodology, the smallest subjects had lower exercise V_E rates (~26 L/min) than the largest subjects (~44 L/min), leading to smaller subjects inhaling lower doses of O_3 than larger subjects. Changes in FEV1, V_T, f_R and symptoms following O_3 exposure were correlated with V_E, and not with lung or body size. These results lead to the conclusion that responses to O_3 exposure are related to total inhaled O_3 dose, and are not a function of lung or body size, in accord with reports by McDonnell et al. (1997), and Messineo and Adams (1990). Figure 11-4, below, illustrates the mean hourly change in FEV1 and total symptoms score at each of the inhaled O_3 doses studied.

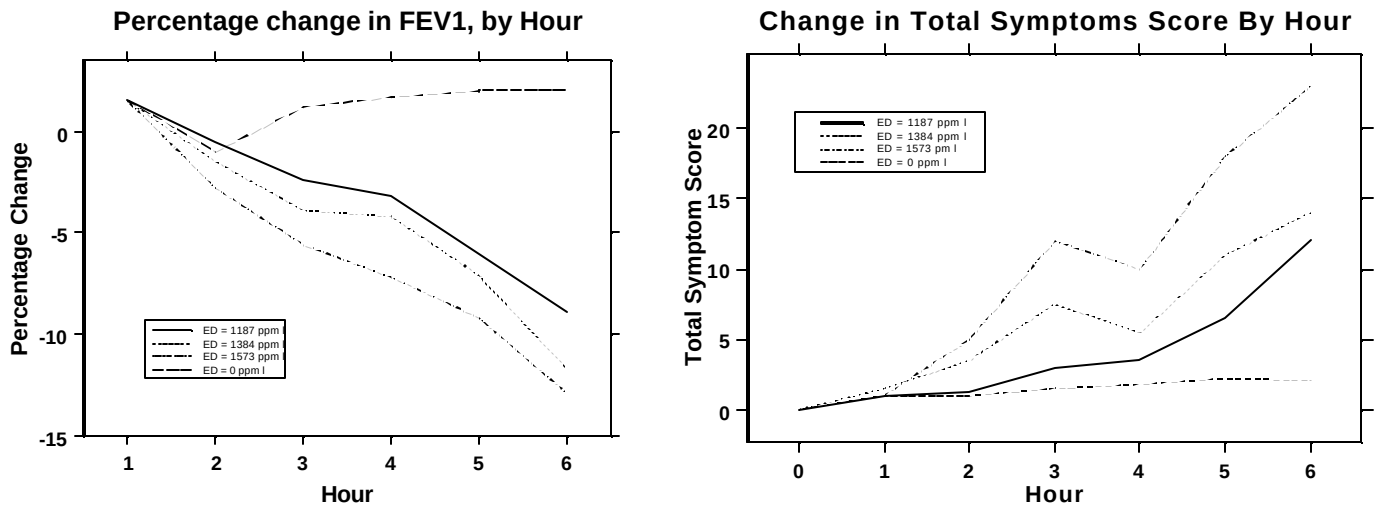

Figure 11-4 Percentage change in FEV1 and total symptoms score by hour with exposure to FA and three inhaled effective doses (ED) of O_3. The O_3 concentration was 0.12 ppm, and effective dose was varied by changing V_E (17, 20, or 23 L/min/m^2 BSA). Derived from Adams, 2000b.

Adams (2002) compared the responses of healthy adults who were exposed to O_3 in an environmental chamber and through a facemask system that allowed natural breathing while they completed the 6.6 hour protocol. The subjects inhaled FA and 0.12 ppm O_3 in the chamber, and 0.04, 0.08 and 0.12 ppm O_3 via the face mask system. Results with the facemask system were comparable to those obtained on the same subjects exposed in an environmental chamber. The changes in FEV1 were +2%, +1%, -4% and -13% for the FA, 0.04 ppm, 0.08 ppm and 0.12 ppm O_3 conditions, respectively (Figure 11-5). The decrement at 0.08 ppm O_3 was significantly larger than that following the FA exposure, and was significantly smaller that that at 0.12 ppm O_3. The change following the 0.04 ppm O_3 exposure was not different from that with FA exposure. However, although the group mean decrement following the 0.04 ppm was not statistically significant, the range of changes in FEV1.0 for the group was +7.8% to –8.2%. Total symptoms score was statistically significant following the exposure to 0.08 ppm O_3, but symptoms following the 0.04 ppm O_3 exposure were not different from following the FA exposure. Pain on deep breath was only significantly greater than following FA exposure for the 0.12 ppm O_3 exposures. The subjects in this study demonstrated somewhat smaller responses to the exposure to 0.08 ppm, compared to similar studies conducted at the USEPA laboratory (Folinsbee et al., 1988; Horstman et al., 1990; McDonnell et al., 1991). This discrepancy may be due to differences in the individual sensitivity of the subjects who participated in the studies, or to the difference in exposure mode (face mask vs. chamber). The subjects in Adams (2002) completed the 0.08 ppm O_3 exposure

with the face mask system only, so the latter issue can not be resolved with the available data.

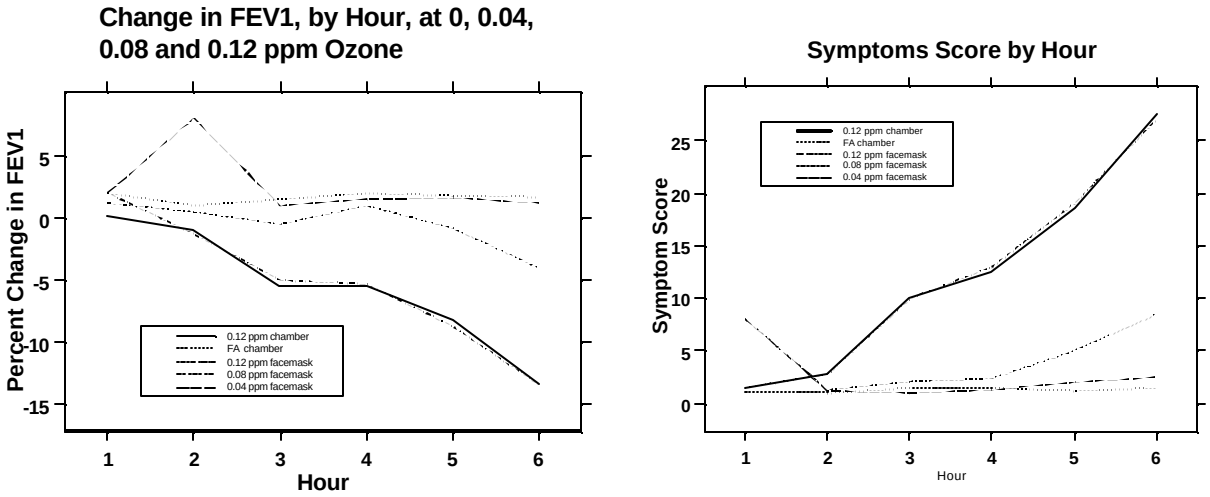

Figure 11-5 Change by hour in FEV1 and total symptoms score with four different inhaled O_3 doses. Derived from Adams, 2002.

Adams (2003b) also compared the pulmonary function and symptoms responses to 0.08 ppm O_3 using the 6.6-hour protocol and a 2-hour intermittent exercise protocol at 0.3 ppm O_3. These endpoints were also followed for 1.4-hour post-exposure to evaluate the time course of recovery. Although the inhaled doses of O_3 were not identical, 946 ppm·L for the 6.6-hour exposure (facemask), and 1385 ppm·L for the 2-hour exposure (facemask), differing by a factor of 1.46, the group mean decrements in FVC and FEV1 following the 2-hour protocol were three to four times those measured following the 6.6-hour exposure, while the difference in symptoms also averaged three to four times greater after the 2-hour protocol (except for pain on deep breath, which differed by about nine times greater after the 2-hour protocol). At 1.4-hour after the 6.6-hour exposure both symptoms and pulmonary function had returned to baseline. In contrast, the decrement in FEV1 and the increase in symptoms were still statistically significant, compared to baseline, at 1.4-hour after the 2-hour exposure to 0.3 ppm O_3. These results highlight the greater role of O_3 concentration, compared to V_E or exposure duration, in inducing adverse responses to O_3 exposure, as well as in the time-course of recovery of both pulmonary function and symptoms once exposure ends.

Table 11-7: Pulmonary Function Effects with Prolonged Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=39 L/min)	Healthy nonsmokers (22 M), 18-33 yrs	FVC and FEV1 decreased progressively as exposure continued. FEV1 decrement at end of exposure was 7%, 7%, and 12% for the three O_3 concentrations, respectively. FEV1 decrements greater than 15% occurred in three, five, and nine subjects at 0.08, 0.10, and 0.12 ppm O_3, respectively. Methacholine responsiveness increased by 56, 89 and 121%, respectively.	Horstman et al. 1990
	See Horstman et al. (1990) and Folinsbee et al. (1988)		Lognormal model fitted to FEV1 data indicates that O_3 concentration is more important than V_E or exposure duration in estimating effects of O_3 exposure.	Larsen et al. 1991
0.08 0.10	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=40 L/min)	Healthy nonsmokers, (38 M), mean age 25 yrs	FEV1 decreased 8.3% with exposure to 0.08 ppm, and 11.4% with exposure to 0.10 ppm O_3. Symptoms of cough, PDI and SB increased with O_3 exposure.	McDonnell et al. 1991
0.08	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=38 L/min). One day of air, two consecutive days of O_3	Healthy nonsmokers (5F, 6 M). 30 to 45 yrs	FVC decreased 2.1% and FEV1 decreased 2.2% after the first O_3 exposure. There were no changes after the second O_3 exposure.	Horvath et al. 1991
0.12	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=42.6 L/min)	Healthy nonsmokers, (10 M), 18-33 yrs	FEV1 decreased 13% after 6.6 hr, while FVC declined 8.3%. Cough and PDI increased with O_3 exposure. Airway responsiveness to methacholine doubled after O_3 exposure	Folinsbee et al. 1988

Table 11-7 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.30	6.6 hr, IE (50 min ex/hr), (V_E=20 l/min/m^2), chamber exposure only 2 hr, IE, (total 1 hr exercise), (V_E=35 l/min/m^2) chamber and facemask exposures	Healthy nonsmokers (15 M/15 F), 18-25 yrs	6.6 hr exposure to 0.08 ppm O_3 induced a mean FEV1 decrement of 3.5±7.4% and total symptom score of 8.2±11.9 compared to a decrement in FEV1 of 12.36±11.94% and a symptom score of 30.9±26.1 following the 2 hr chamber exposure to 0.30 ppm O_3. The total inhaled O_3 dose for the 2 hr exposure was 1358 ppm·L, compared to 946 ppm·L for the 6.6 hr protocol, a difference of 1.44 times. The difference in the changes in FEV1 for the two protocols was 3.5 times greater for the 2 hr exposure. These findings demonstrate the greater importance of the O_3 concentration compared to V_E and exposure duration in predicting responses. Recovery from exposure to 0.30 ppm O_3 was not complete at 1.4 hr post-exposure, and FEV1 and symptoms had different recovery time courses.	Adams 2003b
0.08 constant 0.03 to 0.15 to 0.05 ramp profile (mean concentration= 0.08 ppm)	6.6 hr, IE (50 min ex/hr), (V_E=20 l/min/m^2), chamber exposure and facemask exposures	Healthy nonsmokers (15 M/15 F), 18-25 yrs	Total inhaled O_3 dose was similar for all four O_3 protocols. FEV1 decrement averaged about 3% at end of exposure, and symptoms were similar for all four O_3 exposures. Decrements in FEV1 and increases in symptoms became significant at 6.6 hr for the constant concentration profile, and at hour 4 for the ramp concentration profile, with no further change from hour 4 through the end of exposure. The results highlight the significance of O_3 concentration.	Adams 2003a

Table 11-7 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
a) flat profile at 0.12 b) ramp profile: 0.00 to 0.24 over 4 hr, followed by decrease to 0.00 over final 4 hr (total dose of inhaled O_3 similar for the two protocols)	8 hr IE (30 min ex/hr) (V_E=40 L/min)	Healthy nonsmokers, (23 M), 20-35 yrs	FEV1 decreased 5% by the sixth hr, and remained at this level through 8 hr of exposure. FEV1 change mirrored O_3 concentration profile, but with a lag time of ~ 2 hr. The maximal decrease of 10.2% was at 6 hr of exposure, followed by recovery over the final two hrs to a final decrement similar to that of the flat O_3 profile.	Hazucha et al. 1992
0.12	6.6 hr on two consecutive days IE (50 min ex/hr; 30 min lunch), (V_E=28 L/min for asthmatics; 31 L/min for normals)	Nonsmokers Healthy (8 M, 7 F), 22-41 yrs Asthmatic (13 M, 17 F), 18-50 yrs	Bronchial reactivity to methacholine increased with O_3 exposure in both subject groups. FEV1 decreased 2% in healthy subjects, and 7.8% in asthmatics. Responses were generally less on the second day. Two healthy subjects and four asthmatics had FEV1 decreases >10%.	Linn et al. 1994
0.12	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=38.8 L/min) 5 consecutive days of exposure	Healthy nonsmokers (17 M), mean age 25 ± 4 yrs	FEV1 decreased by 12.8%, 8.7%, 2.5% and 0.6% on exposure days 1 through 4, respectively, and increased by 0.2% on day 5, compared to preexposure. Methacholine responsiveness increased by >100% on all exposure days. Symptoms were increased on the first O_3 day, but were absent on the last three exposure days.	Folinsbee et al. 1994
0.16	4 hr, IE (50 min ex/hr), (V_E+38.9 L/min)	Healthy nonsmokers (17 M), mean age 25 ± 4 yrs	FVC decreased 9.5% and FEV1 decreased 16.6%. FEV1/FVC ratio decreased from 0.79 to 0.73	Folinsbee et al. 1994

Table 11-7 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.16	7.6 hr, IE (50 min ex/hr), (V_E=26-32 L/min)	Moderate asthmatics, (7 M, 10 F) Healthy nonsmokers, (N=13, gender not given) All 18-35 yrs	FEV1 decreased 19% in asthmatics, compared to 10% in nonasthmatics. High responders had worse baseline airway status. Asthmatics had more wheeze after O_3 exposure.	Horstman et al. 1995
0.16	7.6 hr, IE (50 min ex/hr), (V_E=25 l/min)	Physician diagnosed mild asthmatics allergic to dust mites, (8 M)	Increased eosinophils and PMN's after O_3 exposure, particularly in the bronchial fraction of BALF. No correlation between eosinophils and PMN's. FEV1 and FVC decreased 14% and 9%, respectively.	Peden et al. 1997
0.18 0.24	4 hr IE (50 min ex/hr), (V_E=31 l/min), facemask only 2 hr, IE, (total 1 hr exercise), (V_E=47 l/min), both mouthpiece and facemask exposures	Healthy nonsmokers, (3 M/3 F), 21-33 yrs	Decrements in FEV1 averaged 23.9% and 25.4% for the 2 hr mouthpiece and facemask exposures to 0.24 ppm O_3, respectively. The FEV1 decrement after the 4 hr exposure to 0.18 ppm O_3 averaged 29.3%. Symptoms scores were similar for the 2 hr and 4 hr O_3 exposures. Effective doses for the two 2 hr O_3 exposures were 890 and 881 ppm·L, and 1216 ppm·L for the 4 hr exposure.	Adams 2000a
0.50 (KI)	6 hr, including 2 exercise bouts (15 min) at 100 W workload	Smokers (9 M/1 F), 22-42 yrs Nonsmokers (10 M), 21-40 yrs	No significant responses in smokers. Nonsmokers had small, statistically significant decrements in FVC, FEV3 and SG_{aw} with O_3 exposure. Chest discomfort and cough were the most common symptoms, with intensities ranging from mild to moderately severe. Subjects with chest discomfort were more likely to experience a reduction in pulmonary function than those without chest discomfort.	Kerr et al. 1975

11.4.3.3 Summary

Collectively, these studies demonstrate that statistically significant group mean decrements in FEV1 occur in healthy adults performing a protocol simulating a day of active outdoor work or play at O_3 concentrations at least as low as 0.08 ppm. The results also indicate that some individual subjects may experience significant impairment in pulmonary function at still lower O_3 concentrations under these exposure conditions. Evaluation of the responses of the individual subjects who participated in these studies (decrements as large as 48%) demonstrates that significant decrements in FEV1 can occur in more responsive individuals when they undergo multi-hour exposure to O_3 exposure at levels at or below the current federal 8-hour standard, and only marginally above nonanthropogenic O_3 background levels. Further, the data indicate that exposures to O_3 concentrations as low as 0.08 ppm can induce non-specific airway hyperresponsiveness.

When interpreting these results, it should be remembered that the study subjects were healthy, young and middle-aged adults. The range of responses in people with compromised health status is largely unknown. However, these results are likely representative of people who are physically able to perform the protocol (i.e., requiring moderate exertion for several hours), and by extension, of people likely to experience the greatest O_3 exposure -- those who work or play actively outdoors for multi-hour periods.

11.4.3.4 Cellular and Biochemical Responses to Multi-Hour O_3 Exposures

Several studies have investigated the cellular and biochemical changes induced in the lungs by multi-hour O_3 exposure. In one study, subjects underwent fiberoptic bronchoscopy with bronchoalveolar lavage 16-18 hours following exposure to FA (N=18), 0.10 ppm (N=10) or 0.08 ppm (N=18) O_3 using the 6.6 hour protocol described above (Devlin et al. 1991). Analysis of the BALF recovered from these subjects showed evidence for neutrophilic inflammation in the alveolar region, epithelial damage, and cell wall disruption. These conclusions are substantiated by the statistically significant increase in neutrophils, PGE_2, IL-6, and a_1-antitrypsin measured in the BAL fluid following exposure to 0.08 ppm O_3, compared to following FA exposure. Exposure to 0.08 ppm O_3 also decreased the ability of alveolar macrophages to phagocytize microorganisms via the complement receptor. This has implications for defense against microbial pathogens in humans. These changes are indicative of pulmonary inflammation, and activation of inflammatory cells. A consideration in interpreting this study is that the data were analyzed with single-tailed paired T-tests (each subject served as his own control), without correction of the P-value for the multiple tests. The investigators addressed this issue by limiting the primary hypothesis tested formally to the change in neutrophils following both concentrations of O_3. The other analyses were performed as exploratory analyses, and the investigators acknowledged that some of these may appear significant at the P=0.05 level due to random chance. The data also suggest

that O_3 exposures at these concentrations could lead to fibrotic changes in the lung tissues, based on the increased fibronectin and protein recovered following the exposure to 0.10 ppm O_3. There was a considerable range of response magnitude between individual subjects in the changes in the cellular and biochemical markers measured, suggesting that there is a sub-fraction of the population that is very sensitive to the inflammatory effects of O_3. Generally, changes after exposure to 0.10 ppm O_3 were larger than those after 0.08 ppm O_3.

Table 11-8: Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.10 0.12	6.6 hr, IE, (V_E=39 L/min)	Healthy nonsmokers, (22 M), 18-32 yrs	33, 47, and 55% decreases in $PC_{100SRaw}$ after exposure to 0.08, 0.10, and 0.12 ppm O_3, respectively.	Horstman et al. 1990
0.12	6.6 hr, IE (50 min ex/hr; 30 min lunch), (V_E=25 L/min/m^2 BSA)	Healthy nonsmokers, (10 M), 18-33 yrs	Approximate doubling of mean methacholine responsiveness after O_3 exposure. On an individual basis, no relationship between O_3-induced changes in airway responsiveness and FEV1 or FVC.	Folinsbee et al. 1988
0.16	7.6 hr, IE (50 min ex/hr), (V_E=25 L/min)	Mild atopic asthmatics, house dust mite sensitive, (5 F, 4 M), 20-35 yrs	Mean 9.1% decrease in FEV1 18 hr after O_3 exposure. Provocative dose of dust mite allergen decreased from 10.3 to 9.7 dose units after O_3 exposure.	Kehrl et al. 1999
0.20	4 hr, IE, (50 min ex/hr), (V_E=25 L/min/m^2 BSA)	Physician-diagnosed mild asthma, no medications before exposure, (6F, 12 M), 18-36 yrs	Decreased FEV1 and FVC after O_3 exposure. Increased SR_{aw}, lower respiratory symptoms, % neutrophils, total protein, LDH, fibronectin, IL-8, GM-CSF, and MPO in BALF after O_3 exposure. Correlation between pre-exposure methacholine challenge and O_3-induced SR_{aw} increase.	Balmes et al. 1997 Scannell et al. 1996
0.20	4 hr, IE (40 min/hr), workload=50 W	Mild atopic asthmatics: (6F, 4 M), 26.6±2.3 yrs. No medications for 8 wks prior to exposure Healthy subjects: (4 F, 6 M), 27.3±1.4 yrs	FEV1 decreased 9.3% in asthmatic, and 6.7% in healthy subjects. Nonsignificant increase in sputum neutrophils in both groups. No change is methacholine airway reactivity 24 hr past-exposure.	Nightingale et al. 1999
0.20	4 hr, IE, (30 min ex/30 min rest), (V_E=25 L/min/m^2 BSA)	Healthy nonsmokers, (4 F, 8 M), 23-47 yrs	Increased total cells, %neutrophils, IL-6, and IL-8 in sputum at 18 hr post-exposure. Increased airway responsiveness to methacholine 2 hr after FEV1 had returned to within 5% of pre-exposure baseline. Treatment with azithromycin before exposure had no antiinflammatory effect.	Criqui et al. 2000

Table 11-8 (cont.): Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.20	4 hr, IE, (50 min ex/hr), (V_E=25 l/min/m^2 BSA)	Healthy nonsmokers, (42 M, 24 F)	FEV1 and FVC decreased 18.6 and 14.6%, respectively, after O_3 exposure. Baseline $PC_{100SRaw}$ for methacholine was not related to changes in FVC or, FEV1. There was a weak association between $PC_{100SRaw}$ and increased SR_{aw}.	Aris et al. 1995
0.08 0.10	6.6 hr, IE (50 min ex/hr plus 35 min lunch), (V_E=40 L/min)	Healthy, (18 M), 18-35 yrs	18 hr after exposure to 0.10 ppm O_3, there were significant increases in BALF PMN's, protein, PGE_2, fibronectin, IL-6, lactate dehydrogenase, and a-1 antitrypsin, compared to 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 noted at both O_3 concentrations.	Devlin et al. 1991 Devlin and Koren 1990 Koren et al. 1991
0.16	7.6 hr IE (50 min ex/hr plus 35 min lunch), (V_E=25 L/min)	Asthmatics sensitive to dust mites, (N=8), age and gender not given	Increased number of eosinophils in BALF after O_3 exposure.	Peden et al. 1997
0.20	4 hr, IE (50 min ex/hr), (V_E=40 L/min)	Healthy, (15 M, 13 F), 21-39 yrs	Bronchial lavage, bronchial biopsies and BAL at 18 hr post-exposure. BALF analysis showed increased cells, LDH, and IL-8.Biopsies showed an increased number of PMN's.	Aris et al. 1993b
0.20	4 hr, IE (50 min ex/hr), (V_E=44 L/min)	Mild asthmatics (12 M, 6 F), 18-36 yrs	Increased PMN, protein, IL-8, LDH in BALF. Inflammatory responses were greater than a group of non-asthmatics (Balmes et al., 1996)	Scannell et al. 1996
0.20	4 hr, IE (50 min ex/hr), (V_E=44 L/min)	Healthy nonsmokers (14 M/6 F), 22-38 yrs	O_3 increased PMN, protein and IL-8 for all subjects. No relationship of inflammation with spirometric responses.	Balmes et al. 1996

Table 11-8 (cont.): Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.22	4 hr, IE (20 min ex/19 min rest, (V_E=39-45 l/min)	Healthy smokers and nonsmokers (31 M/7 F)	Post-O_3 exposure FEV1 was decreased an average of 13.9% in smokers, 1.4% in nonresponders, and 28.5% in responders. PMN's increased immediately and at 18 hr post-O_3 exposure in all groups. Eosinophils and lymphocytes increased after O_3 exposure. Greater increase in IL-6 in nonsmokers. Neither symptoms nor pulmonary function correlated with inflammation. Nasal lavage indicators were not predictive of bronchial or alveolar inflammation.	Frampton et al. 1997 Torres et al. 1997
0.30 (9-12 AM) 0.15 (12-2 PM) 0.30 (2-5 PM)	8 hr/d for 5 consecutive d, IE (protocol and V_E not given)	(24 M), 12 exposed to O_3, 12 exposed to air	Subjects were inoculated with type 39 rhinovirus prior to exposures. NL was performed on the mornings of Days 1 through 5, 8, 15 and 30. There was no difference in the virus titers in NLF of air and O_3 exposed subjects at any time tested. There were no differences in PMNs or interferon gamma in NLF or in blood lymphocyte proliferative response to viral antigen.	Henderson et al. 1988
0.50	4 hr at rest on 2 consecutive d	Healthy nonsmokers O_3 exposure: (21 M), air exposure (20 M), 18-35 yrs	NL done immediately before and after each exposure, and 22 hr 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	4 hr, resting	Allergic rhinitics, (6 M, 6 F), mean age 31.4 ±2.0 (SD) yrs	NL immediately after exposures. Increased upper and lower respiratory symptoms and increased levels of PMNs, eosinophils and albumin in NLF after O_3 exposure.	Bascom et al. 1990

11.4.3.5 Responses of Allergic Asthmatic Subjects to Multi-Hour O_3 Exposures

Four studies have investigated the responses of allergic asthmatics to multi-hour O_3 exposures. The first, Linn et al. (1994), utilized the 6.6-hour protocol in an evaluation of the responses of 15 healthy and 30 asthmatic subjects to FA, or 0.12 ppm O_3, and 100 µg/m^3 sulfuric acid, singly and combined, with each atmosphere inhaled on two consecutive days. When the pulmonary function changes were analyzed on a percentage change basis to remove the influence of differences in baseline lung function, the responses of the asthmatic and nonasthmatic groups were similar. However, the changes in the asthmatic group were superimposed onto generally reduced pulmonary function at baseline, the asthmatic subjects having reduced FEV1 even with FA exposure. The reduction in FEV1 and increase in SR_{aw} became significant after the third hour of exposure, and the subjects reported increased symptoms during the last two hours of the day-1 O_3 exposure. Both the normal and asthmatic groups had small, statistically significant mean decrements in FEV1 following the day-1 O_3 exposure, which were accompanied by increased nonspecific airway responsiveness. However, there were no statistically significant differences between the decrements in FEV1 of the normal (-1.7%) and asthmatic groups (-7.8%) due to the wide range of variability between the individual subjects in both groups. FEV1, SR_{aw} and symptom responses were smaller on day-2 of exposure, in agreement with the findings of Horvath et al. (1991) and Folinsbee et al. (1994). The smaller mean decrements observed on day-1 in this study compared to Folinsbee et al. (1988;1994), and Horstman et al. (1990) may be related to previous ambient O_3 exposure in these Los Angeles residents, as discussed in Section 11.4.9.7, or to a larger number of O_3-insensitive subjects in the group.

Horstman et al. (1995) extended the findings on responses of asthmatics to multi-hour exposures by comparing the pulmonary function responses of male and female asthmatic and nonasthmatic subjects (18-35 yrs.) exposed to FA or 0.16 ppm O_3 for 7.6 hours. The asthmatics experienced greater percentage reductions in FEV1 and in FEV1/FVC than the nonasthmatics. One and nine asthmatics reported wheeze with the FA and O_3 exposures, respectively. None of the nonasthmatics reported wheeze with either exposure. Six of 17 asthmatics requested ß-agonists prior to and/or during exposure. Interestingly, the benefit of ß-agonists was only temporary, and was followed by substantial decrements in FEV1 and FEV1/FVC. In fact, the total decrements in subjects who used ß-agonists before and/or during exposure were larger than observed in those who did not use medications before or during exposure. Although Horstman et al. describe their asthmatic subjects as having mild disease, the asthmatic group was not homogeneous. That five subjects used theophylline and nine reported daily asthma episodes suggests that at least some should have been considered to have moderate persistent asthma. The extent to which these factors affected the results and implications of the study is unclear. However, overall, the results support the view that irritant receptors are stimulated in both asthmatic and nonasthmatic subjects. This mechanism leads to reflex inhibition of inhalation, and thus reduced FVC, which in turn reduces FEV1, although

bronchoconstriction was evident to a greater degree in the asthmatic than nonasthmatic subjects.

Peden et al. (1997) studied eight asthmatic subjects (age and gender not specified). All were allergic to dust mites, and used only ß-agonists as treatment for their asthma. The subjects were exposed to 0.16 ppm O_3 for 7.6 hour while exercising for 50 minutes of each hour at a light workload (~25 L/min). The protocol was that used in the USEPA studies discussed above, except that it was extended for an additional hour. The subjects underwent fiberoptic bronchoscopy with bronchoalveolar lavage 18 hours after both the FA and O_3 exposures. The decrement in FEV1 averaged 13.6%, with a range of +4% to -20%. There were no differences in the measured chemical mediators of neutrophil or eosinophil activation between the two exposures, although there was an increase in both neutrophils and eosinophils in BALF after the O_3 exposure compared to the FA exposure. This is the first study to report an increase in eosinophils with O_3 exposure, although the subjects had eosinophils in their BALF after the FA exposure as well. The authors suggest that the findings indicate an exacerbation of the eosinophilic inflammatory component of the subjects' asthma, although the results are subject to alternative interpretations. That the subjects had eosinophils in their BALF after FA exposure is consistent with an active allergic inflammatory process in the subjects' lungs, even at baseline, which is characteristic of atopic asthma. Comparison of the biochemical results suggests that the inflammatory cells had not been activated by the O_3 exposure. One characteristic of asthma is an increased number of eosinophils in the lungs, even when the disease is in a quiescent state. However, when these cells are exposed to a substance to which the individual is allergic, they are activated, and release a variety of cytokines and biochemicals that mediate the immediate-hypersensitivity response pathway. The observation that the number of eosinophils was increased, but the concentrations of chemical mediators were not indicates that the O_3 inhaled did not affect allergic pathways in these subjects. However, Vagaggini et al. (2002) recently reported that when O_3 exposure (two-hours) occurred on the day after asthma exacerbation was induced by allergen challenge, subjects showed increased eosinophilic airway inflammation compared to following O_3 exposure without preexisting asthma exacerbation. Given that Peden et al.'s (1997) subjects did not use anti-inflammatory or antihistamine medications and showed evidence of allergic activation even with FA exposure, it is possible that the O_3 exposure interacted with an ongoing allergic process in a manner similar to that described by Vagaggini et al. (2002).

Kehrl et al. (1999) extended the results of the Peden et al. (1997) study, using the same protocol with nine mild allergic asthmatics exposed to FA and 0.16 ppm O_3. The subjects used only ß-agonists. All of the subjects had positive responses to multiple allergens in an 18-allergen test panel. Changes in FEV1, which averaged +2% (range: -5.1% to +9.3%) with FA exposure and –9.1% (-2% to –26.3%) with O_3 exposure, were significantly different. The morning after each exposure the subjects returned to the laboratory for an allergen inhalation challenge, followed the next two mornings (days 3 and 4) by challenge with

aerosolized histamine and lung function testing, in order to assess the duration of enhanced airway hyperresponsiveness. Seven of the nine subjects responded to a lower PD20 of allergen after O_3 exposure (0.58 mean dose shift in the doubling concentration of allergen; P=0.03). After the allergen challenge, subjects were monitored in the laboratory until their pulmonary function was stable, at which time they were admitted to the University General Clinical Research Center, where their lung function was monitored hourly. Three of the subjects had late-phase responses, defined as a greater than 20% decrement in FEV1 more than four hours after the allergen challenge, after both allergen challenges (i.e., allergen following FA or O_3), and one had a late-phase response only after the FA/allergen exposure. The investigators had hypothesized that airway inflammation consequent to O_3 exposure and allergen challenge would increase nonspecific airway hyperreactivity, providing a possible mechanism for epidemiological findings suggesting a correlation between asthma exacerbation and O_3 concentration on the previous day. However, contrary to expectations, airway reactivity, measured as bronchial reactivity to the nonspecific bronchoconstrictor histamine, was increased after the FA/allergen exposure, but not after the O_3/allergen exposure, although the subjects received less allergen after the O_3 than the FA exposure. Histamine reactivity was not assessed immediately after the FA and O_3 exposures (or before the allergen challenge), so the study does not speak to whether or not O_3 exposure alone alters bronchial reactivity.

11.4.3.5.1 Summary

Collectively, the asthmatic subjects in these studies had changes in pulmonary function and nonspecific airway responsiveness that were in the same range as nonasthmatic subjects studied using similar protocols. Although the O_3 concentrations were similar, the asthmatic subjects typically exercised at a lower workload, and as a result inhaled less O_3, which is reflected in the somewhat smaller range of individual responses among the asthmatics. The finding of larger pulmonary function decrements following post-O_3 allergen challenge, compared to those following the FA exposure, suggests that O_3 may enhance ongoing allergic processes. These findings provide plausible support for epidemiological observations that some asthmatics experience asthma exacerbation on the day after elevated ambient O_3 concentrations. In addition, although asthmatics tend to have similar percentage changes in pulmonary function as nonasthmatic subjects, such changes may be superimposed on already depressed pulmonary function.

It is difficult to extrapolate these findings to the broader population of asthmatics for several reasons. First, for ethical reasons only subjects with relatively mild disease can be studied in the laboratory, and therefore it is unclear how the results might apply to those with more serious disease. On the other hand, since responses to O_3 are related to inhaled dose, it is likely that more severe asthmatics would have lower overall exposure due to physical limitations on their capacity for outdoor work or exercise. Second, little is known as to the effect of commonly used asthma medications on responsiveness, although Horstman et

al. (1995) reported that subjects who asked for fast-acting inhalers (ß-agonists) before or during O_3 exposures tended to have larger decrements in pulmonary function than those who did not. Gent et al. (2003) reported a significant association between asthma-like symptoms and ambient O_3 concentration in asthmatic children who used asthma medications but no relationship in asthmatic children who did not use medications. These two studies raise the so far unaddressed question as to whether and, if so, how the biological mechanisms of common asthma treatments interact with the mechanisms mediating responses to O_3. This is in contrast to SO_2 exposure, where medications seem to mitigate pulmonary function responses. The protocols used in the existing studies have typically required the subjects to refrain from taking their asthma medications within a set time period of the exposure sessions, contrary to their situation in normal daily life. There is no information on asthmatics who have been studied while taking their medications, and standards of treatment have altered during the time period over which the few controlled exposure studies of asthmatics have been conducted, factors which add some uncertainties to the conclusions that can be drawn from this literature.

11.4.3.6 Responses of Animals to Controlled Concentrations of Ozone

11.4.3.6.1 Introduction

This section presents a brief summary of the relevant toxicological findings in laboratory animals that have been published since the previous review of the California ambient air quality standard for O_3 in 1987. Unlike studies in humans, animal studies can provide information on the effects of O_3 exposures at high concentrations or for extended durations and permit the use of important invasive techniques that could not be applied in human studies. However, due to the availability of human studies, coupled with the inherent uncertainties in extrapolating quantitatively from animal studies to human exposures, we are not using the animal studies directly in estimating the appropriate level of the standards. The results of laboratory animal studies are reviewed here to support the findings of epidemiological and controlled human exposure studies presented elsewhere in this document. Only animal studies conducted at O_3 concentrations of 1.0 ppm or less are discussed, which more than adequately brackets the highest concentrations found in urban areas of California. For a more in depth discussion of the animal findings presented here, please refer to Appendix A.

Numerous studies have examined the effects of O_3 exposure on pulmonary function in animals. These animal studies have used a variety of exposure regimens to examine the effects of acute (less than a day), short-term repeated (less than a week) and chronic continuous and intermittent O_3 exposures on pulmonary function. For this brief review the O_3-induced pulmonary function responses will be divided into effects on breathing pattern and gas exchange, dynamic and static lung mechanics, and airway reactivity. In addition, this review will be limited to those studies that use 1.0 ppm O_3 or less and used whole animal exposure. Overall the pulmonary function responses induced by acute and short-term O_3 exposure in experimental animals are similar to those seen in human subjects. These pulmonary function responses include rapid shallow

breathing, mild increases in airway resistance, decrements in forced expiratory end-points and increased airway reactivity to various stimuli with all of these responses showing attenuation with repeated exposures of three days or more. As a result animal studies provide an opportunity to examine the pathophysiology underlying these responses and provide a better understanding of the possible mechanisms and health relevance of these responses when observed in human subjects. In addition, chronic exposure studies provide an opportunity to better understand the long-term effects of O_3 on lung health and development. This section provides a short review of this topic divided into three subsections. These subsections cover O_3-induced ventilatory and breathing pattern responses, pulmonary mechanics responses and airway reactivity responses with each subsection including a discussion of the effects of acute (less than a day), short-term repeated (less than a week) and chronic continuous and intermittent O_3 exposure regimens. For a more extensive review of this topic the reader is referred to Appendix A, as well as the 1996 United States Environmental Protection Agency Air Quality Criteria Document for Ozone and Related Photochemical Oxidants (US EPA 1996).

11.4.3.6.2 Ventilatory and Breathing Pattern Responses

Studies that examined ventilatory and breathing pattern responses induced by the inhalation of O_3 concentrations of 1.0 ppm or less are summarized in Table 11-9. Ventilatory and breathing pattern responses produced by acute exposures lasting nine-hour or less have been examined in three species, the dog, rat and mouse. In dogs and rats, acute O_3 inhalation results in a rapid shallow breathing pattern that is characterized by a decrease in tidal volume (V_T) and an increase in breathing frequency (f_b) (Sasaki et al. 1987; Mautz 2003; Mautz and Bufalino 1989; Schelegle et al. 2001; Shore et al. 2002; Vesely et al. 1999b). In conscious rats, acute inhalation of O_3 concentrations of 0.6 ppm and greater have been shown to induce a decrease in minute ventilation (V_E) (Mautz and Bufalino 1989) while anesthetized rats that inhaled 1.0 ppm for eight-hour did not demonstrate this response (Vesely, et al., 1999a; Vesely, et al., 1999b). The decrease in V_E observed in conscious rats by Mautz and Bufalino (1989) was associated with a decrease in metabolic rate. In mice, acute O_3 inhalation has been shown to result in a slow, shallow breathing pattern (Currie et al. 1998; Shore et al. 2002) that is age dependent, with young mice (2 wks of age) showing no ventilatory response to O_3 (Shore et al. 2002). Evidence obtained from dogs and rats is consistent with these responses being initiated in large part by vagal lung C-fiber afferent nerves with their receptors in the conducting airways and/or lung parenchyma (Sasaki et al. 1987; Schelegle et al. 2001; Vesely, et al., 1999b). In rats, rapid shallow breathing induced by the acute inhalation of 1.0 ppm O_3 is not dependent on the recruitment of neutrophils into the airway (Vesely, et al., 1999a).

In rats, repeated daily inhalation of O_3 of concentrations up to 1.0 ppm have shown that the first and second day of exposure are characterized by a rapid shallow breathing pattern and that this breathing pattern response is reduced on each consecutive day of O_3 inhalation (Tepper et al. 1989; Schelegle et al. 2003). If this cycle of five consecutive days of O_3 inhalation is made part of a repeated

episode regimen with 5d of exposure followed by 9d of recovery in filtered air, the same pattern of initial rapid shallow breathing, followed by response attenuation, was reproduced with up to 4 consecutive episodes (Schelegle, et al., 2003). Interestingly, this pattern of breathing pattern response consistently occurred in each cycle of O_3 inhalation, despite the observation that the pattern of injury and inflammation was different with each consecutive episode (Schelegle, et al., 2003). When daily O_3 exposure was continued for 1, 3, 13, 52 and 78 weeks, Tepper et al. (1991) observed a decreased ventilatory response to CO_2 challenge that was associated with an increase in expiratory resistance at all time points, but was greatest at 78 weeks. Wiester et al. (1995) used a similar daily exposure for 52 and 78 weeks and observed that the rapid shallow breathing response induced by a 2 hour inhalation of 1.0 ppm O_3 was attenuated compared to FA controls. In addition, Wiester, et al. (1995) observed that the rapid shallow breathing response to a similar acute O_3 challenge returned when the rats were allowed to breath filtered air for 4 months following the 52 or 78 week daily exposure protocol.

Table 11-9: Ventilatory and Breathing Pattern Responses

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Acute	1.0	2 hr		Dog	Tachypnea with inspiratory time and expiratory time equally shortened. No increase in ventilatory drive 1- and 24-hr PE.	Sasaki et al. 1987
	2.0	0, 2, 4, 6, 8 hr		Mice, M, 7 wk	Decrease in f_b and V_t up to 6-hr of exposure.	Currie et al. 1998
	0.2 -0.8	3.75 hr		Rat, (Sprague-Dawley), 7 wk	Tachypnea, V_E, gas exchange, and R_L increased: C_{dyn} decreased.	Mautz et al. 1985
	0.6 alone and in combination with 10 ppm formaldehyde	3 hr at rest and with light exercise		Rat, M, (Sprague-Dawley)	At rest: decreased V_T; increased f_b. In combination with 10ppm formaldehyde; increased V_T, decreased f_b. Exercise data not reported.	Mautz 2003
	0.2 - 0.8	3 hr		Rat, M, (Sprague-Dawley), 7 wk	Maximum 0_2 consumption decreased, tachypnea at 0.2 ppm, tachypnea observed at 0.4 ppm, and ventilation and core temperature decreased at 0.6 ppm.	Mautz and Bufalino 1989

Table 11-9 (cont.): Ventilatory and Breathing Pattern Responses

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Acute	1.0	8 hr	Vagal Perineural Capsaicin	Rat, (Wistar)	Blocking vagal lung C-fiber conduction abolished 0_3-induced rapid shallow breathing.	Schelegle et al. 2001
	0.3 - 3.0	3 hr		Mice, M/F,(A/J), 2-4-8-12 wk	Decrease in f_b and V_t. Response present in 4 wk old mice, but not in 2 wk old mice.	Shore et al. 2002
	1.0	8 hr	a-chloralose: urethane	Rat, M, (Wistar), 43 d	Decreased V_T; increased f_b; V_E unchanged. Response unaffected by neutrophil depletion.	Vesely et al. 1999b
	1.1	9 hr	a-chloralose: urethane	Rat, F, (Wistar)	Decreased V_T; increased f_b; V_E unchanged. Response abolished by neonatal capsaicin treatment.	Vesely et al. 1999a

Table 11-9 (cont.): Ventilatory and Breathing Responses

	Ozone Conc. (ppm)	Expos. Protocol	Drugs	Species, (Strain), Age, Sex,	Observed Effects	Reference
Short-term	0, 0.35, 0.5, 1.0	2.25 hr, 5 d, 8 hr, 5 d with 9 d recovery. Up to 4 cycles.		Rat, M, (Fisher-344)	Attenuation of tachypnea with consecutive exposures; BAL antioxidants and protein did not adapt with exposure, histopathology increased in severity.	Tepper et al. 1989
Long-term Intermittent	1.0			Rat, M, (Sprague-Dawley), 70 day	Each cycle resulted in rapid shallow breathing on days 1 and 2 of exposure that was absent on days 3, 4 and 5 of exposure.	Schelegle et al. 2003
	Base 0.06, Spike rising to 0.25	13h/d, 7day/wk; 5d/wk; 1, 3, 13, 52, 78 wks 13 hr/d, 7days/wk, 12-18 mo		Rat, male, (Fisher-344), 60 day-old	Decreased V_E with CO_2 challenges. Increased expiratory resistance observed at all time points, but mostly at 78 weeks.	Tepper et al. 1991
	0.06, 0.25			Rat, M, (F-344), 60 days	Prolonged exposure resulted in attenuated f_b and V_T response to an acute 2 hr challenge with 1.0 ppm 0_3. 4 mo recovery returned 0_3 responsiveness to control levels.	Wiester et al. 1995

11.4.3.6.3 Lung Mechanics Responses

Studies that examined lung mechanics responses induced by the inhalation of O_3 concentration of 1.0 ppm or less are summarized in Table 11-10. Acute inhalation of O_3 has been shown to result in a significant increase in pulmonary flow resistance (R_L) and decrease in dynamic compliance (C_{dyn}) in rats (Mautz et al. 1985; Yokoyama et al. 1987) and guinea pigs (Miller et al. 1987; Miller et al. 1988). In rats, decreased Cdyn was associated with a decrease in static lung compliance (C_{st}), and peak expiratory flow (PEF), while functional residual capacity (FRC) and residual volume (RV) increased (Yokoyama, et al., 1987). In guinea pigs these changes in lung mechanics were associated with decreased total lung capacity (TLC), vital capacity (VC), FRC and RV (Miller, et al., 1988). The decreases in FRC and RV were blocked with indomethacin and cromolyn (Miller, et al., 1988), indicating a contribution of inflammation and more specifically, cyclooxygenase products of arachidonic acid metabolism, to the responses.

In mice, five consecutive daily exposures to 1.0 ppm O_3 (3 hour in duration) did not affect lung carbon monoxide diffusing capacity (DL_{CO}), nitrogen washout or lung volumes (Selgrade et al. 1988). In contrast, Yokoyama et al. (1989) found that seven daily 6 hour exposures to 1.0 ppm in rabbits increased lung resistance (R_L) 17 hour following the end of exposure (animals were not studied immediately after the end of the exposure). Kotlikoff et al. (1984) found that seven days of continuous exposure to 0.64 ppm O_3 increased peripheral airway resistance and decreased lung effective reactance at high oscillatory frequencies. Daily exposure of rats for three to 78 weeks (O_3 concentrations ranging from 0.25 to 1.0 ppm) have shown increases in airway resistance (Tepper et al. 1991; Yokoyama 1984). Additionally, daily exposure of rats for four to 52 weeks to O_3 concentrations ranging from 0.2 to 0.7 ppm resulted in a decrease in DL_{CO} and increases in FRC, RV and fixed lung volumes (Gross and White 1987; Martin et al. 1983; Moore and Schwartz 1981). The O_3-induced decrease in DL_{CO} and increases in FRC and RV were reversed one to three months following exposure (Gross and White, 1986; Gross and White, 1987).

Table 11-10: Lung Mechanics

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Acute	0.5	5 min	Ketamine and Fluoro-diazepam	Baboon, M, Adult	Brief exposure caused increased R_L, but NOT Mch reactivity. Increased R_L was blocked by cromolyn.	Fouke et al. 1988
	0.5	5 min	Ketamine and Fluoro-diazepam	Baboon, M, Adult	Brief exposure caused increased R_L that was partially blocked by cromolyn, but no effect on stable prostanoids.	Fouke et al. 1990
	0.2 -0.8	3.75 hr		Rat, (Spraque-Dawley), 7 wk	Tachypnea, V_E, gas exchange, and R_L increased: C_{dyn} decreased.	Mautz et al. 1985
	1.0	1 hr	Ketamine and Xylazine	Guinea pig, M, (Hartley)	R_L increased at 2 hr not 8 hr. Lung volumes, DLco, and alveolar ventilation increased at 8 and 24 hr PE.	Miller et al. 1987
	1.0	1 hr	Ketamine and Xylazine	Guinea pig, M, (Hartley)	Decreased TLC, VC, FRC, and RV; Increased R_L. Indomethacin and cromolyn blocked change in FRC and RV at 2 and 24 hr PE. DLco increased, blocked by cromolyn.	Miller et al. 1988
	0.13, 0.22, 0.45	3 hr	Pentobarbital and Gallamine	Dog, (Foxhounds)	Positron camera indicated non-uniform distribution of ventilation in small airways; no change in R_L, C_{dyn}, or forced expiratory flow.	Morgan et al. 1986
	1.0	24 hr	Pentobarbital	Rat, M, (Wistar), 6 wk	Decreased C_{dyn}, C_{st}, V_{max}; increased FRC, RV; no additional effect in elastase-treated animals.	Yokoyama et al. 1987

Table 11-10 (cont.): Lung Mechanics

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Short-term	1.0	3 hr, 5 days	Pentobarbital	Mice, F, (CD-1), 3 wk	O_3 had no effect, but in combination with virus decreases DLco, N2 washout, and lung volume were observed greater than virus alone 6, 9, and 14 days PE.	Selgrade et al. 1988
	1.0 and 2.0	6 hr, 7 days (1ppm) or 3 days (2ppm)	Pentobarbital	Rabbit, M, (albino)	At 17 hr PE, 1 ppm increased RL; 2 ppm trapped air, decreased Cdyn and forced expiratory volume flows, and increased R_L.	Yokoyama et al. 1989
	0.64	7 or 20 days		Rat, M, (Sprague-Dawley)	Increased peripheral resistance in rats exposed for 7 days but not 20 days; decreased lung effective reactance at high frequencies in both groups.	Kotlikoff et al. 1984

Table 11-10 (cont.): Lung Mechanics

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Long-term Continuous	0.2	24 hr/d, 28-32 days		Rat, M, (Spraque-Dawley), 3-4 wk	Increased lung distensibility at high lung volumes (95-100%TLC) during inflation with air or saline.	Bartlett et al. 1974
	0.7	20 hr/d, 28 days	Halothane	Rat, M, (Fisher-344), 14 wk	Decreased DLco and increased FRC immediately PE, no effect at 4 weeks PE, decrease in forced expiratory flow at 9 weeks PE.	Gross and White 1986
	0.5	20 hr/d, 7 days/week, 52 weeks		Rat, M, (Fisher-344)	Increases in FRC and RV at 6 and 12 mo; DLco decreases over same period. The 3 mo recovery period resulted in reversibility of the functional lesion. Inflammation was mildly correlated with function and also reversed 6 mo PE.	Gross and White 1987
	0.4	7h/d, 5 days/week, 6 weeks		Rabbit, M/F, (New Zealand white), 10 wk	Increased alveolar wall extensibility, increased hysteresis ratio, and decreased stress at moderate extensions. Fixed lung volume increased 150%.	Martin et al. 1983
	0.5	24 hr/d, 180 days		Rat, M, (Sprague-Dawley), 70 d	At 180 days, but not 62 days, fixed lung volume increased.	Moore and Schwartz 1981

Table 11-10 (cont.): Lung Mechanics

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Long-term Continuous	Base 0.06, Spike rising to 0.25	13h/d, 7day/week; 5day/week; 1,3,13,52,78 weeks		Rat, M, (Fisher-344), 60 d	Increased expiratory resistance observed at all time points, but mostly at 78 weeks.	Tepper et al. 1991
	0.5 and1.0	3 and 6 hr/d, up to 60 days		Rat, M, (Wistar), 7 wk	Increased resistance of central airways after 3 hr daily exposures to 1.0 ppm for 30 days; increased resistance of peripheral airways after 6 hr daily exposure to 0.5 ppm 0_3 for 60 days. No effect of age of rats (4 vs. 10 weeks old).	Yokoyama et al. 1984

11.4.3.6.4 Airway Responsiveness

Studies that examined changes in airway responsiveness induced by the inhalation of O_3 at concentrations of 1.0 ppm or less are summarized in Table 11-11. There are relatively few studies that demonstrate an increase in airway responsiveness following the acute inhalation of O_3 concentrations of 1.0 ppm or less. Ozone inhalation has been shown to increase responsiveness of the airways to inhaled methacholine or histamine in dogs, guinea pigs and Fischer 344 rats, but not Sprague-Dawley rats (Fouke et al. 1991; Gross and Sargent 1992; Sumitomo et al. 1990; Tepper et al. 1990; Uchida et al. 1992). The studies that used Fischer 344 rats demonstrate that the increase in airway responsiveness was to inhaled challenges and not intravenous challenges (Tepper, et al., 1990; Uchida, et al., 1992), and was blocked by the cyclooxygenase inhibitor indomethacin (Gross and Sargent, 1990). Repeated O_3 exposures have also been shown to increase airway responsiveness in the rhesus Macaca (Johnson et al. 1988) and guinea pig (Vargas et al. 1998). In guinea pigs exposure to 0.3 ppm O_3 caused increased responsiveness to intravenous substance P (SP) on days 12, and 24, but not on day 48. This increase in responsiveness to SP was not due to impairment in neutral endopeptidase or superoxide dismutase (Vargas, et al., 1998).

Table 11-11: Airway Responsiveness

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Acute	0.5	2 hr	Chloralose	Dog, (Mongrel)	Increased reactivity to INH Mch, no change in BAL prostanoids.	Fouke et al. 1991
	0.3, 0.5, 0.7	1, 2, 24 hr		Rat, (Fischer-344)	Increased reactivity produced at C * T of 1.5 ppm * hr blocked by indomethacin, but not by another cyclooxygenase inhibitor.	Gross and Sargent 1992
	1.0 and 3.0	30, 90 and 120min	Propranolol	Guinea pig, F, (Hartley)	Increased reactivity to INH Mch with 90 min, 1 ppm and with 30 min, 3 ppm. At 2 hr, 3 ppm reactivity occurred at 0 and 5 hr, but not 24 hr PE.	Nishikawa et al. 1990
	0.8	2 hr		Guinea pig, M/F, (Hartley)	No increased reactivity to acid; O_3 alone increased gas trapping.	Silbaugh and Mauderly 1986
	1.0	3 hr	Pentobarbital	Rat, (Sprague-Dawley)	No significant effect on response to INH Mch.	Takebayashi et al. 1998
	0.12, 0.25, 0.5, or 1.0	2 hr	Urethane	Rat, M, (Fischer-344), 60day	Increased reactivity to INH Hist, but not IV Ach.	Tepper et al. 1990
	1.0	4 hr		Rat, (Fischer-344), 12mo	Increased reactivity to INH Mch, not IV Mch challenged rats.	Uchida et al. 1992

Table 11-11 (cont.): Airway Responsiveness

	Ozone Concentration (ppm)	Exposure Protocol	Drugs	Species, Sex, (Strain), Age	Observed Effects	Reference
Long-term Continuous	1.0	6 hr/d, 5 d/week, 12 weeks	Ketamine and Xylazine	Cynomolgus, M, Adult	No R_L, C_{dyn}, or flow volume changes associated with exposure.	Biagini et al. 1986
	1.0	2 hr/ week, 19 weeks	Pentobarbital	Rhesus Macaca, F, Adult	5-lipoxygenase inhibitor blocked the development of reactivity.	Johnson et al. 1988
	0.3	4 hr/d, 1, 3, 6, 12, 24, or 48 days		Guinea pig, M, (Hartley)	0_3 caused increased responsiveness to intravenous SP on days 1, 3, 6, 12, and 24, but not on day 48. Increased SP was not due to impaired neutral endopeptidase or superoxide dismutase.	Vargas et al. 1998

11.4.3.6.5 Host Defense – Animal Models

The host defense system in the respiratory tract of humans and animals protects against infectious and particulate matter deposition. Ozone exposure has been shown to induce changes in all areas of lung host defense including mucociliary clearance, which clears the conducting airways of inhaled substances, and functions of alveolar macrophages and other immune system cells, which protect the lower lung from damage by inhaled particles and infectious microorganisms. Recent work supports previously reviewed studies, in that compromised immune system defense in experimental animals occurs at O_3 concentrations as low as 0.1 ppm while mucociliary clearance is relatively resistant to O_3. Chronic exposure of rabbits to 0.1 ppm O_3 did not affect mucociliary clearance (Schlesinger et al. 1992a), but short-term exposure of newborn sheep to 1.0 ppm O_3 resulted in a prolonged reduction in mucociliary clearance (Mariassy et al. 1990). Ozone-induced decreases in alveolar particle clearance, which may be associated with inflammatory cell and permeability changes in the centriacinar region, has been observed in rodents with prolonged exposure to an urban pattern of O_3 (0.06 ppm with daily peaks of 0.25 ppm) (Pinkerton et al. 1989) and repeated subchronic exposure to 0.3 ppm O_3 (Cohen et al. 1997). Alveolar macrophages are one of the most sensitive indicators of O_3 exposure, and certain O_3-induced human and murine alveolar macrophage function alterations have been shown to be similar (Selgrade et al. 1995). Reductions in alveolar macrophage phagocytic function and bacterial killing occurred following acute exposure to 0.1-0.4 ppm O_3 (Driscoll et al. 1987; Schlesinger et al. 1992b; Gilmour and Selgrade 1993; Oosting et al. 1991). Bacterial lung infections are enhanced with acute O_3 exposures to 0.4 ppm (Gilmour and Selgrade 1993) or 5-day repeated exposures to 0.1 ppm (Cohen et al. 2001; Cohen et al. 2002). The severity of lung infection was related, at least in part, to the degree of O_3-induced reduction of alveolar macrophage bacterial killing and the strength of inflammation-induced neutrophil influx (Gilmour et al. 1991; Gilmour and Selgrade 1993). The lung response to viral infection challenge appears dependent on the timing of exposure to O_3. The acute phase of viral infection in mice is reduced by subsequent exposure to O_3 (0.5 ppm), but continued O_3 exposure for up to 3 months resulted in greater long-term lung damage (Jakab and Bassett 1990). Repeated O_3 exposure (3 hour/day for 2 days) prior to viral infection enhanced infection (Selgrade et al. 1988). But this response was attenuated beyond two days of exposure. As with the inflammatory and permeability effects, continued, repeated O_3 exposure results in an attenuation of the O_3-induced effects on the host defense system. Specifically, attenuation of the deleterious responses on alveolar macrophage phagocytic function, intrapulmonary bacterial killing, and viral-induced lung injury has been observed with repeated O_3 exposure (Cohen et al. 2001; Cohen et al. 2002; Gilmour et al. 1991; Selgrade et al. 1988; Driscoll et al. 1987).

11.4.3.6.6 Inflammatory and Biochemical Responses in Animal Models

Pulmonary biochemical changes following O_3 exposure include altered synthesis and content of structural proteins and changes in cellular and extracellular levels of anti-oxidant enzymes and substances.

Long-term O_3 exposures indicate that excess collagen deposition in lungs does not occur below an O_3 concentration of about 0.4-0.5 ppm for continuous or near-continuous exposures, and likely only occurs in younger animals (Last et al. 1993). The focal nature of the injury may prevent detection with biochemical analysis of the collagen content in whole lung. However, increased alveolar tissue density suggestive of increased collagen deposition has been observed with subchronic O_3 exposures at concentrations as low as 0.12 ppm (Last and Pinkerton 1997). It is unclear if functional consequences result from deposition of excess collagen at ambient levels of O_3, but irreversible changes in lung collagen structure have been shown in monkeys at higher exposures (0.61 ppm, 8 hour/day, for 1 year) (Reiser et al. 1987). Acute O_3 exposure may deplete or enhance airway epithelial concentrations of glutathione and other anti-oxidant enzymes, depending on O_3 concentration and airway level, suggesting that the ability of the epithelium at specific sites to replenish anti-oxidant enzymes as they are used may be a factor in site-specific O_3-induced injury. Antioxidant enzyme activities of superoxide dismutase (SOD) and glutathione (GSH) increase in response to O_3 with repeated exposure (6 hour/day, 5 days/wk for 3 months) to concentrations as low as 0.12 ppm (Plopper et al. 1994b). The increased activities are site-specific, occurring chiefly in lung regions that are most susceptible to O_3-induced injury. The antioxidant substances ascorbate and uric acid increase in response to prolonged, but not acute, O_3 exposures (Long et al. 2001; Kodavanti et al. 1995; Kodavanti et al. 1996; Grose et al. 1989). Ascorbate, in particular, increased in lavaged cells and fluid with repeated exposures as low as 0.2-0.25 ppm (Wiester et al. 1996a; Kodavanti et al. 1996). Adaptation to inflammatory effects of repeated O_3 exposures corresponded with the increase in ascorbate levels in BAL fluid (Wiester et al. 1996a; Wiester et al. 2000).

11.4.3.6.7 Effects of Ozone on Lung Development

Prolonged exposure to O_3 in animal studies results in airway remodeling in adult experimental animals. This remodeling is characterized by epithelial cell hyperplasia and hypertrophy, and interstitial fibrosis (Barr et al. 1990; Schelegle et al. 2003). Part of the underlying biochemical responses involved in airway remodeling is the altered release of epithelial growth factors. This lead to concerns regarding the impact of O_3 on the developing lung. There is evidence in humans of decreased lung function in young adults who were raised in areas with typically high O_3 relative to those raised in low O_3 areas (Kunzli et al. 1997; Galizia and Kinney 1999).

A series of studies conducted in infant rhesus monkeys indicates that O_3 exposure alone and especially in combination with allergen results in altered lung development. This series of studies is particularly important because of

concerns that the O_3 standards recommended adequately protect infants and children. Lung development in the infant rhesus monkey parallels that in humans. Thus, although the concentrations employed in the studies were higher than attained in current ambient exposures, the implications are quite important. The studies are described briefly below, although they do not directly contribute to the evaluation of the level of the standard.

Cyclic exposure to O_3 and to O_3 plus house dust mite allergen (HDMA) alters the development of the tracheal basement membrane zone (BMZ). The BMZ is important to the tracheal epithelial functioning as it serves as the attachment point for the epithelial cells, functions as a barrier to foreign substances, and is intimately involved in cell-to-cell communication. The BMZ is important to normal growth and development of the airway including storage and release of growth factors. The development of the BMZ occurs postnatally in rhesus monkeys. (Evans et al. 2003) evaluated the development of the BMZ in infant rhesus monkeys exposed to filtered air, O_3 or O_3 plus HDMA during the first postnatal 6 months of life. Twenty-four infant rhesus monkeys (30 days old) were exposed to 11 episodes of either filtered air, O_3 (0.5 ppm for 8h/day), HDMA (aerosolized, 2 hour/day for days 3-5 of the cycle) or O_3 plus HDMA; each episode was 5 days followed by 9 days of filtered air. Half the monkeys had been sensitized to HDMA. Ozone and O_3 plus HDMA exposures resulted in increased areas of the BMZ which were thin compared to controls. Immunochemical staining for perlecan, a proteoglycan involved in basement membrane function and storage of growth factor, indicated a reduction of this BMZ component in the O_3 and O_3 plus HDMA groups relative to filtered air. The BMZ content of fibroblast growth factor-2 (FGF-2), the main growth factor stored in the BMZ, was also significantly decreased by O_3 exposure. FGF-2 is thought to be important in the regulation of a number of biological molecules associated with airway remodeling. Upregulation of the cell surface proteoglycan syndecan-4 appeared to result from O_3 and O_3 plus HDMA exposures. Syndecan-4 is rapidly upregulated in injured tissues, and tissue injury stimulates shedding of the portion of the molecule that binds FGF-2 into the extracellular matrix. Syndecan-4 is thus involved in response to tissue injury and repair. Downregulation of the fibroblast growth factor receptor FGFR-1 in the BMZ was also noted in the O_3-exposed animals. The changes in these cellular macromolecules involved in tissue repair and growth reflected the poor development of the tracheal BMZ that resulted from exposure to O_3 and to O_3 plus allergen.

Other publications resulting from this series of experiments indicated that O_3 in combination with aerosolized allergen (HDMA) results in a marked increase in airway eosinophilia, and HDMA-specific serum IgE as well as altered structural development that resulted in increased airway resistance (Schelegle et al. 2003). The volume fraction of parenchyma in the right middle lobe was greater and the volume fraction of conducting airway and blood vessels were significantly less in the HDMA plus O_3 groups relative to filtered air or O_3 or HDMA groups alone. The results indicate that O_3 exposure can amplify the immune response to allergens in sensitized infants, resulting in an allergic phenotype airway. This phenotype was characterized by increased HDMA-induced histamine release as

measured by serum histamine, elevated BAL eosinophils, and increased airway resistance and reactivity. The increased levels of serum HDMA-specific IgE is consistent with the concept that O_3 may prime the developing immune system towards a Th2-type response.

Also of import is the recent publication from this study of changes in airway epithelial innervation induced in the developing rhesus monkey by exposure to O_3 and to O_3 plus HDMA (Larson et al. 2004). These changes were measured by immunohistochemical staining for the presence of nerve cells. The changes noted included significant decreases in the density of epithelial nerves in the midlevel airways (between the sixth and seventh intrapulmonary airway generations) upon O_3 exposure; these decrements increased in the O_3 plus HDMA treated group. These changes were also accompanied by the appearance of abnormal streaks and clusters of nerve cells in the airways just proximal to the midlevel generations. The authors conclude that these effects represent either neural regression or stunted nerve development in the airway.

11.4.3.6.8 Summary

The studies cited in this section support the notion that laboratory animals demonstrate similar responses to the acute inhalation of O_3 as human subjects. These responses include rapid shallow breathing, mild increases in airway resistance, decrements in forced expiratory end-points and increased airway reactivity to various stimuli with all of these responses showing attenuation with repeated exposures of three days or more. In addition the cited studies are supportive of a link between O_3-induced functional decrements and underlying injury and/or inflammation. The ability to make this link between O_3-induced functional decrements and underlying injury and/or inflammation in animal studies is especially important in chronic studies that utilize continuous or episodic exposure regimens in that such controlled laboratory studies are prohibitive in human subjects. As such, they provide valuable insights into the pathophysiology underlying human functional responses to the acute and prolonged inhalation of ambient O_3 concentrations. Of specific concern are the recent findings of epidemiologic studies that report an adverse effect of living in regions with high oxidant levels on lung function growth in children (Frischer et al., 1999; Galizia and Kinney, 1999; Peters et al., 1999; Calderon-Garciduenas et al., 2000; Gauderman et al. 2002) and the observation in animals that these changes are the result of altered postnatal development and growth of the airways (Schelegle et al. 2003).

11.4.4 Subjects with Pre-Existing Disease

11.4.4.1 Subjects with asthma

Asthma is a multi-factorial disease characterized by chronic airway inflammation and hyperresponsiveness, and episodic airflow obstruction, accompanied by respiratory symptoms, which can include wheeze, cough, dyspnea, excess phlegm, and chest tightness. In the U.S. and throughout much of the developed world, asthma prevalence and morbidity has substantially increased in the past couple of decades. Asthma is the most common chronic disease of childhood,

and has been estimated to be responsible for about 14,000,000 missed school days per year (Centers for Disease Control and Prevention National Center for Environmental Health 2004). Because of the underlying airway inflammation and hyperresponsiveness characteristic of this disease, asthmatics may have exacerbations of their disease upon exposure to respiratory irritants, such as O_3. As a result, asthmatics have been considered a potentially susceptible subpopulation in setting air quality standards, and there have been a considerable number of studies intended to examine differences in response to O_3 between asthmatics and nonasthmatics.

Controlled exposure studies of asthmatics involve several issues of interpretation distinct from studies of healthy subjects. First, many asthmatics experience exercise-induced bronchospasm (EIB), or constriction of the airways in response to vigorous exertion, even in the absence of exposure to respiratory irritants or aeroallergens. EIB can result in both decreased lung function and respiratory symptoms. Therefore, when evaluating the results of controlled O_3 exposure studies in which exercise is incorporated into the study protocol, it is particularly important to examine the differences (e.g., lung function or symptoms) between O_3 exposures and filtered air exposures, as the latter act as a "control" to account for the effect of EIB. Second, most asthmatics take inhaled medications, including bronchodilators, which can be taken on an as-needed basis to control symptoms or on a regular schedule, and anti-inflammatory medications, which generally are taken chronically to reduce the underlying inflammation and lessen the overall severity of disease. Theoretically it is possible for both types of medication to blunt the respiratory impacts of O_3; therefore, most investigators have tried to remove this potential "extraneous" influence by having asthmatic study subjects refrain from taking their medications for varying time periods prior to the test exposures. While it might be argued that such tests could overestimate O_3's effects on asthmatics whose disease is well managed, there are many individuals with poorly controlled asthma who rarely take their medications as directed. Thus, both from a scientific standpoint and from the perspective of conducting experiments that may represent "real-world" individuals, it is reasonable to withhold asthma medications in these studies.

Many of the initial controlled O_3 exposure studies focused on identifying differential symptom reporting and lung function changes between asthmatics and nonasthmatics. However, in the 1987 review of the O_3 standard, the Staff Report concluded that, based on controlled exposure studies published up to that time, one could not draw definitive inferences regarding whether individuals with asthma (or COPD, see below) were more sensitive to the acute effects of O_3 than healthy individuals. Less demanding study protocols (resulting in lower breathing rates, and hence lower effective doses for asthmatics) and, in some studies, failure to control for medication use made it difficult to compare the results of these one- and two-hour exposure studies with those involving healthy subjects (California Department of Health Services 1987). Moreover, no studies involving such potentially susceptible populations had examined inflammatory responses. Studies conducted since 1987 have confirmed that low-level O_3 exposures of short duration do not apparently result in lung function or symptomatic changes

that differentially affect asthmatics participating in such comparative studies. In this regard it should be borne in mind not only that asthmatics have lower baseline lung function, which may be overlooked if one is only examining percentage changes in FEV1, for example. In addition, controlled exposure study protocols in general favor the selection of individuals who are not representative of the asthma spectrum. For instance, anyone who has had a recent respiratory infection will not be tested; on the other hand, in real life such infections are common causes of prolonged airway responsiveness in asthmatics. More importantly, there is likely to be a self-selection bias among asthmatics willing to be exposed to a known respiratory irritant.

Despite these limitations, several studies have demonstrated that asthmatics, whose airways already exhibit ongoing inflammation, experience a more vigorous O_3-induced inflammatory response, in both upper and lower airways, than nonasthmatics (Scannell et al. 1996; McBride et al. 1994). Higher effective doses, resulting from higher exposure concentrations and/or longer exposure durations, may cause greater airway obstruction in asthmatics relative to nonasthmatics. In addition, O_3 exposure may potentiate the effects of exposure to other pollutants or to aeroallergens. Some of these results, particularly those indicating increased inflammation, may help explain the consistent epidemiological findings of an association between ambient O_3 levels and increased asthma morbidity (See Sections 12.1, 12.2)

11.4.4.2 Controlled exposures to ozone since 1987 examining pulmonary function and symptoms

Investigations published since 1987 are consistent with the earlier reports in demonstrating that, for short exposures (30 min to 1 hour), asthmatics participating in controlled exposure studies with O_3 concentrations modestly higher than the California standard do not appear to show lung function or symptomatic effects greater than nonasthmatics. Koenig et al. (1988a) exposed 24 adolescents, including 12 with mild asthma, to FA or 0.12 ppm O_3 and 0.30 ppm NO_2, alone or in combination, for one hour of intermittent moderate exercise (mean ventilation rate = 32.5 L/min). No significant O_3-related changes were observed for FEV1, FVC, total respiratory resistance, peak flow, or maximal flow at 75% of FVC. However, a significant decrease was observed for maximal flow at 50% of FVC in asthmatic subjects only. In contrast, after the combined O_3-NO_2 exposure no significant changes were observed for any of the lung function metrics in either group. The investigators suggested that the single statistically significant finding might have been due to chance (multiple comparisons). In another study, Koenig et al. (1987) studied responses of 10 healthy and 10 asthmatic adolescents exposed to 0.12 and 0.18 ppm O_3 for 30 minutes at rest, followed by 10 minutes of moderate treadmill exercise. While there was an effect on total respiratory resistance in both groups at 0.18 ppm, there were no consistent responses in other lung function measures (FEV1, FVC, peak flow, or maximal flow at 50 % and at 75% of FVC) at either concentration.

Several other studies intended to examine joint effects of O_3 with other exposures (aeroallergens, exercise, or sulfur dioxide,) have also demonstrated

no effect of low O_3 exposure concentrations on lung function or symptoms in asthmatics (0.12 ppm x 1 hour or less -- Ball et al. 1996; Molfino et al. 1991; Hanania et al. 1998; Fernandes et al. 1994; Koenig et al. 1990).

At a relatively high effective dose, Kreit et al. (1989) reported that asthmatics demonstrated greater airway obstruction than nonasthmatics. Nine mildly asthmatic and nine nonasthmatic adults (aged 18-35) were exposed to either FA or O_3 (0.4 ppm) for two hours, involving alternating 15-minute intervals of rest and exercise (exercise ventilation rate range = 48 –70 L/min). The asthmatics had discontinued all medications at least 12 hours prior to the exposures. Symptoms, lung function, and airway reactivity to methacholine were recorded before and after each exposure; lung function measurements were also taken during each 15-minute rest period. The asthmatics experienced significantly greater percentage O_3-associated declines in FEV1, FEV1/FVC, and FEF25-75% than the nonasthmatics. Interestingly, the decrements in these measures grew progressively with increasing duration of exposure. Consistent with these observations, the asthmatics' SR_{aw} increased more than that of the nonasthmatics after O_3 exposure. Both groups also experienced significant decreases in FVC, as well as increases in both symptom scores and airway hyperresponsiveness. The investigators indicated that there were no significant differences between the groups with respect to the percentage changes in these latter metrics; however, in absolute terms the asthmatics started from lower baseline lung function and substantially greater airway hyperresponsiveness, so that even if the relative changes in these measures were similar, the potential clinical implications of the absolute changes are markedly different. In any case, this study demonstrated a clearly differential response in measures of airway constriction (FEV1, FEV1/FVC, and FEF25-75%) between asthmatics and nonasthmatics, unlike studies conducted at lower exposure concentrations.

Since the publication of the Kreit study, most studies examining primarily the effects of O_3 on asthmatics' lung function have involved larger effective doses than earlier investigations. However, the evidence regarding whether asthmatics show greater O_3-associated evidence of airway obstruction is mixed. For instance, Stenfors et al. (2002) found modestly greater effects on FVC and FEV1 in healthy relative to mildly asthmatic subjects exposed to 0.20 ppm O_3 for 2 hour, with alternating 15-minute periods of rest and exercise, though there were no significant between-group differences in the magnitude of the effects on lung function. Several others (reviewed in detail in Section 11.4.3.5) have used a 6.6-hour or 7.6-hour protocol. While not entirely consistent, these papers suggest differential susceptibility of asthmatics to an effective dose of O_3 greater than in the earlier studies. For instance, Horstman et al. (1995) found greater spirometric changes in asthmatic than in nonasthmatic adults exposed to 0.16 ppm O_3 for 7.6 hour, with intermittent exercise. Several of the asthmatics (6/17) required rescue medication to treat symptoms of bronchoconstriction. In contrast, Linn et al. (1994) did not find a difference between asthmatic and healthy subjects in a multi-hour exposure study in Los Angeles. In that study, the investigators exposed 15 healthy and 30 asthmatic subjects, aged 18-50, to FA or 0.12 ppm O_3 and 100 $\mu g/m^3$ sulfuric acid, singly and in combination, for 6.5

hour on two successive days per exposure regimen. At least seven of the nonasthmatic subjects were allergic (by skin test) to common aeroallergens. Although asthmatic subjects withheld inhaled bronchodilators for at least 4 hour prior to the exposures, those taking oral bronchodilators or inhaled corticosteroids continued to do so. The well recognized anti-inflammatory effects of corticosteroids could well have blunted some O_3-associated effects. This investigation took place in the Los Angeles basin during the spring, and ambient O_3 levels intermittently exceeded the experimental O_3 exposure concentrations. Therefore, it is likely that, for at least some of the asthmatics and healthy subjects, the overall daily effective doses of O_3 were greater than those designated by the study protocol (especially on the FA exposure days), which could have modified any effects of the experimental exposures. Moreover, the overall O_3-associated effects could well have been attenuated by repeated ambient exposures, even among asthmatics (Gong et al. 1997a). Overall, the percentage decreases in FEV1 and increases in SR_{aw} and symptom scores were similar in the asthmatic and the nonasthmatic subjects; however, the former group's lung function was lower and symptom scores higher at baseline. Graphically, the changes in lung function appeared to be progressive over the course of the exposure.

Basha et al. (1994) exposed five asthmatics and five nonasthmatics (all between the ages of 18 and 45) to 0.20 ppm O_3 or FA for 6 hour, with alternating 30-minute periods of rest and exercise (target ventilation rate = 5 L/min/L vital capacity). All subjects were nonsmokers and had not had a recent respiratory infection. Information on regular medication use by the asthmatics was not provided, though they did not take inhaled medications for 12 hour or oral medications for 24 hour prior to exposures. The investigators measured lung function before, at hour intervals during, and shortly after the exposures; and measured SR_{aw} before and after the exposure. Symptom scores were measured before, once during, and immediately after the exposures. In contrast to other published studies, there were no significant changes in any measure of lung function (FEV1, FVC, FEV1/FVC, and FEF25-75%) before, during, or after the exposures. Results for SR_{aw} were not reported. The nonasthmatics recorded more O_3-related symptoms (itching or burning of the nose, dry mouth or throat, burning throat, urge to cough, and nausea) than the asthmatics, though the mean symptom scores indicated that all of these were in the range of "perceptible" or "distinctly perceptible." The only symptom in asthmatics that was significantly increased was "urge to cough." Analysis of BAL fluid in this experiment is discussed below. The effective doses in this study were smaller than in the North Carolina and Los Angeles studies, which might explain the absence of any spirometric effects in either the healthy or asthmatic subjects. Moreover, the sample size was small, and the investigators did not provide sufficient baseline data on the subjects to allow for comparison with other studies.

Peden et al. (1997) and Kehrl et al. (1999) both studied small groups of allergic asthmatics without healthy subjects as controls, using 7.6-hour exposures to 0.16 ppm FA or O_3. These groups both experienced significant O_3-associated spirometric changes that were in the range of those experienced by healthy

subjects doing moderate exercise using a 6.6-hour protocol (See Section 11.4.5.3). As their ventilation rates were lower than those of the healthy subjects (25 L/min during exercise versus about 40 L/min, respectively), the results of these studies are not directly comparable. However, these studies illustrate that asthmatics demonstrate spirometric responses at O_3 concentrations observed in California, with an extended but plausible exposure duration for individuals doing outdoor labor. Again, O_3-associated effects on lung function are superimposed on pre-existing lung function deficits.

Some controlled exposure studies, as well as several field studies, suggest that there may be some interaction between O_3 and other pollutants at low ambient concentrations; however, in general, most studies do not show effects greater than those attributable to O_3 alone (Koenig et al. 1990; Koenig et al. 1994; Linn et al. 1995; Linn et al. 1997; see also Sections 11.4.12; 12.2.5; and 12.4.2).

11.4.4.3 Ozone and inflammation in asthmatics

Several studies have examined the inflammatory impact of O_3 exposure in asthmatics. Taking into account their underlying chronic airway inflammation, nonetheless, asthmatics still appear to demonstrate a greater inflammatory response to O_3 exposure than nonasthmatics. Scannell et al. (1996) exposed 18 adult subjects (mean age = 26; range 18 – 36) with mild asthma to FA or 0.20 ppm O_3 for four hours, with 50 minutes of moderate exercise alternating with 10-minute rest periods. The subjects had taken no asthma medications for at least 24 hours prior to the exposures, and no inhaled anti-inflammatory agents for at least 48 hours. The subjects completed symptom questionnaires and underwent lung function testing immediately before and after each exposure; lung function was also measured during the first few minutes of the rest periods. Fiberoptic bronchoscopy was conducted 18 hours after the exposures, with subsequent analysis of fluids obtained by proximal airway lavage (PAL) of the left mainstem bronchus and bronchoalveolar lavage (BAL, which was further subdivided into bronchial and bronchoalveolar fractions) of the right middle lobe. There were significant O_3-associated effects on lung function (FEV1, FVC, and SR_{aw}), lower respiratory symptoms, and inflammatory markers in the PAL (percent PMNs) and BAL (percent PMNs, total protein, IL-8, LDH, and several others) among the study subjects. Compared with 81 healthy subjects who had participated in earlier phases of the same study, there were no significant differences in symptoms or lung function changes; however, the increases in percent PMNs and total protein in BAL were significantly greater among the asthmatics. In addition, the mean O_3-associated increase in SR_{aw} was more than twice as large in the asthmatics than in the nonasthmatic subjects (4.82 versus 2.24, respectively), but this difference was not statistically significant.

In a study of five atopic asthmatic and five nonasthmatic adults described above, Basha et al. (1994) reported significant differences between asthmatics and nonasthmatics with respect to inflammatory markers in BAL fluid. The subjects underwent bronchoscopy 18 hours after a six-hour exposure to either FA or 0.20 ppm O_3. Increases in PMNs/mL lavage fluid, percent PMNs, IL-6, and IL-8 were significantly greater in the asthmatics exposed to O_3 compared with FA exposure

and with the healthy subjects after either exposure. TNF and albumin were also increased among the asthmatics exposed to O_3, but these were not significantly different from the FA exposure or from the healthy subjects' results. Thus, despite this study's small size, the lack of significant changes in lung function and the relatively minor symptomatic responses, O_3 exposure resulted in inflammatory changes in the asthmatics' lungs that were significantly greater than in the nonasthmatics.

Recently, Stenfors et al. (2002) examined early inflammatory responses to O_3 in 15 mild asthmatics (mean age 24, range 19-31) and 15 healthy subjects (mean age 29, range 21-48), who were exposed to 0.20 ppm O_3 or FA for two hour, with alternating 15-minute periods of rest and exercise (ventilation rate 20 L/min/m^2 body surface area). All subjects were never-smokers without a recent respiratory infection. All asthmatics were atopic, and used only bronchodilator medication, as needed. Though the manuscript indicates that the subjects did not take any anti-inflammatory or anti-oxidant drugs throughout the study, it is not clear whether bronchodilator medications were also withheld. The investigators measured lung function (FEV1 and FVC) pre- and post-exposure, and also had the subjects undergo bronchoscopy at six hours post-exposure (in contrast to the 18-hour interval in the other studies). During this procedure, the investigators performed bronchial biopsies, two bronchial washings (BW), as well as BAL. As noted above, there were no between-group differences in the magnitude of lung function changes. There were significant increases in PMNs in BW, but not BAL, in both groups. The PMNs of the asthmatics were also "activated", indicated by a significant O_3-related increase in the enzyme myeloperoxidase in their BW. The bronchial biopsies showed a significant increase in PMNs in the airways of the healthy but not the asthmatic subjects. At baseline, the asthmatics had higher levels of lymphocytes and mast cells than the healthy subjects; however, O_3 exposure did not affect the numbers of these cell types in BW or BAL in either group. Eosinophils were present in BW, BAL, and the tissue samples of asthmatics, but were not affected by O_3 exposure. Expression of vascular endothelial adhesion molecules (P-selectin and ICAM-1), which facilitate the recruitment of inflammatory cells to the airways, was upregulated in the healthy subjects, but not the asthmatics. Healthy subjects also experienced O_3-associated increases of PMNs and mast cells in their airway walls, while the asthmatics did not. Under the conditions of this experiment, the asthmatic subjects did not experience greater inflammatory responses to O_3 exposure than the nonasthmatics. However, the overall exposure was shorter and involved a lower dose of O_3 than other studies examining inflammatory responses; moreover, the samples were taken much earlier after the exposures (at six hours rather than at 18 hours). Therefore, in the study by Stenfors et al. (2002), insufficient time might have elapsed prior to the BAL to be able to detect full inflammatory responses. In addition, they apparently did not measure levels of IL-8, a chemoattractant for PMNs, which might have given an indication of early inflammatory differences between the groups.

A follow-up experiment demonstrated that O_3-associated inflammatory responses in asthmatics might follow a slower course than in healthy subjects, which helps

explain the differences observed in the study by Stenfors et al. (2002), above. Bosson et al. (2003) performed bronchoscopy and obtained bronchial biopsies six hours after exposing 15 healthy, nonatopic adults (mean age 24, range 19-31) and 15 atopic asthmatics (mean age 29, range 21-48) with mild disease to FA and 0.20 ppm O_3 for two hours, with intermittent exercise, on two occasions. The biopsies were examined for the presence of a variety of cytokines associated with inflammation. Comparing the results after O_3 versus FA, there were no significant differences among the healthy subjects; however, the asthmatics showed significant increases in IL-5 and GM-CSF, both of which promote eosinophilic inflammation in the airways, and ENA-78, which assists in attracting PMNs. They also found an increase in IL-8 that was of borderline significance (p=0.06). Between-group comparisons showed significant differences between the asthmatics and the healthy subjects for these four cytokines, as well as for GRO-α, another pro-inflammatory cytokine, but not for several others (IL-6, IL-10, TNF-a, and fractalkine).

Peden et al. (1997) studied eight allergic asthmatics exposed to FA or 0.16 ppm O_3 for 7.6 hour (see Section 11.4.3.5). All subjects had mild asthma and all were sensitized to dust mite allergen (*Dermatophagoides farinae*). Bronchoscopy and BAL were performed 18 hours after the FA and O_3 exposures. Ozone exposure was associated with increased PMNs and eosinophils in BAL, but not lymphocytes, macrophages, or biochemical inflammatory mediators. In the BAL fluid, the mean PMN percentage nearly tripled between FA and O_3 exposures (2.5 to 8.6), while the percentage of eosinophils increased by a factor slightly greater than two (1.8 to 3.6). These effects were more pronounced when the investigators examined the initial fraction of the BAL (mean percentage increases from 2.8 to 11.5 for PMNs, and from 2.9 to 17.8 for eosinophils). While other studies had previously demonstrated an influx of PMNs (or neutrophils) after O_3 exposure, this study demonstrated such an effect with eosinophils, as well. Unlike the previous studies by Scannell et al. (1996) and Basha (1994), the subjects in this study also had a small percentage of eosinophils present in BAL fluid after air exposure. Eosinophilic infiltration into the airways and airway nerves is characteristic of allergic asthma, and plays an important role in asthma exacerbations (Costello et al. 2000; Green et al. 2002). Therefore, to the extent that O_3 exposure may exacerbate pre-existing eosinophilic airway inflammation among asthmatics, it may have important clinical implications. Eosinophilic inflammation in asthmatics has no equivalent in healthy, nonatopic individuals; therefore, when this phenomenon occurs it is by definition more severe in asthmatics than nonasthmatics.

Hiltermann et al. (1999) compared the values of inflammatory markers in induced sputum versus BAL fluid of 16 asthmatics exposed to 0.40 ppm O_3 on two occasions for two hour with alternating 15 minute periods of exercise (20 l/min/m^2 body surface area). At this concentration, the investigators observed significant increases in several indicators of inflammation: total cells (by 1.6-fold), eosinophilic cationic protein (ECP – 1.8-fold), and neutrophil elastase (5-fold). Comparing BAL fluid obtained 18 hour after either FA or O_3 exposure, they observed significant increases in albumin, ECP, elastase, percentage PMNs, and

percentage lymphocytes. The O_3-associated changes in ECP and IL-8 (which increased in both induced sputum and BAL fluid, though these increases were not statistically significant) were highly correlated, as were the numbers and percentage changes in eosinophils in sputum and BAL fluid. The percentage change in eosinophils, but not PMNs, in induced sputum was also correlated with airway hyperresponsiveness (which was also measured in this study). This study demonstrated that induced sputum could be used in place of the more invasive bronchoalveolar lavage to measure O_3-associated inflammation in asthmatics.

Using induced sputum to measure inflammatory markers, Vagaggini et al. (2002) recently reported that O_3 exposure can magnify eosinophilic responses caused by allergen challenge in allergic asthmatics. Previously, these investigators had found that exposure to O_3 alone at 0.27 ppm for two hour resulted in enhanced neutrophilic, but not eosinophilic, inflammation in induced sputum of 11 subjects with mild asthma, which is consistent with the results of other investigations (Scannell et al. 1996; Basha et al. 1994 ; Vagaggini et al. 2001). However, in contrast to other studies examining whether O_3 pre-exposure enhances the response to allergen in asthmatics (see below), these investigators reversed the order of exposure. In a randomized, single-blind study, 12 mild asthmatics (age range 18-31) were exposed to aeroallergen challenge, followed 24 hours later by either FA or O_3 (0.27 ppm) for two hours with intermittent exercise (20 min/hour, ventilation rate 25 L/m^2 body surface area). The subjects had mild persistent asthma, but used only bronchodilators as needed (i.e., they did not take anti-inflammatory medications). Presumably medication was not withheld during this experiment, as the aeroallergen challenges alone caused substantial changes in lung function. Aeroallergens were dust mite (n=8), timothy grass (n=3) and a pollen (*parietaria*) (n=1). Six hours after the FA or O_3 exposure, the investigators collected sputum induced with hypertonic saline. They reported that, while there were no differences in IL-8 concentrations or percentages of PMNs in induced sputum, there was a significant difference between the percentage of eosinophils after O_3 exposure (27.5% [2.3-72.8%]) compared with FA exposure (9.9% [3.5-71.5%]) ($p = 0.04$). It is well established that allergen challenge elicits an eosinophilic influx into the airways of asthmatics (Sulakvelidze et al. 1998; Gauvreau et al. 1999). Vagaggini et al. (2002) found that subsequent O_3 exposure could in fact augment this eosinophilic response. These results may also help explain the enhanced eosinophilic response in the study by Peden et al. (1997), in that the subjects in that investigation were likely to have had ongoing regular household exposure to house dust mite allergen, which may in turn have been at least in part responsible for the underlying eosinophilic inflammation. Along with the study by Peden et al. (1997), this finding may have important clinical ramifications, as sputum eosinophil counts are a good predictor of asthma (Green et al. 2002). However, the O_3 concentration used in this study is substantially greater than those typically found in the U.S. today. It would be useful to know whether such effects can be observed at lower O_3 concentrations as well.

Ozone exposure has also been reported to increase inflammation in the upper airways of asthmatics. In a double-blind, randomized study, McBride et al.

(1994) exposed 10 asthmatic and eight nonasthmatic adults to FA, 0.12 or 0.24 ppm O_3 for 90 minutes, with alternating 15-minute periods of rest and exercise (ventilation rates approximately 7 and 23 L/min, respectively). Nasal lavage was performed 10 min, 6 hour, and 24 hour post-exposure. At the 0.24 ppm O_3 exposure level, asthmatic subjects showed significant increases in epithelial cells (an indicator of damage to the upper airways) and PMNs in nasal lavage at 10 minutes after exposure and in PMNs at 24 hour post-exposure. PMNs increased from 2.08 x 10^4 cells/mL at baseline to 66.4 x 10^4 at 24 hour. No significant changes were observed at 0.12 ppm among asthmatics, nor after either O_3 concentration among the nonasthmatics. To the extent that upper airway responses reflect events in the lower respiratory tract (Graham and Koren 1990), these results are consistent with several studies cited above, demonstrated neutrophilic inflammatory responses to O_3 exposure.

11.4.4.4 Interactions with other exposures

The effects of O_3 plus other exposures, including exercise, respiratory irritants, and allergens have been conducted in controlled settings. Investigations of the effects of O_3 plus other air pollutants are reviewed in Section 11.4.12. One potential concern among asthmatics sensitized to aeroallergens is that O_3 could lower the "threshold" of response to a given allergen, potentially increasing individuals' susceptibility to the effects of aeroallergens. There have been several studies examining this phenomenon, with somewhat inconsistent results. All such studies, however, used O_3 doses that exceeded the current California O_3 ambient air quality standard by at least 33%.

11.4.4.5 Ozone and allergen exposure

Allergy plays a large role in most cases of asthma. Among individuals sensitized to an aeroallergen, such as pollen, airway narrowing begins to develop within minutes of inhalation of allergen. This reaches a maximum within about a half-hour and often resolves within one to three hours. About half of adult asthmatics and 70-85% of children who experience this "early asthmatic response" to allergen also develop a more, prolonged "late asthmatic response', in which airway constriction recurs after several hours, reaching a peak about 6 to 12 hours after the initial exposure (O'Byrne 1998). While both early and late asthmatic responses to allergen inhalation are mediated by IgE, the late response is associated with increased airway inflammation, notably with eosinophils, but PMNs and lymphocytes may also be increased, depending on the initial stimulus (O'Byrne 1998).

In an experiment conducted at the University of Toronto, Molfino et al. (1991) first reported that O_3 could lower asthmatics' threshold of response to aeroallergens. Seven adults with mild asthma (mean age = 40, range 21-64) were exposed at rest to four exposure combinations: 0.12 ppm O_3 or FA for an hour, followed by breathing either the allergen diluent (phosphate-buffered saline placebo) or the allergen. Allergens were selected according to the results of skin-prick tests for each individual. PFTs were measured for six hours (presumably once an hour) following each exposure. Airway responsiveness was measured the day before

and the day after each exposure. Though the original study design called for a random allocation of exposures, the second subject experienced an increase in asthma symptoms and airway hyperresponsiveness that lasted three months after the O_3/allergen exposure. Therefore, the investigators administered this combination last to all the subsequent subjects, resulting in a nonrandomized, single-blind exposure protocol. For both allergen and the O_3/allergen combination, 3/7 subjects experienced a 15% decline in FEV1 three to six hours after the exposures. Overall, however, there did not appear to be any significant early- or late-phase change in FEV1 associated with exposure to the O_3-allergen combination in contrast to the allergen alone. However, the O_3 pre-exposure resulted in a reduction of the dose of allergen required to elicit a given degree of airway responsiveness by more than half (mean (SD) PC_{15} = 0.013 (0.017) mg/mL after air-allergen and 0.0056 (0.0062) mg/mL after O_3/allergen (p = 0.041). However, as noted by Ball et al. (1996, discussed below), it is possible that the effects that Molfino and colleagues attributed to O_3 might have been due to persistent effects of allergen on airway hyperresponsiveness, because the air/allergen exposure in this study preceded the O_3/allergen exposure for 6/7 subjects, and the time interval between the two exposures was four weeks or less for 6/7.

Investigators at the University of North Carolina (Chapel Hill) and the U.S. EPA attempted to repeat the work reported by Molfino (Ball et al. 1996), but could not reproduce the Toronto results. Following a double-blinded design, 15 adults (mean age 25.3, range 19-34) with mild asthma and allergy to grass or ragweed pollen were exposed at rest for one hour to either FA or 0.12 ppm O_3, followed by challenge with aerosolized allergen. These exposures took place outside of the pollen season. The subjects withheld bronchodilator use for 12 hour before and during testing. The subjects underwent lung function testing and answered symptom questionnaires before and after exposure to air or O_3, and were then administered an aeroallergen challenge. The next day their airway responsiveness was tested using progressively larger histamine doses until PD_{20} was attained. There was no O_3 effect on FEV1 or SR_{aw}. Though the dose of allergen required to elicit an early asthmatic response was lower in the O_3-exposed group, this difference was not statistically significant. There were no significant differences between the O_3 and air exposures with respect to late asthmatic responses to allergen or airway hyperresponsiveness to post-exposure histamine challenge. Thus, Ball et al. (1996) were unable to confirm the results of Molfino et al. (1991) using a one-hour resting exposure to O_3. This may have been due, as noted above, to the nonrandom order of exposures plus the relatively short inter-exposure period in the Toronto experiment. In addition, though the subjects were generally similar in the two studies, those in the Molfino study had greater baseline airway hyperresponsiveness.

In light of the results reported by Ball et al. (1996), the University of Toronto research group repeated their experiment (0.12 ppm O_3 or FA at rest for one hour, followed by aeroallergen challenge) with 15 mildly allergic asthmatic subjects rather than seven, as well as randomized and better controlled O_3 exposures (0.11 to 0.13 ppm in the latter experiment versus 0.085 to 0.175 in the

first) (Hanania et al. 1998). Under these conditions, they reported no effect of pre-exposure to 0.12 ppm O_3 on airway responsiveness resulting from allergen challenge.

In contrast to the results reported by Ball et al. (1996) and Hanania et al. (1998), three other investigations using higher effective doses both found that O_3 pre-exposure could potentiate airway responsiveness after subsequent allergen challenge (Jorres et al. 1996; Kehrl et al. 1999; Jenkins et al. 1999). Jorres et al. (1996) studied 24 subjects with mild allergic asthma (mean age 26, range 21-35), 12 with allergic rhinitis but not asthma (mean age 25), and 10 healthy subjects without any allergies (mean age 23). The exposure protocol involved assessing baseline airway responsiveness to methacholine and after O_3 exposure, as well as responsiveness to allergen challenge. Then the subjects underwent randomized, single-blind exposures to FA or O_3 (0.25 ppm for three hour, with intermittent 15-minute periods of exercise, ventilation rate approximately 30 L/min), followed by a methacholine challenge test one hour later, and then aeroallergen (pollen or house dust mite) challenge one hour after that. These FA and O_3 exposures were separated by at least four weeks to avoid "carry-over" effects from either O_3 or the allergen challenge. None of the asthmatics had used anti-inflammatory medications in the three months preceding the study; those that used bronchodilator inhalers refrained from using them at least eight hours prior to the exposures. Compared with the FA exposure, O_3 resulted in a marked, statistically significant increase in airway responsiveness to subsequent allergen challenge. More specifically, Jorres et al. (1996) reported that, on average, 3.3 times less allergen was required to attain a 20% fall in FEV1 after O_3 exposure than after FA.

Kehrl et al. (1999) reported results similar to those of Jörres et al. (1996). In a double-blind, counterbalanced design, nine mildly asthmatic subjects (aged 20 – 35) were exposed to FA or to 0.16 O_3 for 7.6 hour, with intermittent exercise (50 min/hour, ventilation 24 L/min), followed 18 hour later by challenge with dust mite allergen (to which all were sensitized). Seven of the nine subjects required less allergen to attain a 20% fall in FEV1. As with the study by Jorres et al. (1996), the O_3 exposure alone also evoked significant decrements in lung function. Therefore, they concluded that "O_3 exposure conditions capable of inducing lung function decrements also increase airway responsiveness to specific allergen in subjects with mild atopic asthma." Moreover, in the Jörres study the increased airway responsiveness to allergen was observed shortly after (about 3 hour) the end of the O_3 exposure; the report by Kehrl and colleagues indicates that this phenomenon can persist for at least 18 hours.

Finally, Jenkins et al. (1999) demonstrated that the O_3 concentration is likely more important than exposure duration with respect to whether atopic asthmatics will experience enhanced airway responsiveness after a subsequent allergen challenge. Using a single-blind, randomized exposure protocol, these investigators exposed 11 mild asthmatic subjects (aged 18-45) to FA, 0.10 ppm O_3, 0.20 ppm nitrogen dioxide, or 0.10 ppm O_3 plus 0.20 ppm NO_2, for six hours with intermittent exercise (for 10 out of each 40 min, ventilation 32 L/min),

followed immediately by challenge with dust mite allergen. Ten of the 11 subjects were also exposed to the same gases, at twice the concentration, but for half the duration (3 hour), also followed immediately by allergen challenge. All exposures were separated by at least two weeks. Only the three-hour exposures to O_3 (0.20 ppm), NO_2 (0.40 ppm), or their combination resulted in significant enhancement of airway responsiveness to allergen. Exposure for six hours at the lower pollutant concentrations did not have any significant effect, though the combination of O_3 and NO_2 was of borderline significance (p=0.067). Thus, even though theoretically the total pollutant doses were equivalent, only the exposures involving the higher concentrations seemed to have had an impact on airway response to allergen. On the other hand, this study only examined immediate response to allergen administered after the gas exposures. In healthy subjects, an inflammatory response is clearly observable 18 hour after a 6.6-hour exposure to concentrations as low as 0.08 ppm O_3 (Devlin et al. 1991). Thus, it is possible that had the allergen challenge been delayed until the day following the lower-concentration O_3 exposure, increased airway hyperresponsiveness to allergen might have been observable. In addition, like many controlled exposure studies, this investigation had limited statistical power. At face value, however, this study suggests that O_3 concentration may be a more important factor than duration of exposure in determining whether allergic asthmatics might experience increased responsiveness to allergen.

It should be noted that all of these studies examined whether O_3 exposure followed by allergen challenge resulted in changes in lung function and airway reactivity. Vagaggini et al. (2002) found that O_3 exposure following allergen challenge could potentiate eosinophilic inflammation, though the O_3 dose used in that experiment was substantially higher than the current California standard.

To the extent that O_3 does potentiate the effects of allergen exposure in allergic asthmatics, there may be several mechanisms. In subjects with allergic rhinitis, high-dose O_3 exposure by itself has been reported to cause an influx of inflammatory cells retrievable with nasal lavage (eosinophils, PMNs, and mononuclear cells), and to augment the nasal inflammatory response to allergen challenge (Peden et al. 1995; Bascom et al. 1990). Allergen challenge can stimulate release of tachykinins by nerves in the airways; these compounds can increase edema in the airways and cause bronchospasm. Ozone may also enhance the release of tachykinins in respiratory epithelial tissue (Schierhorn et al. 2002). Finally, it is well established that O_3 exposure can increase the permeability of the airway epithelium, in part via tachykinin release from airway nerves (Fu et al. 2002). In atopic asthmatics, such an increase in permeability would allow both an increased presentation of antigen to airway mast cells, which could then trigger both immediate responses and amplify the late asthmatic response via subsequent infiltration by inflammatory cells. Ozone may also augment mast cell degranulation in response to allergen via increased fibronectin levels (Devlin et al. 1991).

11.4.5 Subjects with Allergic Rhinitis

Allergic rhinitis is a common chronic disorder estimated to affect up to 30% of adults and 40% of children (Berger 2003). Symptoms include nasal congestion and discharge, itchy eyes, tearing and sneezing, usually occurring in response to exposure to pollens, molds, animal danders and other aeroallergens. Individuals with allergic rhinitis may experience symptoms only in response to seasonal exposures, while up to 20% may be symptomatic most of the year (Berger 2003). While most individuals with allergic rhinitis do not have concomitant asthma, the majority of asthmatics also have allergic (Nayak 2003). Both disorders involve chronic inflammation of different segments of the respiratory tract. Airway hyperresponsiveness, a hallmark of asthma, occurs more commonly among individuals with allergic rhinitis than among healthy individuals. Thus, it has been postulated that subjects with allergic rhinitis could constitute an O_3-susceptible subpopulation. The few small studies that have been conducted involving participants with allergic rhinitis do not allow for definitive conclusions. However, they suggest that such individuals may be as responsive as nonallergic subjects (or possibly slightly more responsive) to the direct effects of O_3 on lower respiratory symptoms and lung function. In addition, subjects with allergic rhinitis appear to experience increased susceptibility to allergen exposure, as measured by airway responsiveness to methacholine, though the magnitude of this increased susceptibility is less than that observed for subjects with asthma.

McDonnell et al. (1987) investigated whether individuals (n=26, age range 18-30) with allergic rhinitis were more susceptible than those without this condition who had been tested earlier using an identical study protocol. The subjects' airway responsiveness was tested with histamine; they were then exposed for two hour to either FA or 0.18 ppm O_3, with alternating 15 minute periods of exercise (ventilation rate = 64 L/min) and rest. Exposures were counter-balanced and double-blinded. None had had symptoms of either allergic rhinitis or respiratory infection in the preceding four weeks. This population of allergic rhinitics had no history of wheeze, nocturnal cough or exertional dyspnea. Their baseline airway responsiveness was similar to that of subjects without allergic rhinitis. The investigators took standard spirometric measurements (FVC, FEV1, FEF25-75%), airway resistance (SR_{aw}), lung volume metrics, and respiratory symptom scores. To validate the diagnosis of allergic rhinitis, the subjects were skin-tested for sensitization to common aeroallergens. FA exposure resulted in no significant changes in spirometric measures or symptoms, whereas O_3 exposure resulted in significant decrements in FVC, FEV1, and FEF25-75%, and significant increases in lower respiratory symptoms (cough, pain on deep inspiration, and shortness of breath), SR_{aw}, and airway hyperresponsiveness. The degree of airway hyperresponsiveness was not associated with the magnitude of O_3-associated effects. Comparing the results of this experiment with a prior set of exposures of healthy subjects, there appeared to be little difference in response to O_3, with the possible exception of modest increases in SR_{aw} (0.46 cm H_2O*sec in the subjects with allergic rhinitis compared with 0.23 in the nonallergic subjects).

Jorres et al. (1996) studied responses to 0.25 ppm O_3 or FA among 24 subjects with mild allergic asthma (mean age 26± 2 yr, range 21-35), 12 with allergic rhinitis but not asthma (mean age 25± 3 yr), and 10 healthy subjects without any allergies (mean age 23± 2 yr). The exposure protocol was somewhat complicated, and involved assessing baseline airway responses to: (1) methacholine, (ii) O_3 exposure, and (iii) allergen challenge. Then the subjects underwent randomized, single-blind exposures to FA or O_3 (0.25 ppm for three hour, with intermittent 15-minute periods of exercise, ventilation rate approximately 30 L/min), followed by methacholine challenge one hour later, and then aeroallergen (pollen or house dust mite) challenge one hour after that. These FA and O_3 exposures were separated by at least four weeks to avoid "carry-over" effects from either O_3 or the allergen challenge. Among the subjects with rhinitis, FEV1 changes after FA and O_3 were -0.8±1.6% and -13.8±3.0%, respectively (p=0.004), while the changes among healthy subjects were +2.9±1.6% after FA and –7.3±2.6% (p=0.018) after O_3. The differences across groups, however, were nonsignificant, which may have been due to the small sample size. Among the subjects with allergic rhinitis, O_3 pre-exposure resulted in significantly greater responses to allergen challenge (-7.8±2.4%) compared with FA pre-exposure (-1.3±1.3%). As expected, no such response was observed among nonallergic subjects. Thus, among this small number of subjects with allergic rhinitis, O_3 appeared to induce a slight increase in airway responsiveness to inhaled allergen. By comparison, the subjects with asthma in this investigation experienced a substantially greater fall in FEV1 (27.9±1.7%) in response to post-O_3 allergen challenge. (Moreover, the asthmatics were exposed to substantially less allergen than the subjects with allergic rhinitis were.) One of the subjects with allergic rhinitis experienced a post-allergen FEV1 decrement of 26.3% after O_3 pre-exposure versus 3.7% after FA. However, with respect to direct symptomatic and functional responses to O_3, there was no significant difference between the subjects with allergic rhinitis and healthy subjects, consistent with the findings of McDonnell et al. (1987). Moreover, the post-O_3 FEV1 decrements in this group of allergic rhinitics was within the range observed in studies of healthy subjects exposed to similar effective doses in other studies (see Section 11.4.2.1.3). Thus, the findings reported by Jorres et al. (1996) suggest that subjects with allergic rhinitis may represent a subpopulation indirectly susceptible to O_3, i.e., via the effect of O_3 on their lower airway responses to allergen exposure.

Holz et al. (2002) examined the effect of single versus repeated O_3 exposures on airway responses to allergen among 11 adults with allergic asthma (mean age 30+10 yr) and 22 with allergic rhinitis (mean age 29+7 yr). The subjects underwent four different exposures, which were administered in random order and were separated by at least four weeks. Each exposure lasted three hour, with alternating 15-min periods of rest and exercise. The four exposure protocols involved single exposures to FA, 0.125 or 0.25 ppm O_3, and one set of exposures to 0.125 ppm O_3 on four consecutive days. Immediately post-exposure, exhaled NO was measured; one hour later, airway response to methacholine was measured (to PD_{15FEV1}). Twenty hours post-exposure, an

aeroallergen challenge was administered with a single allergen dose (previously determined to result in PD_{15FEV1}). Exhaled NO was measured again one hour after the allergen challenge, and sputum induction took place six to seven hours post-allergen. PFTs were then measured hourly in the laboratory, followed by peak expiratory flow at home (for an unspecified duration). Early-phase response to allergen (measured by fall in FEV1) was significantly greater among subjects with allergic rhinitis after pre-exposure to 0.25 ppm O_3 or after the repeated exposures to 0.125 ppm O_3, compared with FA pre-exposure. Ten of the 22 subjects with allergic rhinitis demonstrated at least a 20% fall in FEV1 in response to allergen after both of these O_3 pre-exposures; one who showed a 29% FEV1 decrease reported "severe" symptoms after 4 x 0.125 ppm O_3. No significant effects were observed with respect to late-phase response to allergen. When compared with FA plus allergen exposure, there was a significant increase in eosinophils in induced sputum after allergen following four days of 0.125 ppm O_3 exposure, but this was the only such finding among the subjects with allergic rhinitis. In contrast, asthmatics showed significant increases in a variety of indicators of inflammation in sputum after this exposure regimen. The investigators pooled the exhaled NO results for both sets of subjects, so one cannot ascertain whether there were O_3-related effects among the subjects with allergic rhinitis without asthma. These results support this group's earlier finding that O_3 may increase susceptibility to inhaled allergen (Jorres et al. 1996) and, in addition, suggest that repeated exposures to a low-level O_3 concentration (0.125 ppm) among individuals with allergic rhinitis without asthma may be sufficient to enhance airway responses to allergen.

11.4.6 Subjects with Chronic Obstructive Pulmonary Disease

There have been few studies of O_3's effects on individuals with chronic obstructive pulmonary disease (COPD), which refers to several diseases that all share the feature of airflow obstruction: chronic bronchitis, emphysema, and sometimes asthma. Recently, the U.S. Centers for Disease Control and Prevention estimated that about 10,000,000 adults in the U.S. had COPD, which was the principal cause of eight million cases of outpatient or physician visits, 119,000 deaths, 726,000 hospitalizations, and 1.5 million hospital emergency departments visits annually (Mannino et al. 2002). In the U.S., COPD is usually caused by cigarette smoking, though genetic predisposition and certain occupational exposures are also important factors in the etiology of this condition. Subjects with COPD have permanent loss of lung function and face varying degrees of disability related to respiratory impairment. Their pulmonary anatomy and physiology are distorted: the more affected regions of their lungs are poorly ventilated, restricting their reserve respiratory capacity, while at the same time the air they inhale is distributed to a limited portion of their lungs, resulting in exaggerated local deposition/absorption of any air pollutants.

Of the few studies examining O_3's impact on individuals with COPD, all have used exposure concentrations higher than the current standard. (Linn et al. 1982) exposed 25 older adults (mean age = 62, range 46-70), all but one of whom chronic COPD symptoms and substantial airway obstruction (FEV1/FVC

mean = 50%, range 32 – 66%) to 0.12 ppm O_3 or FA for one hour with alternating 15-minute periods of rest and mild exercise (mean V_E = 20 L/min during exercise). There were no O_3-associated changes in respiratory symptoms or pulmonary function. However, there was a slight but statistically significant difference in arterial oxygen saturation (SaO_2) measured by ear oximetry, comparing the mean change in SaO_2 on the O_3 versus the control days. The biological significance of this change is uncertain; on average the subjects showed mild oxygen desaturation in both sets of exposures, suggesting that interference with blood oxygenation could carry clinically meaningful implications. However, as noted, there were no significant changes in symptoms in this experiment. Moreover, in a subsequent study of 28 subjects (mean age 60, range 45 –68) with COPD (FEV1/FVC mean = 59%, range 36 – 75 (Linn et al. 1983) were unable to replicate the finding of decreased SaO_2 using a similar study protocol with higher concentrations of O_3 (0.18 and 0.25 ppm). Though this latter study group had somewhat less severe disease than in the earlier study by Linn et al. (1982), and the authors speculated that the subjects may have used a greater quantity of medication, nonetheless these subjects did receive markedly greater O_3 doses, weakening the evidence of an O_3-associated effect on SaO_2.

In a study involving 13 subjects (mean age 56, range 43-69) with previously diagnosed COPD (FEV1/FVC mean = 57.5%, range = 46-70%), Solic et al. (1982) reported no significant O_3-associated effects on lung function or symptoms after 2-hour exposures involving 7.5 minutes of exercise (V_E = 20-30 L/min) at the end of each half hour. Like Linn et al. (1982), these investigators also observed a statistically significant difference in SaO_2 between the O_3 and FA exposures. However, unlike the study by Linn et al., in which SaO_2 was measured before and at the end of each exposure, in this study, SaO_2 was measured only at the end of the exposure. Therefore, even though Solic et al. reported a small, but statistically significant exposure-related effect on SaO_2 (94.8 versus 95.3%, p=0.008), one cannot be sure that this difference can be entirely attributed to O_3 because the investigators did not obtain pre-exposure oxygen saturation data. Subsequently, the same investigators conducted a similar experiment involving 13 subjects (mean age 58, range 44 –67) with mild-to-moderate COPD (mean FEV1/FVC = 55.5%, range 37 –65) using an exposure concentration of 0.30 ppm O_3. As before, there were no significant changes in symptoms or lung function, but they did observe a similar exposure-related difference in SaO_2 (95.1% versus 94.2% at the end of the air and O_3 exposures, respectively; p = 0.07 [because of an equipment malfunction only 8 of 13 had acceptable SaO_2 data]) (Kehrl et al. 1985). These investigators believed that the observed differences in end-exposure SaO_2 was due to O_3 toxicity, and speculated that the difference in oxygen saturation was likely due to exaggeration of the pre-existing ventilation-perfusion mismatch characteristic of COPD.

Gong et al. (1997b) exposed nine men with severe COPD (FEV1/FVC mean = 36%, range = 20-73%) and 10 healthy age-matched controls to 0.24 ppm O_3 or FA for four hours, with light exercise (V_E = 20/L/min) alternating with rest every

15 minutes. The subjects were selected to have COPD that was predominately emphysemic rather than predominately chronic bronchitic, because the latter disease characteristic would be more likely to have result in mucus secretion in the airways that could absorb inhaled O_3 before it reaches the target tissue. Subjects were requested to withhold medications prior to the exposures, but some could not do so. Slightly more than half the subjects were exposed during conditions of high ambient O_3 levels. Under these experimental conditions, O_3 exposure resulted in a 19% FEV1 decline among COPD subjects, compared with 2% in healthy subjects (p=0.0002); however, because the COPD subjects also showed a decline of 11% during FA exposure, the overall between-group effect of O_3 was not statistically significant. There were no significant O_3-associated effects on FVC, SR_{aw}, airway reactivity, SaO_2, or respiratory symptoms, though the COPD subjects' total symptom scores were modestly greater than those of the healthy subjects. The COPD subjects had lower overall mean SaO_2 (93.4% vs. 96.4%), and O_3 did appear to reduce SaO_2 in both groups during the exposure period by about 1%; however, this difference had been reversed by the end of exposure. This experiment involved effective doses of O_3 that were substantially greater than in the previous studies of individuals with COPD, and demonstrated a clear relationship with decreased FEV1 and an equivocal one with SaO_2. However, given the small number of individuals, the lack of detail about the extent to which medications were withheld, and the observation that more than half of the exposures occurred during a high O_3 season, this study cannot be considered definitive.

In toto, these experiments involving individuals with COPD indicate that, even at concentrations substantially higher than the current standard, individuals with COPD are unlikely to experience marked respiratory effects. However, this generalization is subject to the caveats expressed in Section 11.4.1.3 about controlled exposure studies generally.

11.4.7 Subjects with Hypertension

In a small study of subjects with stable hypertension, (Gong et al. 1998) reported that 3-hour exposure to 0.30 ppm O_3, with intermittent 15-minute periods of rest and exercise, resulted in several O_3-associated changes in lung function and hemodynamics. In this study (ten subjects with hypertension and six healthy subjects), these investigators measured hemodynamic variables after catheterization of the right heart and a radial artery. Though other studies have examined some cardiovascular variables in healthy subjects and some with cardiovascular disease, this was the first study to involve direct measurements using such invasive procedures. The subjects were catheterized in a hospital setting and exposed to FA on day one of the experiment, and to O_3 on day two. There were no significant O_3-associated effects on numerous indicators of cardiovascular function, including blood pressure, ECG, serum enzymes (creatine phosphokinase, lactate dehydrogenase [LDH-1], and troponin T), blood hormones (norepinephrine and atrial natriuretic factor), vascular resistance (pulmonary and systemic), pulmonary artery pressure, ventricular function, arterial oxygen saturation (SaO_2), or cardiac index. However, there were

statistically significant O_3-associated increases in heart rate and the rate-pressure product (product of heart rate x systolic blood pressure), and decreases in blood oxygenation, as measured by the alveolar-arterial oxygen difference (AaP_{O2}) and plasma epinephrine. In addition, the subjects experienced approximately 6% O_3-related decrements in both FEV1 and FVC. All O_3-associated changes did not differ between the hypertensive and healthy patients. The increased heart rate and AaP_{O2} suggest that, at least at this relatively high effective dose, O_3 exposure may adversely affect pulmonary gas exchange, which theoretically could result in acute arterial hypoxemia for individuals with compromised oxygenating capacity. In these subjects, who had normal baseline oxygenation, no clinically meaningful untoward effects occurred. Gong et al. (1998) cite several earlier studies involving healthy subjects with inconsistent results regarding changes in AaP_{O2} (Islam et al. 1979; Linn et al. 1979); this study cannot resolve whether such changes are likely to occur in patients with cardiovascular disease at lower O_3 exposure levels.

Table 11-12: Controlled Studies of Subjects with Pulmonary or Cardiovascular

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.10 and 0.20	6 hrs at 0.10 and 3hrs at 0.20 (as well as in combination with NO_2) exercise (10/40 minutes, V rate 32L/min) followed with challenge dust mite allergen	11 (9 M, 2 F, age 22-41) mild atopic asthmatics (10 at 0.20 ppm)	6 hrs at 0.10 ppm produced no significant effect on airway responsiveness to allergen. 3 hrs at 0.20 ppm significantly decreased the allergen dose required to decrease FEV1 by 20%.	Only examined immediate response. Limited statistical power. May indicate O_3 concentration more important than duration of exposure.	Jenkins et al. 1999
0.12	1 hr, resting	Atopic asthmatics (n=10); healthy subjects (n=10)	No consistent functional changes. No differences between exposure groups.	Small sample size. Asthmatics studied in afternoon allowed to take morning medications.	Koenig et al. 1985
0.12	1 hr, alternating 15 min. moderate exercise (four to five fold increase in minute ventilation) and rest	12 asthmatic (9 M, 3 F) and 12 healthy (4 M, 8 F) adolescent subjects	Demonstrated a significant decrease for maximal flow at 50% of FVC, No significant decrease noted for FEV1, FVC, peak flow, total resistance, or maximal flow at 75% FVC.	Authors suggest finding may be related to multiple comparisons	Koenig et al. 1988a Koenig et al. 1988b
0.12	One hr resting exposure to FA or 0.12 ppm O_3 followed by challenge w/ allergen diluent or by allergen	7 adults w/ mild asthma (4 M, 3 F, ages 21- 64)	O_3+allergen exposure significantly reduced the amount of allergen required to elicit a given PC15. Otherwise no difference in lung function or symptoms attributable to O_3 exposure.	Results may be due in part to consistent air +allergen exposure preceding O_3 + allergen exposure in 6/7 subjects. Exposures single-blinded only.	Molfino et al. 1991

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.12	One hr resting exposure to FA or 0.12 ppm O_3 followed by challenge w/ grass or ragweed pollen	15 adults w/ mild asthma (5 F, 10 M, age 18- 35 yrs.)	No significant difference between O_3 and FA exposures with respect to lung function, symptoms, or response to allergen challenge	Strong experimental design - double-blind, randomized exposure protocol.	Ball et al. 1996
0.12	Separate days (3 weeks apart) exposed to FA or O_3, 1 hr at rest followed by allergen challenge to determine concentration leading to 15% fall in FEV1	15 adults with mild asthma (9 M, 6 F) 18 to 49 yrs. (mean 32.5)	No significant effect of pre-exposure to 0.12 ppm O_3 on airway responsiveness to allergen challenge. No significant changes in spirometry after O_3 exposure at rest.	No patients on anti-inflammatory medications. Bronchodilators withheld at least 6 hrs. Single blinded randomized. Tighter control of exposure than Molfino 1991.	Hanania et al. 1998
0.12 and 0.24	90 minutes alternating 15 min. exercise (23 L/min) and rest. Nasal lavage 10 min., 6 hr, and 24 hr post exposure.	10 atopic asthmatic (18-41 yr.) 8 non-asthmatic adults (18-35 yr.)	At 0.24 ppm asthmatics demonstrated significant increases in epithelial cells and PMNs at 10 min. and PMNs at 24 hr. At 0.12 ppm no significant changes were observed among asthmatics.	No significant changes were noted at either dosage among non-asthmatics.	McBride et al. 1994
0.12 and 0.18	30 minutes at rest	10 healthy and 10 asthmatic adolescents	Significant increase in total respiratory resistance in both groups at 0.18 ppm. No significant effect on FEV1, FVC, peak flow, or maximal flow at 50 or 75% FVC.		Koenig et al. 1987

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

0.125 and 0.25	Single exposures to FA, 0.125 O_3, and 0.25 O_3, and 4 consecutive days to 0.125. 3 hr, w/ alternating 15-min intervals of exercise and rest. V_E=8.4 L/min (rest); 28.6 L/min (exercise)	11 asthmatics, 22 w/ allergic rhinitis	Significant decrease in FEV1, but not FVC after 0.25 ppm O_3 only. No changes in methacholine airway responsiveness for any O_3 dose. Increased numbers of cellular inflammatory indicators in induced sputum for 4x0.125 ppm O_3. No significant changes in early- or late-phase lung function responses to post- O_3 allergen challenge.		Holz et al. 2002
mean 0.156	Ambient air (mean O_3 = 0.156 ppm) , 1 hr, V_E= 38 L/min (mean)	Asthma (n=50) Healthy (n=48)	PFT changes not associated with O_3 levels. Asthmatics had greater symptom scores. Variation in FEV1 increased with increasing O_3.	No discussion of asthmatics' medication status.	Avol et al. 1998

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.16	7.6 hr, IE (50 min light exercise/hr) (13 L/min/m^2) Allergen exposure 18 hr post O_3	Mild atopic asthmatics, house dust mite sensitive, (5 F, 4 M), 20-35 yrs	Provocative dose of dust mite allergen to elicit 20% decrement in FEV1 significantly decreased after O_3 exposure.	7 of 9 patients. required less allergen after O_3. Mean dose shift 0.58 attributable to O_3 in doubling conc.	Kehrl et al. 1999
0.16	7.6 hrs. Alternating 50 min. exercise (V_E= 13L/m^2) 10 min. rest. Bronchoscopy performed 18 hrs after air and O_3 exposure	8 asthmatics sensitive to dust mite	Mean PMN percentage tripled between air and O_3 (2.5 and 8.6) and eosinophils more than doubled (1.8 and 3.6) Significantly higher counts noted in the initial lavage fluid (ILF) PMNs (2.8 and 11.5) and eosinophils (2.9 and 17.8)	Authors suggest that increase in cells in ILF indicate response of proximal airways (portion with eosinophilic inflammation in asthmatics)	Peden et al. 1997
0.16	7.6 hr IE, alternating 50 minutes light exercise (V_E= 14.2& 15.3 l/min/m_2 asthmatics and non-asthmatics) and 10 min rest	17 atopic asthmatic and 13 healthy adults 18 to 35 yrs.	Decrements in FEV_1 and FEV1/FVC significantly greater in asthmatics. 9 of 17 asthmatics experienced wheezing with O_3 while only 1 did with air. 6 asthmatics required bronchodilator treatment. No difference noted in FVC.	Some asthmatics treated during exposure for symptoms.	Horstman et al. 1995
0.20	2 hr with 7.5 min. exercise (V_E= 20-30 L/min) at end of each half hr	13 white males with mild to moderate COPD (age 43-69 yrs.; mean 56)	Reported no O_3 associated changes in lung function or symptoms. Did note a statistically significant decrease in S_aO_2 between O_3 and FA.	S_aO_2 measured at end of exposure only.	Solic et al. 1982

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.20	4 hr, IE, (50 min ex/hr), (V_E=25 L/min/m^2 BSA) Results compared with prior studies of 81 non-asthmatics	Physician-diagnosed mild asthma, no medications before exposure, (6F, 12 M), 18-36 yrs	Decreased FEV1 and FVC after O_3 exposure. Increased SR_{aw}, lower respiratory symptoms, % neutrophils, total protein, LDH, fibronectin, IL-8, GM-CSF, and MPO in BAL and bronchial washing after O_3 exposure. Correlation between pre-exposure methacholine challenge and O_3-induced SR_{aw} increase.	When compared with changes in nonasthmatics, only inflammatory markers in BAL (% PMNs, total protein) showed significant differences.	Scannell et al. 1996
0.20	Day 1 – purified air; Day 2 – 0.08 O_3 for 3 minutes (odor sham); Day 3: 0.20 ppm O_3 2 hr; alternating 15 minute periods of rest and light exercise	Asthmatics (n=22)	No significant O_3-associated PFT changes. Significant O_3-associated small changes in blood measurements (increased RBC fragility and decreased hemoglobin.)	No control group. Order of exposure not randomized. Subjects allowed to take oral, but not inhaled, medication on test days.	Linn et al. 1978
0.20	2 hr randomized FA and O_3 >3wks apart. Alternating mod. Exercise (V_E=20L/min/m^2) rest 15 min. Bronchoscopy 6 hr after exposure	15 healthy non-atopics (6 M, 9 F; 19-31 yr.) and 15 atopic asthmatics (9 M, 6 F; 21-48 yr.)	Biopsies examined for cytokines associated with inflammation. Asthmatics showed significant increases in IL-5, GM-CSF, ENA-78, and borderline increase in IL-8. No significant between group results for IL-6, IL-10, TNF-a, and fractalkine)	Cytokines found elevated promote inflammation and attract PMNs. Study noted baseline differences in addition to accentuated response.	Bosson et al. 2003

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.20	6 hr, alternating periods of 30 minutes of rest and exercise (5 L/min/L vital capacity)	5 adults w/ mild asthma; 5 healthy subjects (age range for all 18 – 45)	No significant changes in FEV1, FVC, FEV1/FVC, or FEF25-75 % in either group. Minor changes in O_3-associated symptoms, slightly greater in healthy than asthmatic subjects. Significant increase in "urge to cough" among asthmatics. Significant O_3–associated effects on inflammatory markers in BALF (% PMNs, PMNs/mL, IL-6 and IL-8).	Small study. Though pre- and post-exposure SR_{aw} was measured, results were not reported. Inflammatory changes occurred even in the absence of significant effects on lung function.	Basha et al. 1994
0.20	2 hr, IE V_E=20 L/min/m^2 BSA 50 min. exercise alternating with 10 minutes rest	15 healthy subjects (mean age 24, range 19-31); 15 mild, atopic asthmatics (mean age 29, range 21-48)	Significant decrements in FEV1 and FVC in healthy subjects, and in FVC in asthmatics. No between-group differences in magnitude of lung function changes.		Stenfors et al. 2002

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.22	2 hr; alternating 15 minute periods of rest and light exercise (twice resting level during exercise). Ambient air (mean O_3 = 0.22 ppm). Purified air >3 wks post- O_3 exposure.	Asthmatics (n=30); Healthy (n=34)	Significant increase in symptoms for normals, but not asthmatics. No significant difference in PFT changes between normal and asthmatics.	No discussion of asthmatics' medication status. Order of exposure not randomized. Many "healthy" subjects had history of allergy, which may have affected their responses to O_3..	Linn et al. 1980
0.22	2 hr resting	17 asthmatics	Slightly lower FEV1 (post- versus pre-exposure) with O_3 compared with filtered air exposure.	Most subjects on medication. No nonasthmatic control group.	Silverman 1979
0.24	4 hrs light exercise (V_E=20L/min) alternating 15 min with rest	9 men with severe COPD (59-71 yr.) 10 age matched control men	FEV1 declined 19 % in COPD patients. vs. 2% in healthy subjects. 11% decline with FA. SaO_2 reduced by 1% during exposure but resolved by end of exposure.	Half of individuals exposed during high ambient conditions. Some unable to withhold medication.	Gong et al. 1997a
0.25	3 hrs, 6 cycles of 10 minutes rest/ 15 min. exercise/ 5 min. PFTs (minute ventilation 29.7L/min ± 5.5L)	24 adults with mild atopic asthma, 12 with allergic rhinitis (no asthma), and 10 healthy subjects mean age 26 (21-35yrs)	Marked, statistically significant increase in airway responsiveness to subsequent allergen challenge. Compared to FA, O_3 exposure required 3.3 times less allergen to attain a 20% fall in FEV1.	FA and O_3 exposure separated > 4 weeks. No anti-inflammatory medications for at least 3 mo prior.	Jorres et al. 1996

Table 11-12 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

O_3 Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.27	Allergen challenge 24 hrs. before 2 hr exposure. Six hrs later induced sputum collected.	12 adults with mild atopic asthma.	Percentage of eosinophils but not neutrophils in sputum was significantly higher after exposure to O_3 compared to air.	O_3 exposure after allergen augments allergen induced influx of eosinophils into airways	Vagaggini et al. 2002
0.30	Rt. heart and radial artery catheterization FA exp. one day, O_3 next x 3 hrs. 15 min. ex. (V_E=30-40 L/min) alternating with 15 min. rest	10 non-medicated hypertensive males, 42-78 yrs. (mean 53, SD 10) 6 control males, 40 – 49 yrs. (mean 44, SD 3)	Mean pre-existing significant changes in rate-pressure product (1,353 beats/min/mm Hg), and heart rate (8 beats/min), FVC and FEV1 (-6%) and increased P_aO_2 with O_3. No significant differences between hypertensive and normotensive groups.		Gong et al. 1998
0.40	2 hr alternating 15 min. periods rest and activity (V_E 30L/min/m^2)	9 asthmatics, 9 normal (5 F, 4 M each group) 18-35 yr	Statistically significantly greater decrease in FEV1, FEV1%, and FEV_{25-75} in asthmatic subjects. No difference noted between groups for FVC (both decreased). Decrements increased with increasing length of exposure. Asthmatics SR_{aw} increased more than controls	Asthmatics discontinued medication 12 hrs prior to exposure	Kreit et al. 1989
0.40	2 separate 2 hr exposures FA and O_3 followed by unilateral allergen challenge D. farinae increasing dosage 10 to 10,000 AU.	11 asthmatics (m/f) sensitive to D. farinae, age 18-35	O_3 had intrinsic effect of influx of PMNs and Eosinophils independent of allergy. Less allergen required to produce nasal symptoms after O_3 exposure.	Randomized, double blinded. Saline applied to contralateral side.	Peden et al. 1995

11.4.8 Influence of Gender, Age, Racial and Environmental Factors

11.4.8.1 Gender Differences

The question as to whether there is a difference in the responsiveness of males and females to O_3 has been debated for some time. Several papers have suggested males and females may differ in their responsiveness to O_3 (e.g., Horvath et al. 1979; Lategola et al. 1980; Gliner et al. 1983; Gibbons and Adams 1984; Lauritzen and Adams 1985). However, (Drechsler-Parks et al. 1989) reported that young and older females had similar responses to 0.45 ppm O_3 exposure as males in the same age ranges following two hour exposures to 0.45 ppm O_3 with intermittent exercise.

The first study to formally examine the comparative responsiveness of men and women to O_3 was published by Horvath et al. (1986). The study compared the responses of healthy nonsmoking female subjects exposed to 0.48 ppm O_3 with a group of male subjects who had previously completed the same two-hour intermittent exercise protocol in the same laboratory (Drechsler-Parks et al. 1984). Pulmonary function and symptom responses were similar for the two groups, although Horvath et al. (1986) noted that the female subjects had inhaled about 22% less O_3 than the male subjects. This led to the suggestion that females might be more sensitive than males. However, when the O_3 dose was normalized to body surface area or to FVC, the female subjects inhaled slightly higher relative doses of O_3 than the males, pointing to the opposite conclusion.

Subsequently, several investigations further examined the issue of the comparative responses of men and women to O_3. Adams et al. (1987) compared the responses of young men and women exposed to 0.3 ppm O_3 while they completed a one-hour protocol, during which they exercised continuously at a strenuous level ($V_E \approx 70$ L/min for males and ≈ 50 L/min for females). These workloads represented the same relative workload (about 62% of maximal) for both genders. Both groups had similar pulmonary function and SR_{aw} responses. However, because the female subjects inhaled a substantially smaller absolute dose of O_3 due to the lower exercise V_E, yet had similar responses, the authors concluded that females are more responsive to O_3 than males. Again, however, if the inhaled O_3 dose for the female subjects is normalized on the basis of BSA, the females inhaled a greater relative dose of O_3 than the males. If the dose is normalized on the basis of FVC, the relative doses for the males and females are similar. Seal et al. (1993), Drechsler-Parks (1987b), Reisenauer et al. (1988) and Drechsler-Parks et al. (1989) reported similar results, with varying conclusions depending on whether the comparison was based on the absolute or relative dose of O_3 inhaled.

Messineo and Adams (1990) hypothesized that the possibly greater responsiveness reported for female subjects was related to the generally smaller lung size of women compared to men. They tested this hypothesis by exposing two groups of young adult women to FA, 0.18 and 0.3 ppm O_3 for one hour while they performed continuous exercise at a mean V_E of about 47 L/min. One group consisted of women with a group mean FVC of 3.74 L, while the other group had

a group mean FVC of 5.11 L. The mean percentage changes in pulmonary function were similar for the two groups, although the changes observed were approximately twice as large as those of a group of young adult males who had earlier completed a similar protocol in the same laboratory. The results suggest that differential responsiveness to O_3 between males and females is not likely related to differences in lung size.

It is unresolved at this time whether there is a difference in the responsiveness of males and females to O_3 exposure. The conclusion reached with available data varies depending on whether or how the inhaled doses of O_3 are normalized, and there is no basis to recommend one approach over another.

11.4.8.2 Hormonal Influences

Progesterone is known to stimulate ventilation, and its systemic concentration is higher during the luteal phase of the menstrual cycle. Because the high progesterone concentration stimulates ventilation during the luteal phase, females inhale more air during that phase of the cycle, leading to the hypothesis that females might be more O_3-responsive during the luteal phase of the menstrual cycle. In addition, progesterone also inhibits prostaglandin production by the uterine endometrium in a pattern parallel to progesterone concentration. Since prostaglandin production in the lungs increases with O_3 inhalation, contributing to airway inflammation with acute O_3 exposure, it has also been hypothesized that the high progesterone concentration during the luteal phase of the menstrual cycle might inhibit prostaglandin production by the lung tissues in response to O_3 inhalation, thereby reducing responsiveness to O_3 during the luteal phase.

Several researchers have investigated whether this increased ventilation might lead to differences in responsiveness to O_3 during different phases of the menstrual cycle. However, two of the three available studies (Fox et al. 1993; Weinmann et al. 1995) were performed with small groups of subjects, and reached opposite conclusions. Seal et al. (1996) studied 150 women, and concluded that menstrual phase had no measurable effect on responses to O_3.

Table 11-13: Gender and Hormonal Differences

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12, 0.18, 0.24, 0.30, 0.40	2.33 hr, (V_E=25 L/min/m^2 BSA), one exposure/subject	Healthy, nonsmokers (30-33 M and 30-33 F per concentration group; total N=372), 18-35 yrs, black and white	Decrements in FEV1, and increases in SR_{aw} and cough were correlated with O_3 concentration. No significant differences between responses of males and females.	Seal et al. 1993
0.20, 0.30	1 hr (mouthpiece) IE (20 min), (V_E=28 L/min for men; 23 L/min for women)	Healthy nonsmokers, (9 M, 10 F), 55-74 yrs	No change in any spirometry measure. Women had 13% increase in R_T after the 0.30 ppm O_3 exposure.	Reisenauer et al. 1988
0.12, 0.24, 0.30, 0.40	2.3 hr, (V_E=20 L/min/m^2 BSA), one exposure/subject	Healthy, nonsmokers (48 WF, 55 BF), 18-35 yrs, black and white	Although the menstrual cycle phase/race interaction was statistically significant for FEV1, there was no menstrual phase effect when blacks and whites were analyzed separately. No menstrual phase effects for SR_{aw} or cough score.	Seal et al. 1996
0.18	1 hr (mouthpiece), CE, (V_E=47 L/min)	Healthy nonsmokers, (14F), 20-24 yrs	Significant reduction in FVC and FEV1 following O_3 exposure, compared to FA. Lung size had no effect on O_3-induced percentage decrements in FVC or FEV1.	Messineo and Adams 1990
0.30	1 hr (mouthpiece), CE, (V_E=70L/min for men; 50 L/min for women)	Healthy nonsmokers, (20 M) 18-30 yrs, (20 F) 19-25 yrs	Significant decrements in FVC, FEV1 and FEF25-75% following O_3 exposure. No significant differences between men and women for spirometry or SR_{aw}.	Adams et al. 1987

Table 11-13 (cont.): Gender and Hormonal Differences

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30	1 hr, CE, (V_E=50 L/min), mouthpiece	Healthy nonsmokers with regular menstrual cycles, (9 F), 20-34 yrs	FEV1 decreased 13.1% during the mid-luteal phase and 18.1%during the follicular phase. Decrement in FEF25-75% 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	1.25 hr, IE, (2 30 min exercise periods), (V_E= 40 L/min)	Healthy, screened to all be O_3 responders, (19 F), mean age = 22 yrs	FVC and FIVC both decreased 13%, FEV1 decreased 20%. O_3 exposure increased airway responsiveness to methacholine. Small effects in both inspired and expired spirometry persisted past 18 hr post-O_3 exposure. Chemoreceptors were not activated, but ventilatory drive was accelerated.	Folinsbee and Hazucha 2000
0.35	130 min	Healthy nonsmokers with regular menstrual cycles, (9 F) 18-35 yrs	Changes in FVC, FEV1 , FEF25-75%, $V_{max50\%}$ and $V_{max25\%}$ were similar during both the follicular and luteal phases.	Weinmann et al. 1995
0.45	2 hr, IE, (mean V_E=28.5 L/min for men, and 26.1 L/min for women)	Healthy nonsmokers, (10 M) 60-89 yrs; (6 F) 64-71 yrs	Mean decrement in FEV1 = 5.7%. Decrements in FVC and FEV1 were the only pulmonary functions significantly altered by O_3 exposure. No significant differences between responses of men and women.	Bedi et al. 1989
0.45	2 hr, IE, (V_E=27.9 L/min for men and 25.4 L/min for women)	Healthy nonsmokers, (8 M), 51-69 yrs; (8 F), 56-76 yrs	Range of responses in FEV1, 0 to –12% (mean decrement was 5.6%). No significant difference in responses of men and women, although women tended to have somewhat larger effects.	Drechsler-Parks 1987 Drechsler-Parks et al. 1987a
0.48	2 hr, IE, (V_E=25 L/min)	Healthy nonsmokers, (10 F), 19-36 yrs	Mean decrement in FEV1 = 22.4%. Significant decrements in all spirometry measurements. Results not significantly different from a similar study on males (Drechsler-Parks et al., 1984)	Drechsler-Parks et al. 1984 Horvath et al. 1986

11.4.8.3 Age Differences on Pulmonary Function Changes with Ozone

11.4.8.3.1 Children

There is evidence that O_3 responsiveness, measured by pulmonary function and symptoms, varies with age. There are few controlled, clinical studies on subjects under age 18, although field and epidemiological studies (see Sections 12.1; 12.2) have suggested that children may be more responsive to ambient air pollution than young adults.

McDonnell et al. (1985a) reported the first controlled exposure study on children. The study included 23 boys between 8 and 11 years of age, and compared their responses to those of young adult males who had earlier completed the same protocol. The subjects completed a two-hour intermittent exercise protocol while exposed to 0.00 or 0.12 ppm O_3. The boys had similar percentage decrements in FEV1 as the young adult males, although the absolute O_3 doses inhaled by the boys were considerably lower. However, normalizing dose to BSA, both groups inhaled similar relative doses of O_3. Assuming that this was an appropriate normalization technique, the boys and young adult males were similarly responsive. However, the children reported few symptoms, while the adults reported a small, but statistically significant increase in cough following O_3 exposure.

Avol et al. (1987) studied a group of 33 boys and 33 girls with mean age of 9.4 years who were exposed in an environmental chamber to purified air or outdoor ambient air that was drawn into the chamber. It should be noted that the exposure included the full range of air pollutants present in the outdoor air mix on the exposure days. The mean O_3 concentration averaged 0.113 ppm on ambient air exposure days and 0.003 ppm on purified air days. These children had similar declines in FVC and FEV1 following both exposures. The results were similar to other studies using the same protocol that were performed by the same investigators, but with adolescent and adult subjects (Avol et al. 1985; Avol et al. 1984). It should also be noted that these studies were performed during the high O_3 season with subjects who lived in a high O_3 region (Los Angeles basin). Given the well-known attenuation of pulmonary function and symptoms responses to O_3 with repeated exposures (see Section 11.4.9.7), it is not surprising that these subjects demonstrated little response consequent to these exposures. In addition, the authors noted that the children had difficulty performing consistent, reproducible pulmonary function tests, a factor that could also have impacted the results.

Koenig et al. have reported several studies (Koenig et al. 1987; Koenig et al. 1988a,b) on the pulmonary function responses of healthy adolescents exposed to filtered air, 0.12 and 0.18 ppm O_3. Tests of pulmonary function showed no alterations following any of the exposures. However, the Koenig et al. (1987, 1988a,b) study protocols were only 40 min and 60 min, respectively, in duration. Furthermore, they included only 10 or 30 min, respectively, of light exercise, resulting in inhaled O_3 doses that were considerably smaller than the McDonnell

et al. (1985) study. Consequently, it is not surprising that Koenig et al. (1987; 1988a,b) reported smaller pulmonary function responses.

The few data available do not identify children or adolescents as being either more or less responsive than young adults who have undergone similar exposure protocols. However, even though the pulmonary function responses of children and adolescents appear to be in the same range as adults, the lack of symptoms reported by McDonnell et al. (1985a) suggests a lower level of somatic awareness or impaired nociception among children, which might result in their failure to curtail exposure in real-life situations.

The effects of O_3 on children have also been the subject of field studies (see Section 12.1), and long-term epidemiologic studies (see Sections 12.3.5; 12.3.6). These sections discuss acute and long-term effects attributed to O_3 exposure in pediatric populations.

11.4.8.3.2 Middle-Aged and Older Subjects

Studies by Adams et al. (1981) and Folinsbee et al. (1985) included a few middle-aged individuals, and raised the question as to whether there might be a reduction in responsiveness with advancing age. This was subsequently investigated and confirmed in several other studies (Bedi et al. 1988; Drechsler-Parks et al. 1987a; Drechsler-Parks et al. 1989; Bedi et al. 1988; Seal et al. 1993) using protocols similar to that of Folinsbee et al. (1985).

Horvath et al. (1991) examined middle-aged subjects (30-43 yrs) exposed to FA and 0.08 ppm O_3 on two consecutive days for 6.5 hours, extending the data base on multi-hour exposures to an age group likely to be well represented among outdoor workers, and among people who engage in prolonged active recreation outdoors. The subjects also underwent a one-hour continuous exercise challenge to 0.35 ppm O_3 to characterize responsiveness. The mean reduction in FEV1 with the challenge test was –9.6% (range: 1-30%). The mean change in FEV1 following the first 6.5 hour O_3 exposure was –2.2%, only 2 of 11 subjects having a decrement greater than 2%. The changes in FEV1 following the second consecutive O_3 exposure were within the variability of the data. Seven subjects reported at least one symptom of respiratory irritation (cough, chest tightness, or pain on deep inhalation) by the end of the first O_3 exposure, but only 2 subjects reported symptoms after the second 6.5 hour O_3 exposure. None of the subjects reported symptoms after the FA exposure. These results are in contrast to those from studies on young adults who completed the same protocol. (See Section 11.4.3). The greater decrement in FEV1 after the 0.35 ppm O_3 challenge test highlights the importance of concentration compared to ventilation rate and exposure duration.

A modeling study by McDonnell et al. (1993) involving normal, healthy subjects between 18 and 32 years of age suggests that responsiveness to O_3 (measured by changes in pulmonary function and symptoms) begins to diminish by age 30, and that the most responsive individuals are likely to be less than 25 years of

age. Similar results were reported by Seal et al. (1996) based on modeling the responses of 371 healthy adults between 18 and 35 years of age.

11.4.8.3.3 Summary

Data addressing the issue of age-related responsiveness to O_3 are limited to studies that investigated effects on pulmonary function and symptoms. Overall, these data suggest that children experience similar percentage pulmonary function responses to O_3 exposure as young adults who undergo similar protocols, although children tend to report fewer symptoms. In contrast, after about age 30 pulmonary function changes due to O_3 exposure become progressively smaller. Middle-aged and older adults tend to report few symptoms, even with exposure to O_3 concentrations in excess of 0.4 ppm, while young adults can be quite symptomatic with similar exposures. There is no information available on other endpoints, such as airway inflammation, other than for young adults.

Table 11-14: Influence of Age on Pulmonary Function Responses to Ozone Exposure

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.08 0.30	6.6 hr IE (50 min ex/hr; 30 min lunch), (V_E=35-38 l/min) 8 of the subjects completed O_3 exposures on 2 consecutive days 1 hr CE challenge test (V_E=35-38 l/min)	Healthy nonsmokers (6 M, 5 F), 30-43 yrs	Mean decrement in FEV1 with the challenge exposure to 0.35 ppm O_3 was 9.6% (range, 1-30%). The mean FEV1 decrement after the first exposure to 0.08 ppm O_3 was 2.2%, with only 3 of 11 subjects having a decrement greater than 2% on the first O_3 day. Changes in FEV1 on the second O_3 day were within the variability of the measurements. Seven subjects reported at least one symptom (cough, chest tightness, pain on deep breath) by the end of the first O_3 exposure, but only 2 reported a symptom after the second O_3 exposure. There were no changes in lung function or symptoms reported after the FA exposure.	Horvath et al. 1991
0.12	2.5 hr, IE (15 min rest/15 min ex first 2 hr), (V_E=35 l/min/m^2)	Healthy, Caucasian nonsmokers, nonasthmatic (23 M), 8-11 yrs	Significant decrement in FEV1 that was not fully recovered by16-20 hr post O_3 exposure. No change in frequency or severity of cough, pain on deep breath or shortness of breath with O_3 exposure compared to FA exposure.	McDonnell et al. 1985a
0.113 + other ambient pollutants	1 hr CE, V_E~22 L/min	Nonsmokers, (33 M, 33 F), mean age 9.4 yrs	No differences between responses of boys and girls. Similar decrements (<5% on average) following both purified air and ambient air (O_3 at average of 0.11 ppm) exposures.	Avol et al. 1987
0.12	1 hr (mouthpiece) IE, V_E=4 to 5 times resting	Healthy nonsmokers, (5 M, 7 F), 12-17 yrs	No significant changes in any pulmonary function.	Koenig et al. 1988a Koenig et al. 1988b

Table 11-14 (cont.): Influence of Age on Pulmonary Function Changes With Ozone Exposure

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 0.18	40 min (mouthpiece) IE, 10 min exercise V_E=32.6 L/min 40 min (mouthpiece) IE, 10 min exercise V_E=41.3 L/min	Healthy nonsmokers (3 M, 7 F), 14-19 yrs	No significant change in FEV1, increased R_T with exposure to 0.18 ppm O_3. Some subjects responded to a lower PD of methacholine after 0.18 ppm O_3 exposure, compared to baseline methacholine challenge.	Koenig et al. 1987
0.0, 0.12, 0.18, 0.24, 0.30	2.33 hr IE (alternating rest and exercise periods), V_E=4-5 times resting	Healthy nonsmokers, (221 M, 150 F), black and white, 18-35 yrs	Decreasing response to O_3 as age increased. The average estimated decline in FEV1 per year over the range of age 20 to age 32, averaged about 1.1%/year. No effect of menstrual phase on pulmonary function. SES may affect pulmonary function, but the association was not consistent.	Seal et al. 1996
0.18, 0.24, 0.30, 0.40	2.3 hr IE (V_E=20 L/min/m^2 BSA)	Healthy nonsmokers, (N=185 M & 187 F), 18-35 yrs	Older subjects had smaller changes in FEV1 than younger subjects. No age-related differences in SR_{aw} or cough score.	Seal et al. 1996
0.20, 0.30	1 hr (mouthpiece) 20 min exercise (V_E~28 L/min for males, and 23 ~L/min for females)	Healthy nonsmokers, (9 M, 10 F), 55-74 yrs	No change in any spirometry measure. Women had a 13% increase in R_T after exposure to 0.30 ppm O_3.	Reisenauer et al. 1988
0.24	4 hr IE, alternating 15 min exercise and rest (V_E = 20 L/min)	Healthy nonsmokers, (10 M), 60-69 yrs COPD, (9 M), 59-71 yrs	Healthy: Average decline in FEV1 < 2% (ns). Small (ns) decreases in both SR_{aw} and arterial SaO_2. COPD: FEV1 decreased 19%. Post-exposure SR_{aw} higher after exposure to FA and O_3. No change in SaO_2.	Gong et al. 1997b

Table 11-14 (cont.): Influence of Age on Pulmonary Function Changes With Ozone Exposure

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.45	2 hr, IE (20 min rest/20 min ex), (V_E=25 l/min)	Healthy nonsmokers, 18-26 yrs (8 M/8 F), 51-76 yrs (8 M/8 F)	Responses of men and women were similar. Older subjects had smaller and fewer significant decrements in pulmonary function than the younger subjects. The mean decrement in FEV1 was 19.2% for the younger subjects, and 5.6% for the older subjects. Older subjects reported less cough, chest tightness and shortness of breath than younger subjects.	Drechsler-Parks et al. 1989
0.45	2 hr IE (mean V_E=26 L/min)	Healthy nonsmokers, (8M, 8 F), 51-76 yrs	Mean decrement in FEV1=5.6 ± 1.3%; range = 0-12%	Drechsler-Parks et al. 1987a
0.45	2 hr IE, (mean V_E=28.6 L/min for men, 26.1 L/min for women)	Healthy nonsmokers, (10 M, 6 F), 60-89 yrs	Mean decrement in FEV1 = 5.7%; eight subjects had a 5% or greater difference between their responses to O_3 and FA, and the other had less than a 5% difference in their responses to the two exposures.	Bedi et al. 1989
0.45	2 hr IE (V_E=26 L/min)	Healthy nonsmokers, (8M, 8 F), 56-69 yrs	13 subjects had decrements in FEV1 after three separate exposures to 0.45 ppm O_3 within 5% of their mean response to the three exposures. The other three subjects did not have reproducible responses. Symptom reports did not correlate well with pulmonary function changes.	Bedi et al. 1988
0.45	1 hr CE (V_E=26 L/min) 2 hr IE (V_E=26 L/min)	Healthy nonsmokers, (7 M, 5 F), 60 to 79 yrs (all except one in their 60's)	Comparison of 1 hr CE protocol and 2 hr IE protocol indicated no difference between the changes in pulmonary function following the two protocols.	Drechsler-Parks et al. 1990

11.4.8.4 Ethnic and Racial Factors

Young white adults, particularly males, are the most frequently studied subjects in controlled studies. Lung size has been hypothesized as a factor possibly leading to gender differences in responsiveness to O_3 (see Section 11.4.8.1), and also as a possible basis for racial differences in responsiveness. African-Americans tend to have smaller lungs than Caucasians for a given standing or sitting height (Rossiter and Weill 1974), a factor related to differences in typical body proportions. Consequently, an equivalent inhaled volume of O_3 could result in a larger O_3 dose per unit of lung tissue in African-Americans compared to Caucasians, potentially inducing greater effects in African Americans. Only one study has compared the responses of Caucasian and African-American subjects (Seal et al. 1993). The primary statistical analysis found no basis for concluding that there were any differences in the responses of African-American and white men and women. Several exploratory analyses using alternate statistical methodologies were also conducted. However the results of these analyses are not wholly consistent by either race or gender, and there was not a clear dose-response relationship when the subjects were compared by race-gender-concentration group. A significant limitation to this study is that although the study included a large number of subjects (n=372), each subject completed only one of 24 possible experimental combinations (ethnicity {n=2} x gender {n=2} x O_3 concentration {n=6}). Consequently, it is possible that differences in the innate O_3 responsiveness of the 24 experimental groups (n=15-17 subjects per group) could at least partially account for the inconsistent dose-response relationships when the 24 experimental groups were compared. In contrast, the primary analysis considered four race-gender groups (n=92-94 per group). The considerably larger group sizes used in the primary analysis would tend to balance out effects related to individual differences in responsiveness.

There are insufficient data available to draw a conclusion as to whether there is a difference in the O_3 responsiveness of African-Americans and Caucasians. There are no data available on other ethnic or racial groups.

11.4.8.5 Environmental Factors

Most controlled air pollution exposure studies have been conducted at typical indoor temperatures and humidities (20º - 22º C, and 50% relative humidity). However, O_3 concentrations are typically highest on hot, sunny, dry days. Several investigators (Folinsbee et al. 1980; Folinsbee et al. 1977; Gibbons and Adams 1984; Gong et al. 1986; Gong et al. 1997a; Linn et al. 1982) have included heat stress in their exposure protocols to more closely simulate ambient conditions. These studies have used both two-hour intermittent exercise protocols (Folinsbee et al., 1977, 1980; Linn et al., 1982, 1988; Gong et al., 1997), and continuous heavy exercise protocols (Gibbons & Adams, 1984; Gong et al., 1986). Generally, these studies reported pulmonary function and respiratory symptoms after O_3 exposures under hot conditions similar to those recorded after similar O_3 exposure protocols completed at room temperature. Although, Folinsbee et al. (1977) found a non-significant trend toward larger decrements in pulmonary function following their most severe ambient condition

(40º C, 50% relative humidity), there is no convincing evidence that ambient temperature or humidity alters pulmonary function or symptoms responses to O_3 exposure.

11.4.8.6 Smoking

A few early controlled O_3 exposure studies included smokers, but the investigators typically made no attempt to evaluate whether the smokers' responses differed from those of nonsmokers. An exception is Kerr et al. (1975), who reported that among a group of 10 smokers who completed six-hour exposures (including two 15-min periods of light exercise) to 0.5 ppm O_3 (KI) one subject reported cough and four reported chest tightness. This was in contrast to 10 non-smokers, seven of whom reported cough and nine of whom reported chest discomfort. As a group, smokers exhibited no significant pulmonary function changes following exposure, while the nonsmokers had significant reductions in FVC and SG_{aw}.

Frampton et al. (1997a,b) evaluated the comparative responsiveness of 34 smokers and 56 nonsmokers who completed four-hour intermittent exercise protocols while exposed to 0.22 ppm O_3. Post-exposure, smokers had considerably smaller decrements in FEV1 and fewer symptoms than nonsmokers. Sixteen of 56 never-smokers had a decrease in FEV1 greater than 15%, compared to 4 of 34 smokers. The investigators performed a multiple logistic regression analysis on various factors thought to be possibly associated with O_3 responsiveness. The results indicated that age, gender and methacholine responsiveness were not predictive of responder status, although smoking history (pack-yr) was significantly associated with decreased O_3 responsiveness. A sub-group of 14 smokers and 25 nonsmokers exposed once to air and twice to O_3 demonstrated the consistent responses in both groups.

Torres et al. (1997) divided smokers and nonsmokers into three groups based on smoking status and response to an O_3 challenge test. The subjects completed four-hour intermittent exercise exposures, once to FA and twice to 0.22 ppm O_3. Bronchoalveolar and nasal lavages were performed immediately after one O_3 exposure, and 18 hour after the other. Half of the subjects underwent bronchoalveolar and nasal lavage immediately after the FA exposure, and the other half at 18 hour after the exposure. Analysis of cells obtained at nasal lavage showed a high degree of variability among subjects, and no statistical difference between subject groups. There was no correlation between the cellular measurements from nasal lavage and bronchoalveolar lavage. Analysis of the bronchoalveolar lavage data indicated that smokers had considerably more inflammatory cells in BALF, even with FA exposure, than did nonsmokers, demonstrating ongoing lung inflammation even without pollutant exposure. All subject groups showed a decrease in cells when BAL was performed immediately after exposure, and an increase when BAL was performed at 18 hour after O_3 exposure. IL-6 and IL-8 were highest immediately after O_3 exposure, and the concentrations of these two cytokines were correlated with the late increase in PMN. The inflammation, as measured in fluid recovered from both the bronchial and alveolar regions of the lungs, was primarily neutrophilic in

nature, although there were small increases in other immune cell types. The investigators also performed logistic regression analysis to attempt to isolate predictors of O_3 responsiveness. The model included age, gender, provocative dose inducing a 50% increase in SR_{aw} (PD50), allergies, PMN's after FA exposure, and subject group. Age was inversely related to responsiveness. None of the other characteristics considered were associated with O_3 responsiveness. Further, the results indicate that the inflammatory time course in smokers is similar to that of nonsmokers, and that O_3-associated inflammation can be present in smokers and nonsmokers in the absence of symptoms or changes in pulmonary function.

Emmons and Foster (1991) examined the influence of smoking on O_3 responsiveness in a group of smokers who were exposed to O_3 before and after they completed a smoking cessation treatment program. The results suggest that active smoking blunts pulmonary function responses to O_3, in that no changes in pulmonary function were found. Cessation of smoking for 6 months led to significant improvement in baseline FEF25-75%, but little change in baseline FVC. When the subjects were re-exposed to 0.4 ppm O_3 six months after they successfully quitting smoking, the group mean decrement in FEF25-75% was statistically significant, suggesting the reemergence of O_3 responsiveness.

The available data suggest that active smoking blunts responsiveness to O_3, in terms of pulmonary function and symptoms, while smokers and nonsmokers develop similar degrees of inflammation.

Table 11-15: Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.0, 0.12, 0.24	2.17 hr, IE (alternating 10 min periods of exercise and rest); (V_E=36-39 L/min)	Healthy nonsmokers, 24-32 yrs	Significant decrease of 8% (mean) in FEV1 with ambient temperature of 22ºC and 6.5% (mean) at 30ºC, still significantly decreased at 19 hr post-O_3 exposure. SG_{aw} significantly decreased at 30ºC, but not at 22ºC. BHR at 19 hr post-O_3 exposure remained significantly increased at both temperatures.	Foster et al. 2000
0.12 0.20	1 hr, CE (mean V_E=89 l/min) followed by an incremental maximal exercise test to exhaustion	Competitive, endurance cyclists (15 M, 2 F), 19-30 yrs	Exposure to 0.12 ppm O_3 did not alter exercise performance-related endpoints, and FVC and FEV1 decreased an average of 7.6% and 5.6%, respectively. Exposure to 0.20 ppm O_3 led to reduction in maximal exercise performance, including maximal workload and exercise time. FVC and FEV1 decreased an average of 19.1% and 21.6%, respectively. Symptoms generally paralleled pulmonary function and exercise responses, and mainly consisted of chest pain/tightness, shortness of breath and cough. With exposure to 0.12 ppm O_3, 13 of 17 subjects had decrements in FEV1 of 10% or less. At 0.20 ppm O_3, only 3 of the 17 subjects had a decrement in FEV1 of 10% or less (range, -10 to –50%), 6 subjects had decrements in FEV1 of 30% or more after exposure to 0.20 ppm O_3. Exposures carried out at 31° C and 35% RH. Airway reactivity to histamine bronchochallenge was increased after exposure to 0.20 ppm O_3.	Gong et al. 1986

Table 11-15 (cont.): Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.15 0.30	1 hr, CE, (V_E=55 l/min), 24° C and 35° C.	Healthy, (10 F), 22.9± 2.5 yrs	Evaluated the effect of heat stress on responses to O_3. V_E unaffected by O_3, but heat increased V_E with time. Exposure to 0.30 ppm O_3 induced significant reductions in FVC, FEV1 and FEF25-75%. No significant pulmonary function changes with exposure to 0.15 ppm O_3. Symptoms increased with increasing O_3 concentration. Heat exposure intensified subject discomfort, but the effects of O_3 and heat were not interactive.	Gibbons and Adams 1984
0.18	2 hr, IE, (15 min ex/15 min rest), (V_E=35 l/min/m^2) 31° C, 35% RH	Pre-screened for responsiveness Responsive (5 M, 7 F) Nonresponsive (8 M, 5 F) 18-40 yrs	Investigated seasonal variability in response to O_3 exposure at 31° C and 35% RH. Nonresponders had similar pulmonary function changes following all exposures. Responders had the largest reductions in pulmonary function in spring, the end of the low O_3 season, and the smallest changes in autumn. Responses in winter were intermediate between spring and autumn. Subjects were reexposed to O_3 the following spring, and responses were similar to those following the initial spring exposure. Symptoms roughly paralleled the pulmonary function changes.	Linn et al. 1988

Table 11-15 (cont.) : Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.02 0.35 0.50 (KI)	hr IE, (15 min ex/15 min rest), (V_E=30 l/min) 35° C, 45% RH Protocol repeated on 5 consecutive days, with FA on days 1 and 5, and O_3 exposure on days 2, 3 and 4	Healthy Three groups of 10 M, each exposed to FA and one O_3 concentration 18-29 yrs Seven subjects had some history of allergy, none asthmatic. Ten subjects were former smokers; none had smoked in at least 1 yr	No effects with exposure to 0.20 ppm on any of the three O_3 days. With exposure to 0.35 ppm O_3, FEV1 and FEF25-75% decreased the most after the second O_3 exposure, although the changes were not statistically different from the change after the first O_3 exposure. With exposure to 0.50 ppm O_3, FVC, FEV1 and FEF25-75% all decreased after the first two days of O_3 exposure, although the decrements after the second exposure were significantly larger than those after the first and the third exposures. FEV1 and FEF25-75% were also significantly reduced after the third O_3 exposure. There was no change in respiratory pattern (V_T or f_R) with any exposure. Symptoms paralleled pulmonary function changes.	Folinsbee et al. 1980
0.0, 0.22	4 hr, IE (20 min exercise/10 min rest); (V_E=40-46 L/min)	90 M, (56 never smokers, 34 current smokers), 18-40 yrs	Smokers had smaller spirometric and plethysmographic responses following O_3 exposure than nonsmokers.	Frampton et al. 1997a Frampton et al. 1997b
0.0, 0.22	4 hr, IE (20 min exercise/10 min rest); (V_E=25 L/min/m^2 BSA)	12 O_3 nonresponders, nonsmokers (10 M, 2 F), mean age=25 yrs; 13 O_3 responder, nonsmokers (10 M, 3 F), mean age=25 yrs; 13 smokers (11 M, 2 F), mean age 28 yrs	O_3-induced airway inflammation was independent of smoking status or of airway responsiveness to O_3. Symptoms and spirometric changes were not predictive of pulmonary inflammation.	Torres et al. 1997

Table 11-15 (cont.): Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	2 hr (15 min rest/15 min ex), (V_E=32 l/min) Week 1: FA on 2 days Week 2: M-F, 0.40 ppm O_3 Week 3: Tues, Fri 0.40 ppm O_3	Nonsmoking (at least 2 yrs), asthmatics who used either no medications, or inhaled ß-agonists only (8 M, 2 F), 19-48 yrs	Asthmatic subjects show a pattern of attenuation and persistence of attenuation similar to that observed in nonasthmatics. Attenuation of pulmonary function and symptoms responses had begun to wane by four days after the fifth consecutive O_3 exposure. Symptoms showed a similar pattern to pulmonary function changes. Magnitude of responses was similar to studies performed at room temperature.	Gong et al. 1997a
0.42	2 hr, including 5 min light ex Exposures before and after completing a 6 mo smoking cessation program	Healthy smokers (8 M, 26 F), 24-58 yrs Smoking history: 10-66 pack/yrs Control group: (FA exposure on both occasions) N=16 O_3 exposure group: (O_3 exposure on both occasions) N=18	Nine subjects successfully completed the smoking cessation program and were reexposed to 0.40 ppm O_3 after 6 mo of abstinence from smoking. Prior to smoking cessation the subjects exposed to 0.40 ppm O_3 had no significant changes in lung function. After 6 mo without smoking, the same subjects had increased sensitivity to O_3, in that FEF25-75% decreased 22.5% from baseline and more symptoms, in conjunction with a nonsignificant decrease in FEV1, and no change in FVC. The results suggest that responsiveness to O_3 exposure can reemerge in smokers who quit smoking.	Emmons and Foster 1991

Table 11-15 (cont.): Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.47	2 hr, including 1 hr ex (V_E=11-31 l/min; mean, 24 l/min) FA on Monday, O_3 on Tues. through Fri., follow-up O_3 exposures every Tues. of second through sixth weeks	Healthy adults, residents of Los Angeles (8 M, 3 F), 21-53 yrs, 5 with history of allergies, 2 smokers	Experiments performed in winter (low O_3 season) to reduce influence of regular ambient O_3 exposure. All pre-exposure pulmonary function measurements were similar, in contrast to other reports suggesting a reduction in baseline lung function with repeated exposures. Largest changes in lung function after the second O_3 exposure, with the FEV1 after the second O_3 exposure about twice that after the first O_3 exposure. The decrements in FEV1 were similar after the first and third O_3 exposures, were within the variability of the data after the fourth exposure, although not all subjects showed the same pattern. Desensitization after the fourth O_3 exposure was substantially reduced by the first follow-up exposure. Symptoms paralleled lung function changes. Responses of one subject never attenuated, while two had little response on any of the consecutive days.	Linn et al. 1982
0.50 (KI)	Three consecutive days : 8 hr (FA on day one), 6 hr (O_3 on day two) with 2 hr FA recovery period, pulmonary function tests on afternoon of day three two 15 min exercise periods (WL=100 W)	Smokers (9 M, 1 F) Nonsmokers (10 M) 21-60 yrs (3 smokers, 1 nonsmoker over 40 yrs)	Subjects refrained from smoking within 24 hr of the first experiment, and throughout the three experimental days. Smokers had no significant changes in pulmonary function or SG_{aw} after O_3 exposure, while nonsmokers had a significant decrease in FVC and SG_{aw}. Six of the nonsmokers had O_3-induced reduction in FVC of = 10%. Among smokers, one reported cough and four reported chest discomfort, compared to nonsmokers, seven of whom reported cough and nine reported chest discomfort. The most symptomatic subjects were not necessarily the same ones who had the largest lung function changes.	Kerr et al. 1975

Table 11-15 (cont.): Influence of Ethnic, Environmental and Other Factors

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.50 (KI)	2 hr, including 30 min ex during either the second or third half hr (40% VO_{2max}); 4 ambient conditions: (1) 25° C, 45% RH; (2) 31° C, 85% RH; (3) 35° C, 40% RH; (4) 40° C, 50% RH	Healthy nonsmokers (14 M), 20-25 yrs	O_3 exposure induced similar decrements in pulmonary function regardless of which half hr subjects exercised. The largest reductions in lung function occurred immediately after the exercise period (826 mL in FVC and 937 mL in FEV1), with some recovery during the balance of the exposure (decrements of 388 mL in FVC and 426 mL at end of exposure). There was a trend toward greater reduction in pulmonary function under the most severe heat/RH conditions, but this was significant only for FVC. Concluded that heat stress may modify the overall effect of O_3 on pulmonary function.	Folinsbee et al. 1977

11.4.8.6.1 Socioeconomic Status

Only one study has investigated whether socioeconomic status (SES) alters responses to O_3 exposure (Seal et al. 1996). These investigators re-analyzed data from an earlier study (Seal et al. 1993) to model predictors of the FEV1 response to O_3 exposure. The subjects, who ranged from 18 to 35 yrs of age, were each exposed once to O_3 at a concentration of 0.0, 0.12, 0.18, 0.24, 0.3 or 0.4 ppm, while completing a 2.33 hour intermittent exercise protocol. Since most of the subjects were college students, father's education was used as the parameter to represent family SES. The results are difficult to explain, in that the middle SES group had the greatest responses to O_3 exposure, and the high SES group had the smallest responses, with the low SES group having an intermediate response. The index of SES used in this study, father's education, provides only a crude index of the socioeconomic status of the subject's families, and is unlikely to adequately account for influences, such as nutrition, genetics, housing quality, exposure to tobacco smoke, and access to medical care, that may impact on O_3 responsiveness. Consequently, the study does not allow inferences as to whether socioeconomic status impacts on sensitivity to O_3.

11.4.8.7 Summary: Gender, Age, Racial and Environmental Factors and Responsiveness to O_3

Most controlled studies investigating whether gender, age, racial and environmental factors affect responses to O_3 exposure have examined only functional and symptomatic responses to acute exposure. Although a variety of factors have been examined in an attempt to explain differences in responsiveness to acute O_3 exposure, only current smoking and increasing age have been convincingly shown to be linked with responsiveness, albeit in an inverse direction. For a given O_3 exposure concentration, children appear to experience percentage decrements in lung function comparable to those observed in adults; however, they report fewer symptoms, suggesting a lower level of somatic awareness or impaired nociception, which might result in their failure to curtail exposure in real-life situations. Depending on how dose is expressed, women may or may not be more responsive than men. There are insufficient data to conclude whether differences in O_3 susceptibility exist in relation to race or SES.

11.4.9 Responses to Repeated Ozone Exposures

11.4.9.1 Responses to O_3 exposures on consecutive days

Early studies suggested that Canadian subjects and new arrivals to Los Angeles appeared to have greater responses to acute, controlled O_3 exposures than subjects who were residents of Los Angeles (Hackney et al. 1975a,b; Bates et al. 1972; Hazucha et al. 1973; Hackney et al. 1976; Hackney et al. 1977). This raised the question of whether repeated exposure reduced responsiveness to O_3, and possibly represented a "beneficial" response. Most studies that have investigated this issue have used daily exposure protocols ranging from two to four hours in duration with intermittent light to moderate exercise, and O_3 concentrations of 0.35 ppm or more. The number of consecutive days of O_3

exposure among these studies ranged from three to five, simulating a severe, multi-day air pollution episode.

The first study to systematically examine responses of human subjects exposed to 0.5 ppm O_3 on consecutive days (KI) was performed by Hackney et al. (1977). One subject had little response on any exposure day, but the other five showed decrements in pulmonary function that peaked following the second O_3 exposure, and returned to near baseline levels following the fourth exposure. However, V_{50} and V_{25}, measures of flow rate at low lung volumes, remained slightly depressed following the third and fourth O_3 exposures. The severity of symptoms paralleled the pulmonary function changes. Farrell et al. (1979) extended the repeated exposures methodology of Hackney et al. (1977) by also studying the subjects through a multi-day control period of FA exposure. The consistent lack of pulmonary function and symptom responses across five days of FA exposure formed the basis for using only one, or in some cases no, FA exposure in subsequent studies designed to investigate responses to consecutive day exposures to O_3. The subjects also completed five consecutive day exposures to 0.4 ppm O_3 (KI), with results similar to those of Hackney et al. (1977).

Folinsbee et al. (1980) investigated the effect of O_3 concentration on responses to repeated O_3 exposures for two-hour with alternating 15 min rest and exercise periods (V_E = 30 L/min). Subjects exposed to 0.50 ppm O_3 (KI) showed the typical pattern of larger pulmonary function changes and symptom responses following the second O_3 exposure, followed by smaller, though still statistically significant decrements after the third O_3 exposure. Those subjects exposed to 0.35 (KI) ppm O_3 had similar small decrements in pulmonary function and increases in symptoms after all three consecutive O_3 exposures. In contrast, subjects exposed to 0.20 ppm (KI) O_3 had no significant pulmonary function or symptom responses following any of the three O_3 exposures. The results highlight the importance of O_3 concentration to the magnitude of pulmonary function and symptom responses induced by O_3 exposure, as well as to the day-to-day pattern of responses with repeated O_3 exposures.

Frank et al. (2001) reported on responses of healthy adults exposed to 0.25 ppm O_3 for 2-hour on four consecutive days. FVC and FEV1 returned toward baseline across the four days of exposure, with the greatest decrement on day 2, consistent with the findings of others. The unique contributions of this study are that the investigators evaluated small airway function following each exposure, and also presented details about pre-exposure lung functions on each day. The results, illustrated in Figure 11-6 below, indicate that small airway function, measured as SAWgp (a derived measure based on several measures of small airway function), followed a similar timecourse as FVC and FEV1 with O_3 exposures on four consecutive days. The figure also illustrates that pre-exposure lung function on days two through five remained depressed, indicating incomplete recovery within a 24 hour period.

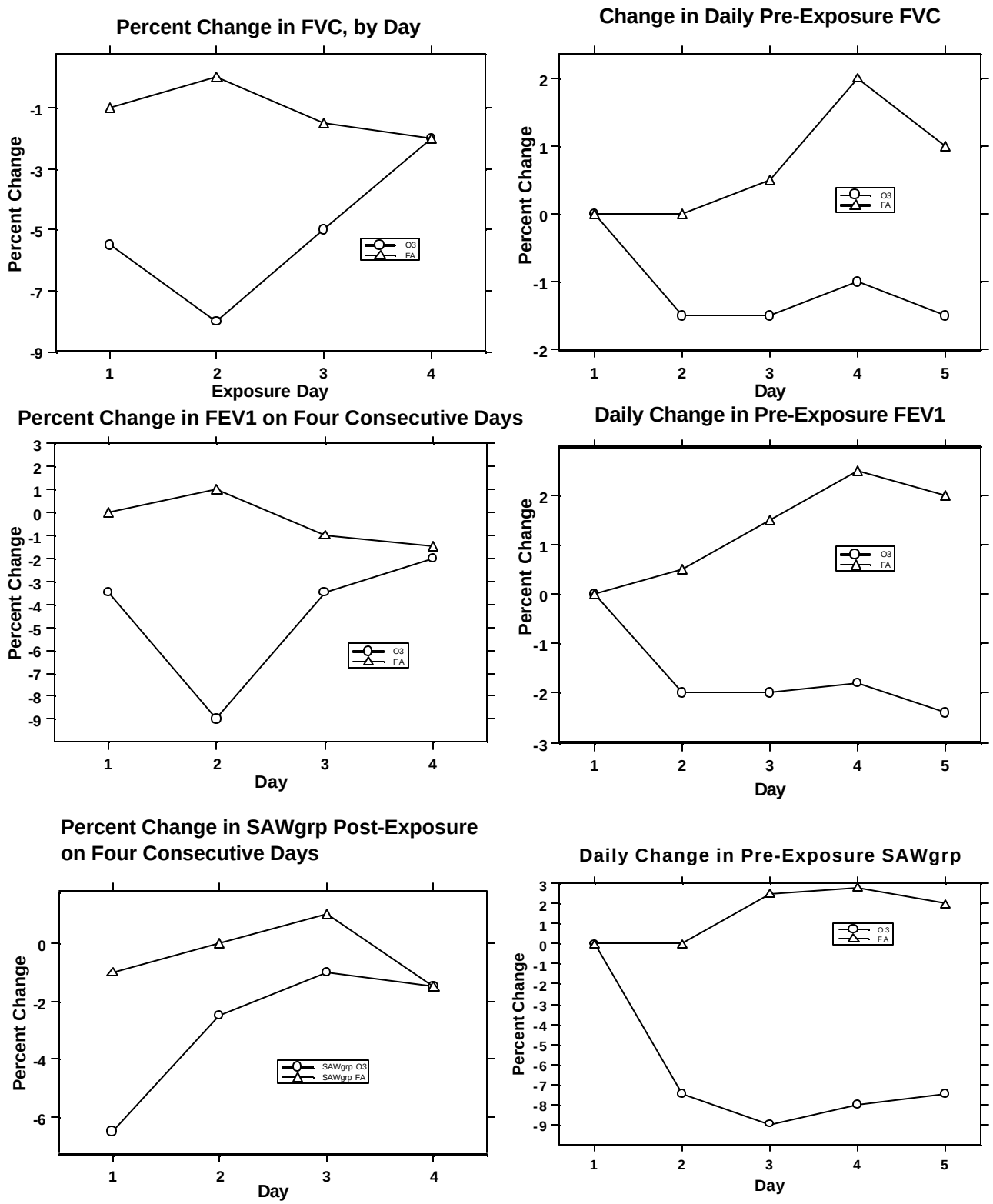

Figure 11-6 Percent change in FVC, FEV1 and SAWgp pre- and post-exposure. Derived from Frank et al., 2001.

Dimeo et al. (1981) sought to determine the lowest O_3 concentration that increased airway reactivity, as measured by increases in SR_{aw} in response to histamine inhalation challenge with repeated O_3 exposures. When subjects were exposed to 0.4 ppm O_3 (KI) on three consecutive days, the response to histamine was enhanced after the first O_3 exposure, but was similar to the FA response following the second and third consecutive O_3 exposures. This was in contrast to the time course of pulmonary function changes reported by others (i.e., larger response on the second day, followed by progressively smaller responses, e.g., Farrell et al. 1979). Although Dimeo et al. did not report pulmonary function data in the paper, the authors did state that the largest decrements in pulmonary function occurred after the second of the three consecutive O_3 exposures. These results underscore that several mechanisms, with different time courses, appear to be involved in the various responses observed following O_3 exposure (see Section 11.2). Further, the authors suggested that the threshold for increased bronchomotor reactivity with O_3 exposure is somewhere between 0.2 and 0.4 ppm (KI). However, the notion of a "threshold" O_3 concentration has been called into question by more recent studies involving lower O_3 concentrations, but greater delivered doses due to longer exposure duration and increased exercise-associated V_E (e.g., Folinsbee et al., 1994). The more recent view is that thresholds likely exist on an individual level, but that the variability among individuals is great enough that on a population-wide basis, the threshold is impossible to determine.

All of the studies discussed above involved relatively young subjects. Bedi et al. (1989) reported a somewhat different response pattern in 16 adults between 60 and 89 years of age who completed exposures to FA on one day, and then to 0.45 ppm O_3 (UV) on four consecutive days. The subjects in this study had similar small, but statistically significant, decrements in FEV1 following the first two O_3 exposures, in contrast to the observations noted above with young adult subjects. There were no significant changes in pulmonary function following the FA or the last two O_3.

The diminished pulmonary function and symptom responses observed in subjects repeatedly exposed to O_3 raised the question as to whether repeated exposures to a low concentration of O_3 might diminish the responses to a subsequent exposure to a higher O_3 concentration. Gliner et al. (1983) reported that repeated exposures on three days to 0.2 ppm O_3 for 125 min each day did not induce pulmonary dysfunction. Responses to exposure to 0.42 or 0.5 ppm O_3 on the fourth day were not different from those measured after a screening exposure to 0.42 or 0.5 ppm O_3. The investigators also noted a small, though not statistically significant, downward trend in pre-exposure FVC over the five consecutive day exposures.

Brookes et al. (1989) investigated responses to the opposite scenario to Gliner et al. (1983), that is, when the O_3 concentration is lower on day two (0.2 ppm) than on day one (0.35 ppm). The data indicate that exposure to a high ambient concentration can increase airway sensitivity such that greater responses occur

following a subsequent exposure to a lower O_3 concentration than would be expected with a single exposure to the lower O_3 concentration.

11.4.9.2 Repeated Prolonged O_3 Exposures

Folinsbee et al. (1994) studied the responses of 17 healthy, young adults who completed 6.6 hour exposures to 0.12 ppm O_3 on five consecutive days. There was a group mean decrement in FEV1 following the first O_3 exposure of 11.9%, which was reduced to 6.2% following the second O_3 exposure. Changes in FEV1 following the final three O_3 exposures were not different from baseline measurements. Methacholine responsiveness, on a group basis, was increased after all five O_3 exposures, although it was greatest on day two. Cough and pain on deep inspiration were statistically increased only after the first O_3 exposure. There was no correlation between symptoms or FEV1 and methacholine responsiveness, indicating that different mechanisms are responsible for each category of responses.

Table 11-16: Changes in Forced Expiratory Lung Volume After Repeated Daily Exposure to Ozone[a]

O_3 Concentration (ppm)[b]	Exposure Protocol[c]	Subjects	Percent Change in FEV1 on Consecutive Days					Reference
			Day 1	Day 2	Day 3	Day 4	Day 5	
0.12	6.6 hr, IE (40)	17 M	-12.8	-8.7	-2.5	-.06	0.2	Folinsbee et al. 1994
0.20	2 hr, IE (30)	10 M	+1.4	-2.7	-1.6	-	-	Folinsbee et al. 1980
0.20	2 hr, IE (18 & 30)	8 M, 13 F	-3.0	-4.5	-1.1	-	-	Gliner et al. 1983
0.20	2 hr, IE (18 & 30)	9 [d]	-8.7	-10.1	-3.2	-	-	Gliner et al. 1983
0.20	1 hr, CE (60)	15 M	-5.0	-7.8	-	-	-	Brookes et al. 1989
0.25	1 hr, CE (63)	4 M, 2 F	-20.2	-34.8	-	-	-	Folinsbee and Horvath 1986
		5 M, 2F	-18.8	-	-22.3	-	-	
0.25	130 min, IE (8 times FVC)	5 M, 3 F	-6.0	-9.0	-5.5	-2.5	-	Frank et al. 2001
0.35	2 hr, IE (30)	10 M	-5.3	-5.0	-2.2	-	-	Folinsbee et al. 1980
0.35	1 hr, CE (60)	8 M	-31.0	-41.0	-33.0	-25.0	-	Foxcroft and Adams 1986
0.35	1 hr, CE (60)	10 M	-16.1	-30.4	-	-	-	Schonfeld et al. 1989
		10 M	-14.4	-	-20.6	-	-	
0.35	1 hr, CE (60)	15 M	-15.9	-24.6	-	-	-	Brookes et al. 1989
0.40	3 hr, IE (4-5 times resting)	13 M [e]	-9.2	-10.8	-5.3	-0.7	-1.0	Kulle et al. 1982
0.40	3 hr, IE (4-5 times resting)	11 F [e]	-8.8	-12.9	-4.1	-3.0	-1.6	Kulle et al. 1982
0.40	2 hr, IE (30)	24 M	-21.1	-26.4	-18.0	-6.3	-2.3	Horvath et al. 1981
0.42	2 hr, IE (27)	1 M, 5 F	-13.3	-	-22.8	-	-	Bedi et al. 1985
0.45	2 hr, IE (27)	10 M, 6 F	-5.8	-5.6	-1.9	-	-	Bedi et al. 1989
0.45	2 hr, IE (3 x resting)	8 M, 2 F [f]	-11.4	-22.9	-11.9	-4.3	-	
0.47	2 hr, IE (30)	8 M	-8.7	-16.5	-3.5	-	-	Folinsbee et al. 1980
0.50	2.5 hr, IE (2 x resting)	6	-2.7	-4.9	-2.4	-0.7	-	Hackney et al. 1977
0.50	3 hr, IE (32)	8 M, 3 F[g]	-34.6	-31.1	-18.4	-12.0	-0.62	Gong et al. 1997a

a See Glossary for abbreviations and acronyms.

b Listed from lowest to highest O_3 concentration.

c Exposure duration and intensity of intermittent exercise (IE) or continuous exercise (CE) were variable; V_E (in parentheses) in L/min or as a multiple of resting ventilation.

d Subjects were especially sensitive on prior exposure to 0.43 ppm O_3, as evidenced by a decrease in FEV1 of more than 20%. These nine subjects are a subset of the total group of 21 subjects used in this study.

e Bronchial reactivity to methacholine challenge was also studied.

f Subjects had mild asthma.

g Seven subjects completed the entire experiment.

Table 11-17: Pulmonary Function Effects With Repeated Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12	6.6 hr, 50 min ex/10 min rest per hr; 30 min lunch (V_E=38.8 L/min)	Healthy nonsmokers (17 M)	FEV1 decreased 13% following the first O_3 exposure 9% following the second O_3 exposure, no change thereafter. Symptoms increased on only the first 2 days. Methacholine responsiveness significantly increased after all O_3 exposures, but maximal on day 2. Trend toward lessened methacholine responsiveness, but not returned to baseline by day 5.	Folinsbee et al. 1994
0.18	2 hr IE, (V_E=60-70 L/min; 35 L/min/m^2 BSA)	Adult, Los Angeles residents (N=59) 18-40 yrs, including 12 responsive, 13 nonresponsive	FEV1 decreased 12.4% at initial screening in late spring in responders, but nonresponders had no change. Responders had nonsignificant responses in late summer and early winter, but were similarly responsive in the following early spring. (Spring 1986, -385 mL; Autumn 1986, -17 mL; winter 1987, +16 m; spring 1987, -347 mL). Nonresponders did not change with season.	Linn et al. 1988

Table 11-17 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.20 0.35 0.50 (KI)	FA on day 1, O_3 on days 2, 3, and 4, FA on day 5 2 hr, IE (15 min ex/15 min rest), (V_E=30 l/min)	Three groups of healthy males (N=10/group), 18-29 yrs, 7 with some history of allergy	Each group was exposed to one of the three O_3 concentrations. Pre-exposure lung function was nonsignificantly decreased across exposure days 2 through 5. The group exposed to 0.20 ppm O_3 had no significant responses on any of the three O_3 exposure days. The group exposed to 0.35 ppm O_3 had the largest changes in lung function after the second O_3 exposure, but the differences were not significantly different from the changes after the first O_3 exposure. The group exposed to 0.50 ppm O_3 had significant decrements in FVC, FEV1 and FEF25-75% after the first and second exposures, and in FEV1 and FEF25-75% after the third O_3 exposure. The decrements after the second O_3 exposure were significantly larger than those after the first and third O_3 exposures. The higher the O_3 concentration, the shorter the time needed for decrements in pulmonary function to become evident. Cough and shortness of breath were greatest after the first two O_3 exposures, followed by attenuation.	Folinsbee et al. 1980
0.20 0.40 (KI)	2 hr, IE (15 min rest/15 min ex), (V_E=twice resting)	Nonatopic, healthy adults, (12 M, 7 F), 21-32 yrs	Study focused on effect of O_3 exposure on airway reactivity. Exposure to 0.20 ppm O_3 did not alter response to post-exposure histamine bronchochallenge, either for the group as a whole or for any individual subject. Exposure to 0.40 ppm O_3 increased response to histamine bronchochallenge after the first day of O_3 exposure, while response to post-exposure histamine bronchochallenge decreased to the baseline level by the third consecutive day of O_3 exposure.	Dimeo et al. 1981

Table 11-17 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.2 0.42 or 0.50	Monday – FA; Tuesday-Thursday 0.20 ppm O_3; Friday – 0.42 or 0.5 ppm O_3 125 min, IE (15 min rest/15 min ex), (V_E=30 l/min for males, 18 l/min for females) Single day challenge to 0.42 or 0.50 ppm O_3	Healthy nonsmokers, (8 M, 13 F), 18-31 yrs	Males and females responded similarly, although females inhaled a smaller effective dose of O_3. Exposure to a low concentration of O_3 did not alter the pulmonary function responses to a subsequent exposure to a higher O_3 concentration. Exposure to 0.20 ppm O_3 did not induce significant reduction in any pulmonary function, or induce greater responses after the second O_3 day on a group basis, although it did for a few individual subjects. Subjects responded similarly to both high O_3 concentration exposures. There was a nonsignificant downward trend in pre-exposure FVC over the 5 experimental days.	Gliner et al. 1983
0.20/0.20 0.35/0.20 0.35/0.35	1 hr CE (V_E=60 L/min)	Healthy aerobically trained nonsmokers (15 M). FVC=4.24 to 6.98 L	Consecutive days of exposure to 0.20 ppm induced similar responses on both days (-5.02%, -7.8%). The 0.35/0.20 pair of exposures caused a somewhat greater response to 0.20 ppm on the second day (-14.4% vs. 8.74%) than observed with the 0.20/0.20 ppm O_3 combination. The 0.35/0.35 combination caused increased response on the second day (-15.9%, -24.6%). Symptoms were worse after the second exposure to 0.35, but not when the second exposure was to 0.20 ppm O_3.	Brookes et al. 1989

Table 11-17 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.25	4 consecutive days of O_3 exposure 4 consecutive days of FA exposure 130 min (30 min rest/30 min ex), (V_E=eight times FVC)	Healthy (5 M, 3 F), 25-31 yrs	FVC, FEV1 and small airway group function showed the expected pattern with repeated O_3 exposures. Decrements in FVC and FEV1 were largest on day 2, with the day 4 post-exposure values were similar to those with four days of FA exposure. Small airway function decrement was largest on day 1 of O_3 exposure, and similar to FA by day 4. Pre-exposure FVC, FEV1 and small airway group function decreased progressively across the 4 days of O_3 exposure, but only small airway group function was significantly depressed by day 4.	Frank et al. 2001
0.35	1 hr CE	Healthy, aerobically trained nonsmokers (8 M), 22.4 ± 2.2 yrs	Largest decrease in FEV1 on the second of four days of O_3 exposure (-40%). Trend toward attenuation of changes in spirometry, but not complete with 4 days of exposure. VO_{2max} decreased on day 1, but not significantly reduced by day 4. Performance time less on day 1 (211 sec) compared to after FA (253 sec).	Foxcroft and Adams 1986
0.35	1 hr CE (V_E=60 L/min)	Nonsmokers, nonresidents of Los Angeles for more than 6 mo, (40 M, in four groups of 10), mean age 25 yrs	No differences in responses to exposures separated by 72 or 120 hrs. FEV1 response at 24 hr after the initial O_3 exposure was larger (-30.4% vs. –16.1%). Second exposure induced a larger FEV1 response with 48 hr separation between exposures (-14.4% vs. –20.6%). Similar trends observed for respiratory pattern and SR_{aw}.	Schonfeld et al. 1989
0.45	2 hr IE (20 min ex/20 min rest), (V_E=26 L/min)	Healthy nonsmokers (M, 8 F), mean age M = 61 yrs; 65 yrs F. (FVC = 4.97 for M; 3.11 for F)	Spirometric responses were not reproducible among three O_3 exposures, while repeated air exposures yielded consistent responses.	Bedi et al. 1988

Table 11-17 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.45	2 hr IE (20 min ex/20 min rest), (V_E=27 L/min)	Healthy nonsmokers, 60 to 89 yrs (10 M, 6 F). Mean FVC=3.99 L; mean FEV1=3.01 L; FEV1/FVC range=61-85%	Overall increase in symptoms, but no single symptom increased significantly. FVC decrements were 111 mL and 104 mL, on days 1 and 2, respectively. FEV1 decreased by 171 mL and 164 mL on days 1 and 2, respectively. FEV3 decreased 185 and 172 mL on days 1 and 2, respectively. Percentage changes in FEV1 were –5.8, -5.6, -1.9, and –1.7 after the four consecutive day O_3 exposures.	Bedi et al. 1989
0.45 (+ 0.30 ppm PAN)	2 hr IE (20 min rest/20 min ex), (V_E=27 L/min)	Healthy nonsmokers (3 M, 5 F), mean age 24 yrs	FEV1 decreased 19% with exposure to O_3 alone, and 15% on day 1 of O_3+PAN exposure. After 5 consecutive day exposures to O_3+PAN, the FEV1 decrement had attenuated to about 5%. After three days without O_3+PAN exposure, the FEV1 decrement was about 7%, and was 15% after nonexposure for 5 days. Maximal decrements occurred after the day 2 exposure. PAN had no apparent effect on attenuation of pulmonary function responses to O_3 exposure.	Drechsler-Parks et al. 1987b
0.40	3 hr, IE (15 min rest/15 ex), (V_E=32 L/min) on 5 consecutive days	Adults with mild asthma (N=10)	FEV1 decreased 15% after the first O_3 exposure, with attenuation to 6% on day 5. Bronchial reactivity increased after day 1, and remained elevated across the five days of O_3 exposures. Attenuation in asthmatics is similar to healthy subjects, but may be slower and less complete.	Gong et al. 1997a
0.50 (KI)	2 hr (+ 30 min for measurements), IE, (15 min ex/15 min rest), (V_E 150-200 kg·m/min) FA on day 1, then O_3 on 4 consecutive days	Healthy, Los Angeles residents, preselected to be responsive to O_3, (N=6, gender not specified), 25-57 yrs, 5 had history of allergy	Maximal reduction in pulmonary functions was on O_3 day 2, with most pulmonary functions returned to near control levels by O_3 day 4. Several measures of flow in small airways remained slightly depressed on O_3 days 4 and 5. Experiments were during the end of the low-smog season. Symptoms generally paralleled pulmonary function responses.	Hackney et al. 1977

11.4.9.3 Exercise Performance

It is well known (see Section 11.4.10) that single O_3 exposures can limit maximal exercise performance. (Foxcroft and Adams 1986) investigated whether the attenuation of pulmonary function decrements and symptoms responses with repeated O_3 exposures would also extend to O_3-induced reduction in maximal exercise performance. Although pulmonary function changes were largest following the second of the consecutive exposures, these subjects had similar decrements in pulmonary function after the first and fourth days of exposure. In both cases the decrements were statistically significant when compared to the FA day. Thus, in terms of pulmonary function, these subjects did not develop the usual pattern of responses to repeated O_3 exposure. However, symptomatology did decrease across the four exposures. Although there was some improvement in exercise performance time between the maximal exercise test following the first O_3 exposure compared to that on the fourth day, exercise time remained significantly less on both days than following FA exposure.

11.4.9.4 Repeated O_3 exposures in subjects with lung disease

Several studies have investigated the responses of individuals with lung disease to repeated O_3 exposures. Since O_3 appears to mainly affect the lungs, people with pre-existing lung disease might be at increased risk of adverse health effects compared to healthy people. Kulle et al. (1984) investigated the pulmonary function and symptom responses of patients with mild chronic obstructive lung disease to repeated O_3 exposures. On a group basis, there were statistically significant decrements in FVC and FEV_3 following the first of five consecutive O_3 exposures and also following an additional O_3 exposure four days after the fifth consecutive exposure. These lung function decrements were not likely to have been functionally significant (+3 to -2%). Although the individual subjects' data were not included in the report, the authors stated that 12 of the subjects showed the typical pattern of pulmonary function responses with consecutive days of O_3 exposure. Among the other 8 subjects, several consistently had small decrements after all O_3 exposures, and several never showed any changes in pulmonary function with O_3 exposure. Symptom reports were minimal, and did not correlate with pulmonary function changes. This study may be of limited general applicability, in that all of the subjects were middle-aged, current smokers (most about two packs/day; range one to four packs/day), with smoking histories ranging from 16 to 36 years. However, the data did show inverse correlations between O_3 response magnitude and both age and smoking history. Others (Drechsler-Parks 1987; Drechsler-Parks et al. 1987a; Drechsler-Parks et al. 1989; Frampton et al. 1997a,b; Torres et al. 1997; Torres et al., 1997) have also reported either or both of these relationships.

Asthmatics also appear to manifest attenuated functional responses with repeated O_3 exposure. Gong et al. (1997a) exposed adult asthmatics twice to FA, and then on five consecutive days to 0.4 ppm O_3 (UV). The subjects showed a nonsignificant decline in preexposure pulmonary function across the five consecutive days of O_3 exposure, in agreement with other investigators examining subjects without respiratory disease (Gliner et al. 1983; Frank et al.

2001). Pulmonary function decrements were similar and statistically significant after the first two O_3 exposures, and returned toward baseline over the following three exposures, a pattern similar to that reported for normal subjects (see Section 11.4.9). Responses after re-exposure to 0.4 ppm O_3 four and seven days after the last consecutive O_3 exposure indicated that response attenuation had begun to reverse after four days without experimental O_3 exposure. Examination of individuals' responses confirms previous findings that those subjects who were initially most responsive showed slower development of response attenuation, and faster loss of attenuation (Horvath et al. 1981). Airway responsiveness to methacholine was at least partly independent of effects on lung function, with excess bronchial reactivity persisting after FEV1 responses had attenuated, in accord with the findings of Folinsbee et al. (1994). Methacholine reactivity was increased after all the O_3 exposures. Although there was a trend toward reduction in airway reactivity to methacholine across the O_3 exposure days, attenuation in this endpoint was not complete with five consecutive day exposures. Since this is the only study of this type with asthmatics, and since it is unknown whether the group studied is representative of the asthmatic population, it is unclear whether the findings can be generalized to the broader population.

11.4.9.5 Persistence of attenuation

As discussed above (Section 11.4.9.5), Gong et al. (1997a) reported that reduced pulmonary function and symptom responses with repeated O_3 exposures only persist for a few days of nonexposure to O_3 in asthmatics. Several studies with normal subjects that used similar exposure protocols have reported similar results (Horvath et al. 1981; Kulle et al. 1982; Linn et al. 1982).

11.4.9.6 Duration of hyperresponsiveness to O_3

The observation that people typically have a larger response to O_3 on the second consecutive day of exposure raised the question of how long the hyperresponsiveness consequent to the first O_3 exposure persists. Three studies have investigated this topic. Bedi et al. (1985) used an intermittent exercise protocol to investigate the responses of 6 healthy young adults when two exposures to 0.45 ppm O_3 (UV) were separated by 48 hours. Sensitization from the first O_3 exposure carried over to the second exposure, in that pulmonary function decrements were significantly larger and appeared earlier in the second exposure than the first, leading to the conclusion that airway sensitization from a single high-level O_3 exposure lasts at least 48 hours.

Folinsbee and Horvath (1986) extended these observations by examining responses to two identical O_3 exposures to 0.25 ppm O_3 separated by 12, 24, 48, or 72 hours . The results showed that re-exposure at 12 or 24 hours induced statistically larger decrements in pulmonary function than had been observed following the initial exposure. Re-exposure at 48 hours after the initial exposure induced larger (but not statistically significant) decrements in pulmonary function than after the first exposure. When the re-exposure was at 72 hours after the initial exposure, the lung function responses to the two exposures were similar.

Symptom reports generally paralleled the pulmonary function responses. Schonfeld et al. (1989) reported similar results for healthy adults who participated in two exposures to 0.35 ppm O_3 separated by 24, 48, 72 or 120 hours. Re-exposure after 24 hours induced the expected increase in pulmonary function and symptom responses, while responses when re-exposure was at 72 or 120 hours were similar to the initial exposure. Re-exposure at 48 hours after the initial exposure resulted in pulmonary function and symptoms responses that were intermediate between those observed with re-exposure at 24 hours and 72 hours, and were not statistically different from those following the initial O_3 exposure.

Collectively, these papers suggest that a single O_3 exposure induces a state of heightened airway sensitivity to a subsequent O_3 exposure that persists for somewhere in the range of 24 to 48 hours. It should be noted that these studies used O_3 concentrations considerably higher than are currently typical of ambient air in California.

11.4.9.7 Repeated natural O_3 exposures and responses to controlled O_3 exposures – seasonal variation

As noted above, it has been proposed that the reason long-term Los Angeles residents might be less responsive to O_3 in controlled exposure studies than Canadians and new arrivals to Los Angeles might be attenuation due to regular exposure to O_3 concentrations typical of Southern California. Linn et al. (1988) addressed one aspect of this hypothesis by evaluating whether there was a seasonal influence on O_3 responsiveness in 38 Los Angeles residents. The subjects participated in exposures to FA and 0.18 ppm O_3 in late spring, autumn, winter, and again the following spring. Subjects who were responsive to O_3 showed seasonal variation in their degree of responsiveness. These subjects had the largest decrements in pulmonary function in the spring when ambient O_3 concentrations had been low for several months, considerably smaller changes in autumn, representing the end of the high ambient O_3 season, and intermediate changes in winter. Subjects who had minimal responses to the initial spring O_3 exposure had similar responses at all follow-up O_3 exposures. The results suggest that, among individuals who are responsive to O_3, long-term natural exposure to comparatively low ambient O_3 concentrations alters responsiveness across seasons. This is in contrast to repeated controlled exposure studies at low concentrations (see Section 11.4.9), which did not induce changes in O_3 responsiveness across the consecutive exposure days. These findings suggest that due to regular, repetitive ambient O_3 exposures, responses of subjects native to the Los Angeles Basin may not be representative of the population whose O_3 exposures are more infrequent and episodic. This must be considered in the interpretation of all O_3 exposure studies conducted in Los Angeles relative to studies conducted elsewhere.

11.4.9.8 Repeated O_3 exposures and airway inflammation

Frank et al. (2001) performed bronchoscopy with BAL 24 hours after subjects had undergone four consecutive daily exposures to 0.25 ppm O_3. Ozone

exposure induced changes in FVC, FEV1 and symptomatology that followed the typical pattern of increased effects on day-two compared to day-one, followed by response attenuation. Frank et al. were the first to specifically examine the effect of repeated O_3 exposure on small airway function (SAWgp -small airway group: a composite index of several measures of small airway function). The results showed the greatest decrements in SAWgp on the first O_3 exposure day. The subjects also demonstrated increased f_R and decreased V_T. Further, daily pre-exposure FVC, FEV1 and SAWgp progressively decreased across the four O_3 exposure days, although only the decrease in SAWgp was statistically significant. The authors concluded that the BALF results after the fourth O_3 exposure indicated pulmonary inflammation. However, since the only statistically significant endpoint in the BALF analysis was an increase in neutrophils, there being no significant changes in albumin, fibrinogen, kinins or other types of cells, this is a tenuous conclusion. Given that there were only 8 subjects, the group may have been too small to determine statistical significance, and thus these results can only be considered qualitatively.

Christian et al. (1998) performed a more extensive analysis of BALF obtained at bronchoscopy about 20 hours after the subjects completed a single four-hour exposure to 0.2 ppm O_3 with intermittent exercise (V_E = 25 l/min/m^2), and after they completed the same protocol on four consecutive days. The pattern of responses in FVC, FEV1 and SR_{aw} was similar to that reported by others who have used similar single- and multi-day. Changes in V_T, f_R and symptoms were also similar to previous reports (see Sections 11.4.2 and 11.4.3). The significance of this paper is the extensive BALF analysis. Contrary to the hypothesis of the study, the BALF results suggested that inflammation did not progress with consecutive days of O_3 exposure, as evidenced by decreased PMNs and reduced fibronectin concentration in the bronchial fraction of the BAL after the 4 consecutive days of O_3 exposure compared to after the single-day O_3 exposure. In the bronchoalveolar fraction, there were significant decreases in the number of PMNs, fibronectin and IL-6 after the four-day exposure, compared to the single-day exposure. These results suggest attenuation of the O_3-induced inflammatory response in both proximal airways and in the distal lung with repeated O_3 exposures. However, even though inflammation appeared attenuated after the four-day exposure, the BALF analysis indicated that inflammation was still present. How long such inflammation persists is unknown. Moreover, chronic inflammation may lead to airway remodeling and structural damage to the respiratory tract. No information addressing these issues is currently available on human subjects, although animal studies (see Section 11.3.3) suggest that chronic exposure to O_3 can lead to morphological changes in the lung tissues, and to reduction in inflammation after a few days of exposure.

Jorres et al. (2000) investigated the effect of repeated O_3 exposures on inflammatory markers in BALF and mucosal biopsies. The study involved 23 healthy adults who underwent single exposures to FA and 0.2 ppm O_3, as well as four-hour exposures to 0.2 ppm O_3 on four consecutive days. BAL was performed and mucosal biopsies were obtained 20 hour after the single and the fourth O_3 exposures. Changes in FEV1 followed typical patterns, that is, similar

responses following both the single O_3 exposure and the first of the consecutive O_3 exposures, and somewhat larger decrements after the second consecutive exposure. This was followed by smaller changes in FEV1 following the third and fourth O_3 exposures. The pro-inflammatory markers IL-6 and IL-8, along with total protein, were increased after the single O_3 exposure, but only total protein remained elevated after the four-day protocol. IL-10, an anti-inflammatory cytokine, was only increased after the four-day protocol, suggesting that it may have a role in the attenuation process. Macrophages in BALF decreased with the single O_3 exposure, compared to FA, but had returned nearly to the FA level after the four-day protocol. Neutrophils increased three-fold after the single O_3 exposure, and decreased toward the FA level with four days of O_3 exposure, although they were still about twice the baseline levels after four days of exposure. The results of the bronchial biopsies showed mucosal inflammation that was greater after the four-day protocol than after the single O_3 exposure, in contrast to the BALF analysis, which suggested that airway inflammation was waning. These results suggest that BALF analysis may not accurately reflect airway tissue effects. It is important to note that the biopsy analysis indicated the presence of increasing airway inflammation, even though pulmonary function changes had attenuated, and some markers of inflammation, as measured in BALF, were returning to baseline levels.

11.4.9.9 Summary

Although the term “adaptation” has been used to describe the reductions in pulmonary function and symptom responses that develop with repeated exposures to relatively high O_3 concentrations (i.e., over 0.2 ppm), the database as a whole suggests that this term is a misnomer. First, research suggests that ventilatory responses and reduced exercise performance do not show response attenuation with repeated exposures to O_3 concentrations that lead to diminution of pulmonary function responses. There is also evidence that some aspects of airway inflammation continue, or even increase, with repeated O_3 exposures. Baseline pulmonary function decreases with repeated O_3 exposure. Attenuation of pulmonary function and symptom responses to a fixed O_3 concentration does not necessarily alter responses to a subsequent exposure to a higher or a lower O_3 concentration. And finally, pulmonary function and symptom response attenuation begins to reverse after only a few days of nonexposure, at least under controlled laboratory conditions. In contrast, Linn et al. (1988) paper suggests that under ambient exposure conditions the attenuation effect may be seasonal in nature. When all the evidence is considered, one must conclude that this pattern represents attenuation of some, but not all, of the responses elicited by O_3 exposure, since some adverse responses persist, even with attenuation of symptom and pulmonary function responses. This indicates that “adaptation” is an inappropriate characterization of these phenomena. In particular, the persistence of airway hyperreactivity and inflammation suggests that long-term exposure to O_3 may result in chronic morphological effects, such as tissue remodeling, and chronic inflammation similar to those changes observed in animals (see Section 11.3). In addition, evaluation of the individual data presented in the papers reviewed above indicates that not all subjects

demonstrated attenuation of pulmonary function and symptom responses across consecutive days of O_3 exposure, suggesting that this response is not universal. Furthermore, it remains unclear whether or not attenuation represents a beneficial response.

11.4.10 Effects of Ozone Exposure on Exercise Performance

11.4.10.1 Human Studies

The first evidence that exercise performance is decreased by inhalation of oxidant pollutants appeared in 1967 in a study of high school cross-country runners (Wayne et al. 1967). The authors suggested that the detrimental effects of oxidant air pollutants on exercise performance may have been related to increased airway resistance (R_{aw}), and to oxidant-induced breathing discomfort, which reduced the runners' motivation and ability to perform at high levels.

Subsequently, Folinsbee et al. (1977) reported reduced work capacity, as indicated by reduction in maximum oxygen uptake (V_{O_2max}), maximum attained workload, maximum ventilation (V_E), and maximum heart rate, in subjects who performed a maximal exercise test after they had completed a two-hour exposure to 0.75 ppm O_3 (KI) with light intermittent exercise. These findings were coupled with a significant reduction in FEV1 and a significant increase in symptoms of cough and chest discomfort. In contrast, Horvath et al. (1979) and Savin and Adams (1979) did not observe changes in exercise performance, however, the exposure protocols in these studies both resulted in substantially lower effective doses of inhaled O_3 than those inhaled by the subjects in the study by Folinsbee et al. (1977). The participants in the study by Horvath et al. (1979) performed the maximal exercise test after a two-hour resting exposure to 0.75 ppm O_3 (KI), while those in the study by Savin et al. (1979) were exposed to 0.3 ppm O_3 only during the maximal exercise test. The differences among these findings can therefore be attributed, at least in part, to the significantly larger dose of O_3 inhaled by the subjects in the study by Folinsbee et al. compared to those of Horvath et al. (1979) and Savin and Adams (1979).

More recent studies by Foxcroft and Adams (1986) and Gong et al. (1986) confirm the findings of Folinsbee et al. (1977) that O_3 inhalation during heavy exercise leads to a reduction in work capacity. Both of these studies also reported significant decrements in various measures of pulmonary function and marked subjective symptoms of respiratory discomfort.

Linder et al. (1988) reported small, statistically significant reductions in exercise performance during a progressive maximal exercise test at O_3 concentrations of 0.06 and 0.12 ppm O_3, compared to clean air. Total exercise time decreased as O_3 concentration increased, with the reductions in exercise time statistically significant under both the 0.06 and 0.12 ppm conditions, compared to the air condition. Subjects also had statistically significant reductions in pulmonary function after both O_3 experiments, although the changes consequent to both O_3 exposures were less than 100 mL, and are within the typical variability of repeated pulmonary function tests and are thus unlikely to be of clinical significance. Subjects also reported a increasing number of symptoms with increasing O_3 concentration. It is significant that the subjects in this study only inhaled O_3 during the exercise tests, which lasted about 18-23 minutes (depending on atmosphere and gender), in contrast to most of the other studies discussed above, in which the exercise test occurred after O_3 exposure.

Several studies have also investigated whether O_3 exposure has an effect on endurance exercise performance in highly trained subjects and endurance athletes (Adams and Schelegle 1983; Folinsbee et al. 1984, Folinsbee et al. 1986; Avol et al. 1984; Gong et al. 1986; and Schelegle and Adams 1986). The subjects in these studies were exposed to O_3 concentrations ranging from 0.08 to 0.35 ppm. The studies used several different exposure protocols, but all were designed to investigate endurance exercise performance at a heavy workload. Collectively, these studies found that symptoms and the magnitude of pulmonary function decrements increased with increasing O_3 concentration. They also reported that increasing numbers of subjects were unable to complete the exercise protocols as O_3 concentration increased from 0.16 ppm to 0.35 ppm. The reduction in endurance performance is thought to be related to the degree of symptomatology, although there is also evidence for neurally mediated reflex reduction in tidal volume, V_T, coupled with an increased sensation of respiratory effort. In contrast, Gong et al. (1988) reported that 15 competitive cyclists who inhaled 0.21 ppm O_3 while performing 60 min of heavy continuous exercise followed by a sprint to exhaustion in simulation of a 25-mile cycle race experienced no statistically significant difference in metabolic data or ride time compared to when they performed the same protocol while inhaling FA. Interestingly, this was coupled with significantly lower peak V_E with O_3 than FA exposure, and O_3-induced decrements in pulmonary function. These disparate results may be related to differences in baseline characteristics: the subjects in the study by Gong et al. (1988) were elite competitive athletes who were accustomed to pushing themselves to maximal performance, whereas most of the subjects in the other studies cited above were highly fit, but non-competitive athletes (Adams and Schelegle, 1983; Folinsbee et al., 1984; Folinsbee et al. 1986; Avol et al. 1984; Gong et al, 1986; and Schelegle and Adams, 1986).

11.4.10.1.1 Summary

Overall, the published literature suggests that exercise performance can be reduced under conditions where O_3 inhalation has induced pulmonary function decrements and/or symptoms of respiratory discomfort. Significant reductions in exercise performance have been reported at O_3 concentrations as low as 0.06 ppm.

11.4.11 Extrapulmonary Effects of Ozone Exposure

11.4.11.1 Animals

Ozone can cause effects in organ systems and tissues outside of the respiratory tract. Due to the highly reactive nature of O_3, it is unlikely that O_3 can directly affect extrapulmonary tissues, although O_3 reaction products may enter the bloodstream and be transported from the lung to affect other organs and tissues. Potential O_3 reaction products have been detected in blood plasma following O_3 exposure and have been implicated in extrapulmonary tissue injury (Rahman et al. 1992). Release of cytokines as a result of O_3-induced pulmonary injury has also been proposed as a potential source of extrapulmonary tissue injury (Laskin et al. 1994; Laskin et al. 1998). The immune system, which protects the body

from damage by infectious microorganisms and neoplastic cells, can be affected by O_3 exposure. O_3 exposures as low as 0.2-0.3 ppm have resulted in immunotoxic effects on T-cell lymphocyte function and immune system organs, including the spleen and thymus, but generally continuous or near-continuous multi-day exposures required to achieve an effect (Van Loveren et al. 1990; Van Loveren et al. 1988; Li and Richters 1991; Dziedzic and White 1986; Li and Richters 1991). A long-term study mimicking urban O_3 exposures (daily spikes of 0.25 ppm) was negative for immune effects (Selgrade et al. 1990). Recent developmental studies in rodents showed that continuous exposures of 0.6 ppm or greater was required to elicit an effect (Petruzzi et al. 1995; Dell'Omo et al. 1995a). Neurobehavioral developmental effects at equivalent or higher O_3 concentrations have yielded ambiguous or negative results (Dell'Omo et al. 1995b; Petruzzi et al. 1999). O_3 has been shown to alter bone marrow erythroid progenitor formation (Goodman et al. 1989). However, similar to developmental effects, requires multi-day continuous exposure at high ambient levels (0.5 ppm) are required to elicit an effect. Central nervous system (CNS) and behavioral effects have been recorded at O_3 concentrations as low as 0.1-0.2 ppm, but are probably indicative of sensory irritation or O_3-mediated products having a direct or indirect effect on the CNS (Umezu et al. 1987; Rivas-Arancibia et al. 1998; Musi et al. 1994; Paz 1997). Cardiac effects, including slowed heart rate and bradyarrythmic episodes were noted in rodents at O_3 levels of 0.1 ppm (Arito et al. 1990; Iwasaki et al. 1998). These effects were transient and likely related to the labile thermoregulatory control of the experimental rodent species (Watkinson and Gordon 1993; Watkinson et al. 2001). These O_3-induced thermoregulatory effects have not been reported in humans.

11.4.12 Interaction of Ozone with Other Pollutants and Bioaerosols

11.4.12.1 Introduction

Typically laboratory investigations of responses to air pollutants have involved exposure to a single pollutant, although some studies have investigated the impacts of exposures to mixtures of two or three pollutants. The reasons for the relative paucity of joint exposure studies include the difficulty of adequately controlling the concentrations of multiple pollutants simultaneously, as well as the large number of pollutants in ambient air, which are subject to a practically infinite number of combinations by location and time. Atmospheric chemistry is complex, and it becomes increasingly difficult to adequately assess the exposure mixture as the number of pollutants increases. Moreover, as opportunities for chemical reactions among various atmospheric chemical species increase, the possibility that observed effects may be related to unknown reaction products increases. In this case, controlled exposure studies using only routinely monitored pollutants cannot evaluate the overall toxicity of ambient mixtures. Moreover, since each chemical species in ambient air has a different time-concentration profile, developing a representative atmosphere presents complex issues as to the most appropriate time-concentration profile for the different pollutants during the exposure, i.e. whether they are presented simultaneously, sequentially, or in an overlapping pattern, and also whether the exposures should take place at

constant or time-varying concentrations. Ideally, the selected pattern should at least approximate one that occurs in ambient air.

11.4.12.2 Ozone Plus Sulfur-containing Pollutants

Hazucha and Bates (1975) found larger decrements in one pulmonary function parameter (FEF25-75%) in eight males exposed to a mixture of 0.37 ppm O_3 (KI) and 0.37 ppm SO_2 than when the same subjects were exposed to 0.37 ppm O_3 alone. The investigators also reported considerable variability in responses among the subjects. However, since FEF25-75% is typically less reproducible between multiple tests than some of the other pulmonary functions, and differences were not apparent in any of the other endpoints studied, the observation may be a chance occurrence or an experimental artifact. As several other larger studies with better control of the experimental atmospheres have not found any evidence for interaction between O_3 and SO_2, it is likely that the finding of Hazucha and Bates (1975) was spurious (Bedi et al. 1979, Bedi et al. 1982; Folinsbee et al. 1985; Bell et al. 1977; Kleinman et al. 1981).

Asthmatic adolescents participated in a study evaluating sequential exposure to O_3 and SO_2, based on the hypothesis that responses to SO_2 would be greater when the SO_2 exposure followed O_3 exposure (Koenig et al. 1990). Changes in FEV1 and thoracic resistance (R_T) were significantly greater when the second phase of the exposure was to SO_2 compared to when the second part of the exposure was to FA or O_3. It should be noted that the SO_2 concentration used in this study, 0.10 ppm, is below the “threshold” range considered to induce bronchoconstriction in exercising (California Air Resources Board 1994). These results suggest that O_3 pre-exposure may potentiate responses to subsequent SO_2 exposure in adolescent asthmatics.

Some investigators have hypothesized that exposure to H_2SO_4 aerosol would potentiate responses to O_3 exposure. An early study by (Kleinman et al. 1981) found no convincing evidence for interaction with exposure to a mixture of O_3, SO_2 and H_2SO_4 aerosol, while Kulle et al. (1982) and Horvath et al. (1987) reached similar conclusions regarding a mixture of O_3 and H_2SO_4 aerosol.

More recently, Linn et al. (1994) used the 6.5 hour exposure protocol (see Section 11.4.3) to investigate the pulmonary function and symptom responses of normal, atopic and asthmatic subjects exposed to FA, 0.12 ppm O_3, 100 $\mu g/m^3$ H_2SO_4 aerosol (MMAD = 0.5 μm), and a mixture of the two pollutants. There were no differences between the responses to FA and H_2SO_4. However, both O_3 and O_3 + H_2SO_4 exposure induced respiratory symptoms and decrements in pulmonary function, and an increase in bronchial reactivity to methacholine. 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 were, however, several subjects who responded to the combined O_3 + H_2SO_4 exposure more strongly than the rest of the subject group, suggesting that there may be some individuals who are more susceptible to joint exposures than to O_3 alone.

Utell et al. (1994) investigated the pulmonary function and symptoms responses of healthy and allergic asthmatic subjects exposed for three hour to sodium

chloride (NaCl) or H_2SO_4 aerosol (100 µg/m^3), followed 24 hour later by a three-hour exposure to 0.08, 0.12 or 0.18 ppm O_3. Each subject was exposed to both aerosols at two of the three O_3 concentrations (four of the six possible exposure conditions) in an incomplete block design. The statistical analysis indicated that exposure to H_2SO_4 aerosol significantly altered the FVC response to exposure to 0.18 ppm O_3 following the H_2SO_4 aerosol preexposure only in the asthmatic but not the healthy subjects. However, although asthmatics reported more symptoms than healthy subjects did, there was no dose-response relationship between O_3 concentration and symptom intensity in either subject group. In most cases, the decrements in FVC for both groups fell within the typical reproducibility with repeated pulmonary function tests (±5%). Interpretation of this study is complicated by the fact that each subject was exposed to only four of the six exposure conditions. Thus, differences in the innate responsiveness of the subjects in the different groups could have impacted the results. Furthermore, the responses of both the asthmatic and nonasthmatic subjects were sufficiently variable that it is difficult to draw conclusions as to whether or not there is a difference in the responsiveness of healthy and asthmatic individuals under these exposure conditions. This difficulty is compounded by the fact that there was no FA control exposure, so it is impossible to determine whether the small responses observed in the asthmatic subjects were related to the exposures or to the underlying disease.

Frampton et al. (1995) used the same protocol (described in the preceding paragraph) as Utell et al. (1994) to investigate the responses of healthy and asthmatic subjects who were exposed for three-hour to 100 µg/m^3 of NaCl or H_2SO_4 aerosol on one day, and 24 hours later to either 0.08, 0.12 or 0.18 ppm O_3. The normal subjects had changes in pulmonary function under these exposure conditions that were very small, and did not follow a dose-response pattern. This led the investigators to conclude that exposure to H_2SO_4 aerosol had no meaningful effect on their responses to a subsequent O_3 exposure. As a group, the asthmatics tended to have larger pulmonary function responses when exposed to 0.18 ppm O_3 subsequent to the H_2SO_4 aerosol exposure, although there was large inter-individual variability in the responses. The O_3-associated decrement in FEV1 was about 3% larger after H_2SO_4 versus NaCl exposure. The small reduction in FEV1/FVC indicated that reduced maximal inhalation, and not bronchoconstriction, was the mechanism for the observed responses. Analysis of several subject characteristics, including age, gender, total IgE blood levels, airway responsiveness, and baseline lung function in asthmatics, showed that none predicted O_3 responsiveness.

Linn et al. (1997) compared the responses of 41 children (9-12 years) to controlled exposure to FA and a mixture of 0.1 ppm O_3 + 0.1 ppm SO_2 + 100 µg/m^3 of H_2SO_4 aerosol utilizing a four-hour intermittent exercise protocol. Contrary to expectations, there were no significant changes in pulmonary function with either exposure, nor was there a meaningful difference in symptoms between the two exposures. However, the subjects participating in this study resided in areas of the Los Angeles Basin (Downey, Rubidoux, Upland and Torrance) that typically have relatively high air pollution levels. The report does

not indicate during which season(s) the subjects were studied, and consequently the possibility of response attenuation (see Section 11.4.9.7) cannot be ruled out as an effect modifying factor.

Kagawa (1983; 1986) investigated responses of Japanese men to various mixtures of O_3, NO_2, H_2SO_4, and SO_2. Although the pollutant concentrations used in these studies are relevant to ambient levels, these data contribute little to selection of an ambient air quality standard for O_3 for several reasons: (1) some of the subjects were smokers, (2) there was no control exposure (Kagawa, 1986), (3) there were two different exercise protocols used (Kagawa, 1986), and (4) of greatest significance, the data were analyzed with multiple t-tests, without evidence for consideration of the effect of multiple comparisons on the P level.

In summary, although there are isolated findings to the contrary, the data do not support the likelihood of clinically meaningful interactions between O_3 and SO_2 or H_2SO_4 aerosols at ambient concentrations in human subjects. Observed responses at the pollutant concentrations studied to date appear to be attributable to the O_3 in the mixture.

Table 11-18: Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm O_3 0.10 ppm SO_2	1 hr, mouthpiece, IE, 45 min (15 min rest/15 min ex/15 min rest) exposure to air or O_3, followed by 15 min exposure (15 min ex) to O_3 or SO_2, (V_E=30 l/min)	Allergic asthmatics, medications withheld for at least 4 hr before exposures, (8 M, 5 F), 12-18 yrs	When the first exposure was to O_3, pulmonary function responses to SO_2 were attenuated. Decrements in FEV1 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 ppm O_3 0.12 ppm O_3 0.18 ppm O_3 100 µg/m^3 NaCl 100 µg/m^3 H_2SO_4	3 hr exposure to NaCl or H_2SO_4 aerosol, followed 24 hr later by 3 hr exposure to O_3, IE, (10 min ex/20 min rest), (V_E=30-36 l/min for asthmatics; 33-40 l/min for nonasthmatics)	Nonsmoking asthmatics (10 M, 20 F; 18-45 yrs) and nonasthmatics (16 M, 14 F; 21-42 yrs)	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 after exposure to 0.18 ppm O_3. Asthmatics had no significant changes in FEV1 with any O_3 exposure, but did have more symptoms than nonasthmatics. Asthmatic subjects had variable severity of disease, as indicated by medication use. The asthmatic group included 2 subjects who used no regular medications, 16 who used inhaled beta agonists only, 11 who used both inhaled beta agonists and oral theophylline, and 1 who used only oral theophylline.	Frampton et al. 1995 Utell et al. 1994
0.1 ppm O_3 0.1 ppm SO_2 100 µg/m^3 H_2SO_4	4 hr IE (15 min ex/15 min rest), (V_E=22 l/min)	Healthy (N=15), Asthmatic (N=26), 9-12 yrs	Spirometry, PEFR and symptoms score showed no meaningful changes between any of the conditions for the total study population. The symptoms scores reported by a subset of asthmatics/allergics were positively associated with the inhaled concentration of H_2SO_4 ($p<0.01$).	Linn et al. 1997

Table 11-18 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm O_3 100 µg/m^3 H_2SO_4	6.5 hr, IE (50 min ex/10 min rest per hr), two consecutive days, (V_E=29 l/min)	Nonsmokers, nonasthmatics (8 M, 7 F; 22-41 yrs), asthmatics (13 M, 17 F; 18-50 yrs),	Exposure to O_3 or O_3 + H_2SO_4 induced similar, significant decrements in forced expiratory function in both subject groups. Differences between O_3 and O_3 + H_2SO_4 were, at best, marginally significant. O_3 was the more important pollutant for inducing respiratory effects. A few subjects in both groups were more responsive to O_3 + H_2SO_4 than to O_3 alone. Asthmatic subjects developed more symptoms of respiratory irritation with O_3 exposure than nonasthmatics.	Linn et al. 1994
0.12 ppm O_3 0.30 ppm NO_2 70 µg/m^3 H_2SO_4 0.05 ppm HNO_3	1.5 hr, IE (15 min rest/15 min ex), on 2 consecutive days, (V_E=23.2 l/min)	Nonsmoking asthmatics, (15 M, 7 F), 12-19 yrs	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 ppm O_3 1200-1600 µg/m^3 H_2SO_4	2 hr, IE (20 min rest/20 min ex), (V_E=30-32 l/min)	Healthy nonsmokers, (9 M), 19-29 yrs	No significant effects of exposure to O_3 alone or combined with H_2SO_4 aerosol.	Horvath et al. 1987
0.20 ppm O_3 0.10 ppm NO_2 127 µg/m^3 H_2SO_4	90 min, IE (15 min ex/15 min rest), (V_E=32 l/min)	Asthmatic adolescents (17 M, 7 F), nonsmokers, 11-18 yrs	Similar responses occurred after $H_2SO_4/O_3/NO_2$, O_3/NO_2 and clean air exposures.	Linn et al. 1997
0.10 ppm O_3 0.10 ppm SO_2 100 µg/m^3 H_2SO_4	4 hr, IE, (15 min ex/15 min rest), (V_E=22 l/min)	Healthy (N=15), Asthmatics (N=5), Allergic without asthma (N=21) All nonsmokers, 9-12 yrs, (22 girls/19 boys)	No spirometric effects with any exposure. Trend for FEV1 to increase with increased acid dose. Symptom scores increased slightly with acid dose in asthmatics.	Linn et al. 1997

Table 11-18 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30 ppm O_3 (KI) 100 µg/m^3 H_2SO_4	2 hr O_3 followed by 4 hr H_2SO_4 15 min ex at 1 hr before end of exposure (V_E=30-35 l/min)	Healthy, (7 M, 5 F), 19-28 yrs, plus one 46 yrs	No significant pulmonary function changes or changes in methacholine reactivity with any exposure. No evidence of synergism or interaction between O_3 and H_2SO_4.	Kulle et al. 1982
0.37 ppm O_3 (KI) 0.37 ppm SO_2 100 µg/m^3 H_2SO_4	2 hr, IE (15 min rest/15 min ex), (V_E=twice resting)	3 smokers, 7 ex-smokers, 9 never-smokers (14 M, 5 F) 19-54 yrs	Compared responses to the mixture to responses to FA exposure. No O_3 alone exposure. Mean decrements in FVC and FEV1 with exposure to the mixture were 2.8% and 3.7%, respectively. This is similar to literature values for a similar exposure to 0.37 ppm O_3 alone.	Kleinman et al. 1981
0.40 ppm O_3 (KI) 0.4 ppm SO_2	2 hr, IE (15 min rest/15 min ex), (V_E=30 l/min)	Healthy nonsmokers, (8 M), 19-32 yrs	Pulmonary function decrements were similar for the O_3 and SO2/O_3 conditions. High temperature (35º C) and humidity (85%) did not alter observed responses.	Bedi et al. 1982
0.40 ppm O_3 (KI) 0.4 ppm SO_2	2 hr, IE (15 min rest/15 min ex), (V_E=30 l/min)	Healthy (9 M), 2 with history of allergies, 1 had asthma as a child, 18-27 yrs	Exposure to O_3 and SO_2/O_3 induced similar decrements in FVC, FEV1, and similar types and intensities of symptoms. During the last exercise period, both O_3 and SO_2/O_3 induced reduction in V_T and a compensatory increase in f_R, with no change in V_E.	Bedi et al. 1979
0.37 ppm O_3 (KI) 0.37 ppm SO_2	2 hr, IE (15 min rest/15 min ex), (V_E=2 to 2.5 times resting), O_3 exposure and O_3/SO_2 exposure on consecutive days	Study 1: Los Angeles residents (4 normal, 4 sensitive) Study 2: Montreal residents (N=4), sensitive Los Angeles residents (N=5) Age, gender, smoking history not given	Study 1: No significant changes in lung function with exposure to O_3 alone. Small, statistically (but not functionally significant) decrements in FEV1 in the sensitive subjects after the SO_2/O_3 exposure. Results probably due to the consecutive day exposure design rather than to an interaction of O_3 and SO_2. Study 2: No significant changes in either the Los Angeles or Montreal subjects.	Bell et al. 1977

Table 11-18 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

O_3 Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30/0.35 ppm O_3 1.0 ppm SO_2	2 hr, IE, (10 rest/30 min ex), (V_E=38 l/min)	Healthy nonsmokers (21 M), 19-28 yrs	Decrements in FVC and FEV1 were larger following exposure to O_3 alone than following exposure to SO_2/O_3 exposure. There was no significant change in SR_{aw} with any exposure.	Folinsbee et al. 1985
0.37 ppm O_3 (KI) 0.37 ppm SO_2	2 hr IE, (15 min rest/15 min ex), (V_E=twice resting)	Nonsmokers (8 M), 19-25 yrs	SO_2 had no significant effect on pulmonary functions. Decrement in FEF25-75% was larger following SO_2/O_3 (~35%) than following exposure to O_3 alone (~15%). There was considerable variability among subjects.	Hazucha and Bates 1975
0.15 ppm O_3 0.15 SO_2 0.15 ppm NO_2	2 hr, IE, (15 min ex/15 min rest), (WL=50 W)	Healthy (7 M), 19-23 yrs, 1 smoker	No differences between responses to exposure to O_3 alone and exposure to O_3 in combination with SO_2 and/or NO_2.	Kagawa 1983

11.4.12.3 Ozone Plus Nitrogen-containing Pollutants

A few early studies (e.g., Folinsbee et al. 1981; Hackney et al. 1975) investigated the effects of exposure to mixtures of air pollutants that included relatively high concentrations of nitrogen species as well as O_3, and found that the observed responses could be attributed to the O_3 alone. Subsequently, others have investigated whether exposure to O_3 and NO_2, either sequentially or concurrently, alters responses to O_3. Adams et al. (1987) reported on the responses of 40 male and female nonsmokers exposed to FA, 0.3 ppm O_3, 0.6 ppm NO_2 and the combination of 0.3 ppm O_3 and 0.6 ppm NO_2. The only significant difference between the O_3 and NO_2 + O_3 exposures was that SR_{aw} was lower following exposure to NO_2 + O_3, compared to that following O_3 alone.

Koenig et al. (1988a) compared the responses of healthy and allergic asthmatic adolescents to one-hour exposures to FA, 0.3 ppm NO_2, 0.12 ppm O_3 and a mixture of 0.3 ppm NO_2 + 0.12 ppm O_3, with intermittent exercise. None of the exposures induced significant changes in any measure of pulmonary function in either subject group. The study was subsequently repeated and extended with a group of adolescent asthmatics who were exposed to FA, 0.12 ppm O_3 + 0.3 ppm NO_2, the two oxidants + 70 $\mu g/m^3$ H_2SO_4, or the two oxidants + nitric acid (HNO_3) for 90 min on 2 consecutive days while performing light, intermittent exercise (Koenig et al. 1994). None of the exposure conditions induced statistically significant changes in any measured parameter of pulmonary function.

Morning fog followed by a moderate O_3 concentration during the afternoon is a common occurrence in coastal California. Aris et al. (1991) hypothesized that exposure to acidic fog followed by O_3 exposure would result in larger decrements in pulmonary function compared to water fog of neutral pH followed by O_3. Healthy adults were exposed for three hour to 0.2 ppm O_3 starting one hour after a two-hour exposure to FA or to fog consisting of either water or 0.54 mg/mL nitric acid. The results suggest that both types of fog ameliorated the pulmonary function effects of the subsequent O_3 exposure.

Aris et al. (1993a) extended their investigation into responses to nitric acid by comparing the responses of healthy, nonsmoking subjects to FA, 500 $\mu g/m^3$ HNO_3 gas + 0.2 ppm O_3, or 0.2 ppm O_3 alone. Although mean FEV1 and FVC decreased, and mean SR_{aw} and respiratory symptom scores increased for both HNO_3 + O_3 and for O_3 alone, there were no significant differences between the two conditions. Similarly, there were no statistically significant differences between the HNO_3 + O_3 and the O_3 exposures in the cellular or biochemical constituents measured in either BAL or proximal airway lavage fluids, or in bronchial biopsy specimens. These results, along with those of Aris et al. (1991) discussed above, suggest that these concentrations of HNO_3, either in gaseous or aerosol form, do not alter responses to exposure to O_3 in healthy adults.

Hazucha et al. (1994) investigated the responses of nonsmoking females who underwent a sequential exposure protocol in which they inhaled 0.6 ppm NO_2 or

FA for two hour, followed three hour later by a two-hour exposure to 0.3 ppm O_3. Subjects rested in ambient air during the inter-exposure period. There were slightly larger decrements in FEV1 and FEF25-75% following the NO_2/O_3 exposure than following the FA/O_3 exposure. There were no differences in the changes in SR_{aw} or symptomatology between the two exposure conditions. However, the $PD_{10}FEV1$ concentration for methacholine following the NO_2/O_3 exposure (1.7 mg/mL), was significantly smaller than that following the FA/O_3 exposure (5.6 mg/mL), with both significantly smaller than the baseline methacholine $PD_{10}FEV1$ of 14.3 mg/mL. The results suggest that preexposure to NO_2 potentiated airway responsiveness to the subsequent O_3 exposure.

More recently, Jenkins et al. (1999) reported on the responses of mild asthmatics with allergy to dust mites who completed bronchial allergen challenges immediately after exposures to O_3, NO_2 and a mixture of NO_2 + O_3 (NO_2/O_3). There were two exposure scenarios: 1) six-hour exposure to FA, 0.1 ppm O_3, 0.2 ppm NO_2 and 0.2 ppm NO_2 + 0.1 ppm O_3, and 2) three-hour exposure to FA, 0.2 ppm O_3, 0.4 ppm NO_2 and 0.4 ppm NO_2 + 0.2 ppm O_3. The total doses of O_3 and NO_2 were equivalent for the two exposure scenarios. None of the six-hour exposures altered responses to the allergen challenge compared to the FA condition. In contrast, all of the three-hour exposure conditions significantly decreased PD_{20FEV1} compared to FA, although there were no differences among the three pollutant exposures, suggesting that interaction between these pollutants and allergens may be dependent on pollutant concentration, rather than on total inhaled pollutant dose. There was no evidence for interaction or synergism between O_3 and NO_2.

In summary, notwithstanding isolated findings to the contrary, the available evidence suggests that any interaction or synergy between O_3 and nitrogen-based air pollutants in the range of ambient concentrations is insignificant. Research also suggests that pre-exposure to fog may mitigate the effects of subsequent O_3 exposure, although inhalation of HNO_3 gas had no effect on responses to O_3.

Table 11-19: Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm O_3 0.30 ppm NO_2	1 hr, mouthpiece, IE (15 min rest/15 min ex), (V_E=4-5 times resting, mean value = 32.5 l/min)	Healthy, nonsmokers (5 M/7 F), 12-17 yrs Asthmatics (9 M/3 F), 13-18 yrs	No significant changes in any pulmonary function with O_3 alone or O_3/NO_2. Two asthmatics used no regular medications, 5 used theophylline alone or in combination with beta agonist, and 5 used only beta agonists.	Koenig et al. 1988b
0.12 ppm O_3 0.30 ppm NO_2 70 µg/m^3 H_2SO_4 0.05 ppm HNO_3	90 min, IE, (15 min rest/15 min ex), (V_E=3 times resting), by mouthpiece	Allergic, asthmatic adolescents (11 M, 7 F), 12-19 yrs	Subject group includes a wide range of apparent asthma severity (0-4 regular medications). Analyzed with paired t-tests, although the significance level was adjusted to account for the multiple tests. No statistically significant changes in any pulmonary function with exposure to any of the pollutant mixtures, compared to FA.	Koenig et al. 1994
0.20 ppm O_3 500 µg/m^3 HNO_3	4 hr, IE, (50 min ex/10 min rest), (V_E=40 l/min)	Healthy, (7 M, 3 F), 19-41 yrs	Compared responses to exposure to O_3 alone with exposure to a mixture of HNO_3 and O_3. No differences between pulmonary function or cellular or biochemical constituents in BALF, or between bronchial biopsies after the two exposures.	Aris et al. 1993a
0.2 ppm O_3 0.4 ppm NO_2 0.1 ppm O_3 0.2 ppm NO_2	3 hr, IE (10 min ex/20 min rest), (V_E=32 l/min) 6 hr, IE (10 min ex/20 min rest), (V_E=32 l/min)	Atopic asthmatics (9 M, 2 F), 22-41 yrs	Exposure to NO_2 alone had minimal effects on FEV1. O_3 alone and O_3/NO_2 induced a greater decline in FEV1 in the 3 hr exposures (higher concentration) than a 6 hr exposure (at lower concentrations). Responses after the 6 hr O_3 and O_3/NO_2 exposures were not significant. Allergen challenge by inhalation significantly reduced the PD_{20FEV1} after the 3 hr exposures, but not the 6 hr exposures to O_3 and O_3/NO_2. There was no evidence that inclusion of NO_2 had an additive or potentiating effect on O_3.	Jenkins et al. 1999

Table 11-19 (cont.) Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.20 ppm O_3 500µg/m^3 HNO_3 or H_2O fog	5 hr total, IE (50 min ex/10 min rest), (V_E=40 l/min) 2 hr exposure to air, or HNO_3 or H_2O fog, followed by 1 hr break, and then by 3 hr exposure to O_3	Healthy nonsmokers, (6 M, 4 F), screened to have a minimum of 10% decrement in FEV1 after 3 hr exposure to 0.20 ppm O_3 with 50 min ex/hr	Exposure to HNO_3 or H_2O for followed by O_3 induced smaller pulmonary function decrements than air followed by O_3.	Aris et al. 1991
0.25 ppm O_3 0.30 ppm NO_2 30 ppm CO	2 hr, IE, (15 ex/15 rest), (V_E=twice resting)	Healthy, (N=6, gender not given) 22-41 yrs, several ex- and current smokers	No consistent changes attributable to exposure with exposure to O_3 alone, or in mixtures. . There was no evidence that inclusion of NO_2 or CO had an additive or potentiating effect on O_3.	Hackney et al. 1975

Table 11-19 (cont.): Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30 ppm O_3 0.30 ppm NO_2 200 µg/m^3 H_2SO_4	2 hr, including 20 min ex, (50 W)	Healthy adults (6 M), 2 smokers	Possible small decrease in SG_{aw}.	Kagawa J 1986
0.15 ppm O_3 0.15 ppm NO_2 200 µg/m^3 H_2SO_4	2 hr, including 60 min ex (50 W)	Healthy adults (6 M), 2 smokers	Possible small decrease in SG_{aw}.	
0.15 ppm O_3 0.15 ppm NO_2 0.15 ppm SO_2 200 µg/m^3 H_2SO_4	2 hr, including 60 min ex (50 W)	Healthy adults (3 M), 1 smoker	Possible small decrease in FEV1.	
0.30 ppm O_3 0.60 ppm NO_2	1 hr, mouthpiece, CE (V_E=70 l/min for men and 50 l/min for women)	Healthy nonsmokers, (20 M, 20 F), 21.4±1.5 yrs for F, 22.7±3.3 yrs for M	No differences between spirometric responses to O_3 and O_3/NO_2. Increase in SR_{aw} with O_3/NO_2 was significantly less that that observed after exposure to O_3 alone.	Adams et al. 1987
0.30 ppm O_3 0.60 ppm NO_2	2 hr exposure to NO_2 or FA, followed 3 hr later by 2 hr exposure to O_3, IE (15 min ex/15 min rest), (V_E=20 l/min/m^2)	Healthy nonsmokers, (21 F), 18-34 yrs	No significant effect of NO_2 exposure on any measured parameter. Sequential exposure of NO_2 followed by O_3 induced small, but significantly larger decrements in FEV1 and FEF25-75% than the FA/O_3 sequence. Subjects had increased airway responsiveness to methacholine after both the FA/O_3 and NO_2/O_3 exposure sequences, although that after the NO_2/O_3 sequence was significantly larger.	Hazucha et al. 1994
0.36 ppm O_3 0.36 ppm NO_2 0.75 ppm NO_2 0.36 ppm SO_2	2 hr resting	Healthy nonsmokers, (6 M, 6 F), 19-33 yrs	The absorbed fraction of O_3 increased relative to baseline with NO_2 and SO_2 co-exposure. The differences were explained by an increased production of O_3 reactive substrate in ELF due to inflammation.	Rigas et al. 1997

Table 11-19 (cont.): Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.50 ppm O_3 (KI) 0.50 ppm NO_2	2 hr, including 30 min ex during third half hr (V_E=40 l/min); 4 ambient conditions: (1) 25° C, 45% RH; (2) 30° C, 85% RH; (3) 35° C, 40% RH; (4) 40° C, 45% RH	Healthy, (8 M), 19-24 yrs	Both O_3 and NO2/O_3 exposure induced decrements in FVC, FEV1 and FEF25-75%. Responses to the mixture of NO_2 + O_3 were not different from those following exposure to O_3 alone. There was some evidence that concurrent heat exposure exacerbated the lung function responses.	Folinsbee et al. 1981
0.50 ppm O_3 (KI) 0.30 ppm NO_2 30 ppm CO	4 hr	Healthy, (4 M), all with history of smoking, 2 active smokers, 36-49 yrs	Few changes in any measured parameter. No evidence for interactive, additive or synergistic effects with the mixtures. Effects could be attributed to O_3.	Hackney et al. 1975

11.4.12.4 Ozone, Peroxyacetyl Nitrate, and More Complex Mixtures

Peroxyacetyl nitrate (PAN) is a photochemical oxidant air pollutant that is known to be a strong eye irritant. Several studies investigated whether or not PAN alters pulmonary function and symptom responses to O_3 when both are inhaled simultaneously compared to separately. Studies by Horvath et al. (1986) and Drechsler-Parks et al. (1984) reported on the responses of young females and males, respectively, exposed for two-hour to FA, 0.48 ppm O_3, 0.27 ppm PAN, and 0.48 ppm O_3 + 0.27 ppm PAN. The results suggested that there was an interaction between O_3 and PAN, demonstrated by the significantly larger (about 10%) decrements in FVC, FEV1 and FEF25-75% following the combined exposure compared to the exposure to O_3 alone. Symptom reports indicated that the combined exposure induced greater subjective stress than did exposure to O_3 alone.

Drechsler-Parks et al. (1989) compared the responses of older men and women (51-76 yrs) with those of young men and women (19-26 yrs) who completed two-hour 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. The observed effects could be attributed solely to the O_3 in the exposures. In contrast to the studies by (Drechsler-Parks et al. 1984) and (Horvath et al. 1986), there was no evidence for an interaction between PAN and O_3. A likely explanation for this is that the PAN concentration in this study was slightly less than half that used in the earlier studies. Assuming that the PAN-induced augmentation of the O_3 response is linear, the expected additional effect (on lung function) of including PAN in the exposure mixture used in this experiment would be expected to be less than 5%, which would be within the variability of the pulmonary function measurements, and therefore not likely to be detectable.

Drechsler-Parks et al. (1987b) investigated the possible influence of PAN on O_3-associated response attenuation in a group of healthy young adults who completed two-hour exposures to 0.45 ppm O_3 + 0.3 ppm PAN on five consecutive days. Attenuation and loss of attenuation of pulmonary function and symptoms responses occurred with the same time course and pattern as has been reported for consecutive daily exposures to O_3 alone (see Section 11.4.9). The results suggest that PAN does not alter development or loss of O_3-induced attenuation.

In summary, based on the results of a few controlled studies, there is evidence that concurrent exposures to high concentrations of PAN and O_3 result in pulmonary function and symptom responses somewhat larger than those observed following exposure to the same concentration of O_3 alone. However, typical ambient PAN concentrations are considerably lower than those utilized in any of these studies. Consequently, even if O_3 and PAN do interact in their effects on pulmonary function at high concentrations, it is unlikely that PAN contributes significantly to adverse health effects in healthy young and older adults at concentrations in the ambient range.

Table 11-20: Responses of Human Subjects to Mixtures of Ozone and Peroxyacetylnitrate

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.485 ppm O_3 0.27 ppm PAN	2 hr, IE (20 min ex/20 min rest), (V_E=25 l/min)	Healthy nonsmokers, (10 F), 19-36 yrs	Exposure to the mixture of PAN/O_3 induced decrements in FVC and FEV1 averaging 10% greater than measured after exposure to O_3 alone.	Horvath et al. 1986
0.43 ppm O_3 0.30 ppm PAN	2 hr, IE (20 min rest /20 min ex), (V_E=27 l/min)	Healthy nonsmokers (3 M, 5 F), mean age 24 yrs	No differences between responses to exposure to O_3 alone and PAN/O_3	Drechsler-Parks 1987
0.45 ppm O_3 0.60 ppm NO_2 0.13 ppm PAN	2 hr, IE (20 min rest/20 min ex), (V_E=25 l/min)	Healthy nonsmokers, (16 M, 16 F), including 16 subjects age 19-26 yrs, and 16 subjects 51-76 yrs	No differences between responses to O_3 alone, NO_2/O_3, PAN/O_3 or PAN/NO_2/O_3.	Drechsler-Parks et al. 1989
0.45 ppm O_3 0.30 ppm PAN	2 hr, IE (15 rest/20 min ex), (V_E=27 l/min)	Healthy nonsmokers (10 M), 18-32 yrs	Both O_3 and PAN/O_3 induced significant decrements in FVC, FEV1, and FEF25-75%. The decrements after PAN/O_3 exposure averaged about 10% larger than after exposure to O_3 alone, suggesting an interactive effect.	Drechsler-Parks et al. 1984

11.4.12.5 Ozone Plus Particulate Matter

There have been few human exposure studies on mixtures of O_3 with particulate matter, with the exception of H_2SO_4 aerosol (discussed above in Section 11.4.12.2). Stacy et al. (1983) published a large study that was designed as a preliminary screening survey intended to direct future research plans. The investigators divided 231 normal male subjects into 20 groups of 9-15 individuals. Each subject participated in only one experiment. Exposure conditions investigated included FA, 0.4 ppm O_3, 0.75 ppm SO_2, 0.5 ppm NO_2, 100 µg/m^3 H_2SO_4, 133 µg/m^3 $(NH_4)_2SO_4$, 116 µg/m^3 NH_4HSO_4, or 80 µg/m^3 NH_4NO_3 as single pollutant conditions, and as mixtures of each gas with each of the aerosol pollutants. The mass median diameter of all aerosol particles was 0.55 µ, with extremes of 0.20 to 0.94 µ. Exposures were four-hour in duration, and the subjects performed two 15-min periods of moderate exercise during exposure. The groups exposed to O_3 or mixtures including O_3 demonstrated significant differences in SR_{aw}, FVC and FEF50%. None of the aerosols, SO_2 or NO_2 alone or mixed with aerosols induced significant alterations in any endpoint measured. There was no indication of interaction between any of the pollutants studied, and observed effects could be attributed solely to O_3.

A recent study (Brook et al. 2002) reported a significant increase in brachial artery vasoconstriction in healthy adults exposed for two hours to a mixture of 150 µg/m^3 concentrated ambient particles (CAPS) plus 0.12 ppm O_3 at rest, compared to following two hours of filtered air exposure. Unfortunately, the study did not include exposures to O_3 or CAPS singly, making it impossible to determine which pollutant was responsible for the observed effect, or whether exposure to both is required.

Table 11-21: Responses of Human Subjects to Mixtures of Ozone and Particulate Matter

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm O_3 153 µg/m^3 PM2.5	2-2.5 hr resting	Healthy nonsmokers, (15 M, 10 F), 18-50 yrs	Neither systolic nor diastolic pressure was affected by the pollutants, despite a significant constriction of the brachial artery, and a reduction in brachial artery diameter, compared to following FA exposure ($p<0.03$). Flow- and nitroglycerin-mediated brachial artery dilatation were unaffected by exposures to FA or the mixture of O_3+CAPS. There was no exposure to O_3 alone.	Brook et al. 2002
FA 0.40 ppm O_3 0.75 ppm SO_2 0.50 ppm NO_2 100 µg/m^3 H_2SO_4 133 µg/m^3 $(NH_4)_2SO_4$ 116 µg/m^3 NH_4HSO_4 80 µg/m^3 NH_4NO_3	4 hr, including two exercise bouts	Healthy, nonsmokers (231 M), 18-40 yrs, divided into 20 groups (N=9-15/group)	Each subject participated in only 1 exposure. Groups exposed to O_3, or gaseous or aerosol mixtures including O_3 had decrements in FVC, FEF50, and significant increases in SR_{aw}. None of the aerosols alone, NO_2 or SO_2 alone, or mixtures of aerosols and SO_2 and/or NO_2 induced significant effects.	Stacy et al. 1983

11.4.12.6 Animal Studies

Since most people are exposed to several air pollutants simultaneously or sequentially, animal studies that reproduce these interactions can represent more realistic environmental conditions than studies with O_3 alone. Pollutants can interact toxicologically in three basic modes: additive, more than additive (synergistic), or less than additive (antagonistic). Taken together, the studies suggest that the types of interactions produced with O_3 and co-occurring pollutants are dependent on many factors. Investigations of sulfuric acid and O_3 co-exposures indicate that the type interaction is dependent on the health endpoint, composition of the aerosol, and size of the aerosol. A further complication is that the magnitude of the sulfuric acid/O_3 interaction is not always related to the exposure concentrations of the constituent pollutants. Striking synergistic interactions have been observed with acute exposures to O_3/ultrafine sulfuric acid aerosol combinations (Kimmel et al. 1997) and O_3/sulfuric acid layered on metal particulate (Chen et al. 1991). Interactions of O_3 and NO_2 have produced antagonistic or synergistic effects, depending on factors such as exposure concentrations used, animal species tested, and health endpoint examined. One of the more sensitive measures of O_3/NO_2 interactions utilized a bacterial infectivity model in which 15-day exposure to a simulated urban pollutant atmosphere (baseline of 0.5 ppm NO_2 with peaks of 1.0 ppm, and a baseline of 0.05 ppm O_3 with peaks of 0.1 ppm) produced a synergistic interaction with regard to mortality in mice (Graham et al. 1987). With particulate matter/O_3 co-exposures, the type of interaction produced has been shown to depend on the O_3 concentration used, the organic content of the particulate, and the endpoint measured. Of note, potentiation of O_3 injury has been observed with co-exposure to urban-type dusts (Vincent et al. 1997; Bouthillier et al. 1998; Adamson et al. 1999), and preexposure to O_3 followed by toxic particle instillation resulted in a marked retention of the toxic particles in small airways (Pinkerton et al. 1989).

11.4.13 Ozone Plus Allergens: Exacerbation of Allergic Airway Disease

11.4.13.1 Introduction

The prevalence of allergic diseases, such as rhinitis ("hayfever") and asthma, has increased markedly during the past fifty years (Nicolai 2002). This is a worldwide phenomenon as indicated by increased "hay fever" incidence in Japan from 3.8% in 1974 to over 10% currently (Salvi 2001). The reported incidence of allergic rhinitis in the UK is currently reported to be 24%. Some references put the percentage of allergic individuals as high as 30% in Western countries. In fact, approximately 10% of schoolchildren in the United States are asthmatic, while in the 1950's only about 1% of children were asthmatic (Salvi 2001). There is evidence that could be considered supportive of the notion that the increase in allergic respiratory diseases, such as asthma and rhinitis may be related to increased atmospheric concentrations of air pollutants, such as O_3 (D'Amato et al. 2002). In fact, the World Health Organization and the National Institutes of Health classifies air pollution as among the possible factors contributing to and triggers that lead to development of asthma (D'Amato and Liccardi 1998).

A brief description of the allergic syndromes of rhinitis and asthma is warranted. Both syndromes are immune-mediated hypersensitivity diseases, in which a non-protective immune response is stimulated by substances in the environment called allergens. The most common allergens are generally plant pollens, dust mites, and molds – substances that are difficult to avoid. Animal danders also comprise a significant source of allergens for many allergic patients. The patient with rhinitis has clinical signs that are related to the nasal cavity, with sneezing a common sequelae. Individuals with “hay fever” often show both ocular and nasal involvement. Asthma is a lung disease characterized by increased bronchial hyperreactivity, pulmonary inflammation, and in the chronic state, airway remodeling. Most forms of asthma are found in individuals who are “atopic”, meaning that they have a genetic propensity to produce IgE antibodies against inhaled allergens.

Allergic diseases are characterized by the development of IgE antibodies that react with specific allergens. These IgE antibodies affix firmly to mast cells in the tissues and upon reexposure to the same allergen that induced the initial IgE synthesis, the mast cells release mediators from their granules. The mediators (histamine, bradykinin, eosinophil chemotactic factors, etc.) cause the resultant clinical signs, such as lacrimation, nasal discharge, itching, and sneezing. In asthmatic patients, additional effects include bronchial smooth muscle contraction, leading to a decrease in expiratory volume. arachidonic acid cascades that are stimulated by allergen interaction with IgE on mast cells result in production of leukotrienes and prostaglandins, which have longer lasting smooth muscle contractile and chemotactic properties than the mediators initially released. The stimulus for IgE production evokes a response from T-lymphocytes, called a T-helper type 2 (Th2) response. This means that certain cytokines are produced that have an effect on B-lymphocytes resulting in further stimulation of IgE production. The presence of a Th2 cytokine profile is considered to be indicative of an allergic response (Gould et al. 2003).

While the initial stimulus for the asthmatic response is IgE-mediated, chronic exposure to inhaled allergens generates cellular influx and remodeling of the bronchial smooth muscle, with increased mucus cell production. A common method to evaluate the presence of IgE antibodies (and hence allergic/atopic sensitization to allergens) is the skin prick or intradermal test. In this test, a small amount of each potential allergen is injected into the skin. If the area develops a wheal within a few minutes, the presence of IgE-mediated mast cell degranulation and consequently allergy to the substance, is confirmed. This is a useful screening procedure to determine to which allergens (if any) an individual is allergic. It does not, however, relate directly to the presence of asthma, as one can be allergic without having asthma. Atopic asthmatics typically have positive responses to one or more allergens in skin test panels (Gould et al. 2003).

As stated above, in recent years the incidence of allergic/atopic asthma has been increasing dramatically in industrialized countries. In fact, the number of asthma cases has more than doubled since 1998, resulting in an estimated 5,500 deaths and many thousands of hospital visits yearly in the United States (Redd and

Mokdad 2002), representing a major public health problem. Despite the recent improvement in air quality and the decrease in public smoking in many areas of the country, exposure to environmental air pollutants is frequently targeted as a factor in asthma exacerbation. In fact, a variety of causes have been suggested for the increase in asthma, including: increases in vehicular exhaust, increased levels of air pollutants (O_3, nitrogen dioxide, and particulate matter), exposure to tobacco smoke, respiratory viral infection, and the recently coined "hygiene hypothesis". This latter hypothesis attributes the increase in asthma to movement of populations away from the traditional farm environment where childhood exposure to microbes modulates the immune response away from the allergic phenotype (Matricardi et al., 2001). While the hygiene hypothesis may provide insight into the immunological control of allergic responses in the lung, it is clearly not the single most important factor causing the "asthma epidemic". One has only to review the historical epidemiological reports that link asthma with air pollution to appreciate the impact of air quality on this disease (Folinsbee 1993; Koren 1995). This section focuses on data that has been published linking air pollution and respiratory allergy, with particular emphasis on O_3.

11.4.13.2 Epidemiological Studies

11.4.13.2.1 Historical Studies

Concern as to whether there is an association between increased incidence of asthma and air pollution is not new. In 1948 in Japan a syndrome called "Tokyo-Yokohama Asthma" was described. This syndrome occurred between September and May in previously normal adults who had recently moved to the highly industrialized area near Tokyo and Yokohama. The disease, characterized by a chronic nocturnal cough, wheezing, and shortness of breath, was rapidly progressive while the patient resided in the area and showed improvement when the patient left the area (Smith et al. 1964). A group of patients composed primarily of U.S. military personnel and their dependents were studied. In all there were 426 reported cases, 28% of which had shown some previous allergic symptoms. Air stagnation had an effect on the syndrome, with disease exacerbation being most evident following days of stable air. Indeed, in Tokyo, the SO_2 often measured 0.072 ppm. and the smog layer frequently reached up to 1,000 feet. In another study, Oshima et al. (1964) examined the indigenous population in the Tokyo-Yokohama (T-Y) area and compared these individuals to those in Niigata area, where air pollution concentrations were much lower. That study concluded that workers in the T-Y area had an increased incidence of respiratory disease. Moreover, the increase in disease among cigarette smokers and patients with a history of allergic disease hinted that air pollution might not only stimulate new allergic disease, but also exacerbate allergic conditions that were already present.

Another early study in Japan focused on school children from three severely polluted cities and compared these with one unpolluted city (Saku city) (Yoshida et al. 1974). The sources of pollution included pulp and paper mills (Fuji city), power plants, and petroleum refineries (Chiba prefecture), and urban transportation (Tokyo). The presence of allergic disease was established using

peripheral blood eosinophil counts, intradermal skin tests, and serum IgE levels. Increases and/or positive responses in all of these parameters are indicative of an allergic response. Results showed that Fuji city had a prevalence rate of asthma that was 2.19% compared with 0.94% in Saku city. Similarly, the prevalence rate for Tokyo was 2.74%. These data are from the early 1970's.

Other early epidemiological studies include a Nashville study, which sought to correlate the frequency, and severity of asthma attacks with the level of air pollution in Nashville, Tennessee. This 1961 study showed that there was a direct correlation between the SO_4 concentration in the air and the asthma attack rate (Zeidberg et al. 1961).

For over forty years the increase in the O_3 concentrations in the Los Angeles area has been followed, and associations between high O_3 levels and acute episodes of respiratory distress, including asthma have been documented. Hospital admissions were correlated with O_3 levels in a 1966 report by Sterling et al. (1966). Another study (Whittemore 1980) focused on asthma patients, and had each of them keep a diary of their asthma symptoms. Air pollution data was supplied by the Los Angeles County Air Pollution Control District. Indeed, on days when oxidant concentrations were high, a significant number of patients reported asthma attacks.

11.4.13.2.2 Recent Epidemiological Studies

To study the relationship between the prevalence of atopy and photochemical air pollutants, a cross-sectional epidemiological survey was performed in 2604 primary school children living in seven communities in France. The gaseous air pollutants (SO_2, NO_2, and O_3) were measured during a two month period. Skin prick tests were performed on the children and used to evaluate atopy. It should be noted that this study did not evaluate the presence of asthma or related pulmonary symptoms, but rather focused solely on skin sensitization. The results did not demonstrate any association between atopy and mean levels of the three air pollutants (Charpin et al. 1999).

In another study of children exposed to different levels of air pollution in Italy, not only skin prick tests for atopy, but also respiratory function tests were performed. Comparison of Milan, with high levels of air pollution (107 children), with Erba, a small rural town with low air pollution (113 children) demonstrated no link between reduced lung function and the presence of atopy (Centanni et al. 2001).

11.4.13.2.3 Controlled Human Exposure Studies

Several studies have purposefully exposed human volunteers to various levels of O_3 and other pollutants (NO_2 and SO_2) and then challenged them with inhaled allergen while monitoring pulmonary function. Several studies have demonstrated an increased airway response to inhaled allergen following exposure to air pollutants. For example, Molfino et al. (1991) showed that exposure to 120 ppb O_3 for 1 hour increased the bronchial sensitivity of asthmatics to ragweed challenge, as demonstrated by a decrease in the amount of allergen needed to induce a defined reduction in the FEV1. In another study,

subjects with mild stable allergic asthma, allergic rhinitis, and normal subjects were exposed to either 250 ppb or air for 3 hours (Jorres et al. 1996). Response to methacholine and response to allergen were measured after 1 and 3 hours respectively. Subjects with mild asthma had decrements in FEV1 averaging 12.5% (+/- 2.2%) after O_3 exposure compared with air-exposed subjects ($p<0.001$). Those with allergic rhinitis also showed a significant difference. In contrast to these studies, Ball et al. (1996) demonstrated that pre-exposure to O_3 did not alter the amount of allergen necessary to induce a 15% reduction in the FEV1.

Jenkins et al. (1999) examined the effect of pollutant dose with exposure to mixtures of O_3 and NO_2. Eleven nonsmoking mildly asthmatic patients completed two protocols. In the first protocol the patients were exposed for 6 hours to air, 100 ppb O_3, 200 ppb NO_2, and the combination of 100 ppb O_3 and 200 ppb NO_2. After exposure, the patients were challenged via aerosol with increasing doses of *D. pteronyssinus* (house dust mite) allergen. In the second protocol, the exposures to pollutants were only 3 hours in duration, but the concentrations of pollutants doubled (200 ppb O_3, 400 ppb NO_2, and 200 ppb O_3 and 400 ppb NO_2), resulting in an equivalent concentration X time product. These experiments demonstrated that exposure of mild atopic asthmatics for 3 hours to 200 ppb O_3 and 400 ppb NO_2 alone or in combination resulted in a significant increase in lung sensitivity to inhaled allergen, although the effects were not additive. Moreover, the exposure for a shorter time to a higher concentration of pollutant was more effective in increasing the airway responsiveness to allergen challenge. Exposure for 6 hours to low pollutant concentrations did not cause any statistically significant difference between pollutant exposures when compared to air. However, the 3 hour exposure to higher pollutant concentrations showed a significant reduction in $PD_{20}FEV1$ for pollutant groups when compared with air exposure.

Accumulation of eosinophils in airway mucosa and/or lavage fluid is considered to be a hallmark of allergic disease. Thus, to determine the effect of 0.16 ppm O_3 on allergic airway inflammation, Peden et al. (1997) exposed eight asthmatic patients with house dust mite sensitivity to O_3 or clean air (with a 4 week washout period) and performed bronchoscopy 18 hours later. Results showed that the O_3 exposure was associated with increased eosinophilic inflammation in the lower airways of these patients, as compared with clean air. This study demonstrated that O_3 exposure can enhance an ongoing allergic exacerbation.

Hiltermann et al. (1999) compared use of induced sputum to bronchoalveolar lavage (BAL) as sampling methods for evaluation of cellular and cytokine responses to O_3 in asthmatics. There was a high correlation between eosinophil counts in the sputum and in the BAL fluid. In both samples, the levels of eosinophils were elevated after exposure to 0.4 ppm O_3 for 2 hours. In addition, IL-8 and ECP were elevated and correlated between the two samples.

The synergy of O_3 and allergen in increasing the duration of the asthmatic response was demonstrated by Vagaggini et al. (2002) in a study that utilized 12 subjects with mild persistent untreated asthma. After initial allergen challenge

tests demonstrated an early and a delayed response to allergen challenge, these patients were exposed to allergen aerosol and then 24 hours after allergen exposure they were exposed to O_3 or air for 2 hours. Pulmonary function tests were performed before and after these air or pollutant exposures. Sputum was examined for IL-8 and for cell numbers and types. The results showed that the exposure to O_3 potentiated the eosinophilia, but did not alter the production of IL-8.

A study (Holz et al. 2002) was performed in human subjects to evaluate and compare the allergic response after either single (125 ppb or 250 ppb) or multiple (4 consecutive days) 3 hour exposures to O_3 (125 ppb) or filtered air as control followed by allergen exposure the next day. In this study mast cell tryptase and histamine, eosinophil enumeration in sputum, and exhaled nitric oxide were evaluated. The subjects included 22 with rhinitis and 11 with mild asthma. The mean concentration of methacholine required to produce a 20% fall in FEV1 was significantly greater in the rhinitis group and the nitric oxide concentration during expiration was significantly lower in the rhinitis group than in the asthma group. Subjects in both groups had varying levels of IgE, with no statistical difference between groups. Results of this study were complex and not all of the parameters reached statistical significance. However, compared with filtered air the 4 consecutive day exposures to 125 ppb. O_3 was associated with a higher total cell count in sputum than the single exposure to 250 ppb O_3; neutrophils and eosinophils comprised the greater percentage of cells in both rhinitis and asthma patients. The early phase FEV1 response to allergen challenge showed that subjects with rhinitis showed a significant decrease in this parameter after 250 ppb ($p=.002$) and 4 times exposure to 125 ppb O_3 ($p=.04$). The subjects with asthma did not have a statistically significant change in this parameter. The authors estimated that the four times exposure led to inhalation of an amount of O_3 likely to be encountered during a week-long summer bike tour, and concluded that this exposure would likely enhance allergen responsiveness.

11.4.13.2.4 In Vitro Studies Using Human Tissues and Cells

To examine the mechanisms involved in pollutant enhancement of asthma studies have been performed using both tissues and cells derived from asthmatic and control subjects. For example, to evaluate the effect of O_3 and nitrogen dioxide on the permeability of bronchial epithelial cells of asthmatic compared with non-asthmatic subjects, Bayram et al. (2002) exposed cultured bronchial epithelial cells from both types of subjects to either air or pollutant for a period of 6 hours. Cell permeability to ^{14}C-labeled bovine serum albumin (BSA) was measured. The results showed that cells exposed to either 10 to 100 ppb of O_3 or 200 to 400 ppb. of NO_2 had increased epithelial permeability, which was significantly greater in asthmatics than in non-asthmatics. These results pose an interesting possibility that pollutants may indeed facilitate sensitization to inhaled allergens by increasing access to underlying dendritic cells required for antigen processing in the lung.

The possibility of interaction of airway sensitization and O_3 exposure for induction of airway hyperresponsiveness has been examined in isolated human airways

(Roux et al. 1999). The human bronchial sections came from cancer patients undergoing lung resection, which were subsequently used for *in vitro* testing. In this study, bronchial sections were sensitized to house dust mite allergen by incubation in serum from allergic patients. Then, the bronchial rings were exposed to 1 ppm O_3 for 20 or 40 minutes and the dose/response relationship between antigen dose and airway contraction was measured and subsequently compared with antigen responses of tissues that had not been exposed to O_3. The results indicated that exposure to O_3 potentiated the contractile response of the human bronchus to allergen challenge. Although *in vitro* studies such as this only involve a small part of the dynamic interactions that occur in asthma, this study demonstrate a synergy between a specific antigen and non-specific irritant on human bronchial responsiveness.

While a number of studies have demonstrated that O_3 is capable of enhancing the reactivity of asthmatic individuals to allergens to which they are sensitized, there is little data demonstrating that O_3 exposure alone can induce allergic sensitization in human subjects (Forster and Kuehr 2000). This topic has been investigated in more detail in studies using animal models.

11.4.13.2.5 Experimental Models

The value of animal models for evaluation of the effects of O_3 and other pollutants on allergic respiratory disease is that animals can be isolated and exposed to known components without concurrent environmental contaminants. In addition, specific sensitization with known, well-characterized allergens can be employed. There are, in fact, two possible effects of O_3 on the allergic/asthmatic response. First, whether O_3 plays a role in facilitation of sensitization. Secondly, whether O_3 elicits or worsens the asthmatic responses. While the latter has been studied with some human populations, animal models are more appropriate for evaluation of the sensitization arm of the allergic response. A variety of models have been used, including the guinea pig, mouse, brown Norway rat, monkey, and dog.

The first studies on the influence of air pollutants on allergic lung diseases/asthma were performed in ovalbumin sensitized guinea pigs by Matsumura (1970a). At that time the guinea pig was considered the preferred animal model for type 1 hypersensitivity, since they are very sensitive to allergen and display a similar shock organ response as humans, i.e. the lung is the primary target of IgE-mediated mast cell mediator release in both species. Matsumura studied the effects of O_3, nitrogen dioxide, and sulfur dioxide on experimentally induced respiratory allergy using this model. Animals were exposed to O_3 at 1, 5, or 10 ppm, nitrogen dioxide at 20, 40, or 70 ppm, or sulfur dioxide at 20, 60, 180, or 330 ppm for 30 minutes. This was followed by inhalation of either bovine serum albumin or ovalbumin for 45 minutes. The process was repeated five to seven times on different days. After the 5th or 6th antigen aerosol anaphylactic symptoms were observed. Mortality in the 10 ppm O_3 and antigen aerosol group was 33.3% compared with 0% in the ambient air control group. Skin tests with antigen showed that allergic sensitization had indeed been accomplished without parenteral sensitization. Results showed that

O_3 concentrations of 5 ppm and greater were able to enhance sensitization to inhaled antigen.

To further investigate the mechanisms responsible for this anaphylactic sensitization, Matsumura (1970b) labeled ovalbumin with ^{131}I and aerosolized it to guinea pigs after a 30 minute exposure to 8 ppm of O_3. After measuring the levels of the labeled ovalbumin in the blood, Matsumura concluded that a single 8 ppm 30 minute exposure to O_3 was sufficient to facilitate absorption of egg albumin from guinea pig lungs into the blood.

Twenty years later Sumitomo et al. (1990) built upon Matsumura's early work and showed that exposure to O_3 at 1, 3, and 5 ppm decreased the threshold for ovalbumin induced airway hyperresponsiveness from 0.5% to 0.02%. In this study O_3 inhalation also elicited enhanced airway hyperresponsiveness in previously sensitized guinea pigs. Thus, the authors concluded that O_3 can not only facilitate sensitization to allergen, but also enhance responses to provocation in the guinea pig model.

Vargas et al. (1994) used the guinea pig model to examine airway hyper-responsiveness induced by O_3, allergen, and combined O_3 and allergen exposure, as measured by the response to histamine (ED50 determination). In the ovalbumin sensitized guinea pigs histamine ED50 significantly decreased after antigen challenge. This decrease was greater in animals, which were exposed for one hour to 3 ppm O_3 compared to air control animals.

In another study using guinea pigs (Schlesinger et al. 2002), the effect of long term O_3 exposure on airway hyperresponsiveness was examined in sensitized and non-sensitized animals. In addition, another group received concurrent O_3 and antigen exposure. Two levels of O_3 were examined (0.1 ppm and 0.3 ppm) and exposures were for 4 hours per day for 4 days per week for 24 weeks. The animals that were not sensitized did not show any effect of O_3 exposure. However, O_3 exposure exacerbated airway hyperresponsiveness in response to both specific and non-specific provocation in sensitized animals. This effect lasted for 4 weeks after termination of the exposures. Moreover, antibody responses to the antigens used in sensitization were significantly correlated with increases in airway hyper-responsiveness.

The guinea pig model was also used to evaluate the effect of O_3 on nasal allergy. (Iijima et al. 2001) exposed guinea pigs to filtered air or to 0.4 ppm O_3 for 5 weeks. Once a week during these exposures a 1% solution of ovalbumin was administered by the intranasal route. By the third week of the protocol, animals exposed to O_3 and ovalbumin showed an increased number of sneezes during a 20 minute period after ovalbumin administration, compared to other groups. By week 5 these animals were sneezing at an average rate of 15 sneezes in 20 minutes. The quantity of nasal secretions was also elevated significantly by the sixth and final ovalbumin administration. Histopathology showed infiltration of eosinophils into the nasal epithelium and subepithelium. In the subepithelium the O_3 plus ovalbumin group showed a significant increase in eosinophils compared with the air plus ovalbumin exposed animals. While O_3 was shown to enhance

the symptoms of nasal allergy in the guinea pig model, the IgE antibody response to ovalbumin did not show a significant difference in O_3 versus air-exposed animals.

The mouse is currently the model of choice by most immunologists when examining the allergic response. Pioneering studies on the effects of O_3 on the development of allergic responses to inhaled ovalbumin in the mouse were performed by (Osebold et al. 1980). To examine the effect of cyclic O_3 exposure on allergic sensitization mice were exposed to 0.8 ppm O_3 for three days in alternate weeks for a total of three cycles of exposure and for four days in the last exposure. Thus, mice were only in the O_3 environment for three days every other week. This protocol mimics acute O_3 injury. On the day after each O_3 exposure mice received a 1% aerosol of ovalbumin for 30 minutes, after which they remained in ambient air until the next O_3 cycle. During the last aerosol exposure mice were observed for signs of atopic reactivity. One week after the fourth and final ovalbumin aerosol, mice were injected with ovalbumin by the IV route and were observed for development of systemic anaphylactic shock. Results indicated that exposure to O_3 enhanced anaphylactic sensitization when compared with similarly sensitized mice housed only in ambient air. One hundred percent of ovalbumin/O_3 exposed mice developed clinical anaphylaxis compared with 74% of ovalbumin/air exposed mice. A second experiment using 0.5 ppm O_3 showed a difference of 85% anaphylaxis for the O_3/ovalbumin group compared with 5% in the ovalbumin/air group (Osebold and Gershwin, 1980; Osebold et al. 1988). IgE producing cells were also examined in the lungs of mice from these and other experiments. O_3 was shown to significantly increase the number of IgE containing cells in lungs of mice exposed to the pollutant and ovalbumin aerosol. The difference in the number of IgE cells in O_3 exposed mice compared with those in ambient air was highly significant ($p < 0.006$) in the 0.8 ppm experiment previously described (Gershwin et al. 1981). Data from these experiments showed that O_3 exposure can enhance aerosol sensitization to antigen.

Exposure to O_3 and ovalbumin using a very different protocol showed that the IgE response to parenterally administered ovalbumin was suppressed when the mice had been exposed to 0.8 ppm O_3 continuously for 1 to 4 weeks, followed by a single ovalbumin aerosol (6 minutes) one week prior to the parenteral injection (Ozawa et al. 1985). This protocol is contrived and less relevant to “real life” exposure than others that demonstrate an enhancing effect of O_3 on the IgE response.

More recently, others have also studied the effect of O_3 on allergic responses using a mouse model. Studies by Neuhaus-Steinmetz et al. (2000) used O_3 exposures ranging from 180 to 500 ug/m^3 (0.09 ppm to 0.25 ppm), Mice were exposed for four hours, three times per week, for four weeks. Some mice also received ovalbumin aerosol five times per week throughout the four week period. Control mice received ovalbumin without O_3 exposure. Skin tests were performed 48 hours after the last O_3 exposure to evaluate allergic sensitization. Other markers of allergic sensitization studied included: total IgE, ovalbumin

specific IgE, IgG1, and IgG2a, Th1 and Th 2 cytokines, and leukotrienes C4, D4, and E4 in bronchoalveolar lung lavage fluid. In BALB/c mice, which are genetically Th2 skewed responders, O_3 exposure induced a dose dependent eosinophil response in BALF in response to ovalbumin challenge. IL-4 and IL-5 were increased in BALF, whereas interferon gamma remained unchanged. In addition, skin test sensitivity and ovalbumin specific IgG1 levels in serum were elevated in O_3 exposed mice. Leukotriene concentrations in BALF were significantly increased in O_3 and allergen exposed mice. The low IgE responder mouse strain, C57BL/6, were also evaluated in this protocol. C57BL/6 mice treated with only ovalbumin showed the expected Th1 antibody response profile, while treatment with O_3 and antigen was associated with a shift towards a Th2 type response. Thus, O_3 appeared to modulate the immune response toward the allergic phenotype in this mouse model. This study is particularly important because it shows that the effect of O_3 could potentially induce allergy in the non-atopic population that would not be expected from genetic analysis to develop an IgE response to environmental allergens.

Another recent study (Depuydt et al. 2002) using C57/BL/6 mice, a low IgE responder strain, concluded that O_3 exposure (0.1 ppm for 4 hours) does not increase allergic sensitization but enhances antigen-induced airway inflammation. In this study, mice were “immunized” by antigen-pulsed syngeneic dendritic cells and then challenged with ovalbumin two weeks later. Other groups were similarly allergen exposed without O_3 but received O_3 (or air for control group) during the ovalbumin challenge. There was an increased eosinophilia in the BAL of the O_3 challenged groups, but not in the groups that received O_3 concurrent with the dendritic cells. Several factors make this study irrelevant to human health concerns. First, it uses a non-allergic mouse strain and thus fails to model the atopic human population. Secondly, administration of dendritic cells pulsed with allergen is contrived and not relevant to “real life” exposure. Thirdly, neither IgE nor airways hyperresponsiveness were examined as indicators of allergic sensitization. However, the enhanced airway inflammation in antigen-induced O_3 exposed mice may be significant in explaining responses in nonallergic humans.

Another rodent model for allergy has been the Brown Norway rat. These rats were used by Wagner et al. (2002) in a recent study that examined the effect of O_3 exposure in an ovalbumin-induced model of allergic rhinitis. The ovalbumin-sensitized rats were exposed to 0.5 ppm O_3 for 8 hours/day on either one or three consecutive days. Following the O_3 exposure, rats received either ovalbumin or saline intranasally. Twenty four hours after this antigen instillation the rats were sacrificed and the nasal tissues were examined. Results indicated that rats receiving O_3 and allergen showed the presence of mucus-containing cells in the epithelium, increased numbers of epithelial cells, and an increase in intraepithelial mucosubstances in respiratory cells lining the septum. There were also increased numbers of eosinophils in the maxilloturbinates of the ovalbumin challenged rats. This study shows that in non-asthmatic subjects, allergic rhinitis “hayfever” can also be exacerbated by inhalation of O_3.

Some O_3-related experiments have been performed using a canine model. The dog is often naturally sensitized to Ascaris antigen due to early infection with the roundworm. This provides a convenient model for aerosol challenge and elicitation of allergic signs. Using this model the effects of O_3 on allergic reactivity have been examined by several groups. One study by Spannhake (1996) using Ascaris sensitive dogs showed that mast cell mediators (histamine and the arachidonic acid metabolite prostaglandin D2) were decreased in mast cells present in lung lavage fluid taken 30 minutes after dogs completed a 5 minute exposure to 1 ppm O_3, compared to dogs breathing only ambient air. The BAL cells were subsequently challenged in vitro by exposure to Ascaris antigen or an ionophore. The data showed that there was a significant decrease in PGD_2 in O_3 versus filtered air exposed airways. This data may have little relevance to actual *in vivo* allergen exposure.

O_3 has been shown to increase airway hyperresponsiveness in dogs, even in the absence of allergen challenge. Janssen et al. (1991) demonstrated that production of prostaglandins, especially E_2, are important in this mechanism. Li et al. (1992) demonstrated that dogs with O_3-induced airway hyperresponsiveness also had a neutrophil influx in BAL. Using a monoclonal antibody to block the leukocyte adhesion to endothelial cells, they showed that the reduction of neutrophils and eosinophils by this treatment did not correlate with a change in O_3-induced airway hyperresponsiveness. This study demonstrates that O_3 has a similar effect as allergen on induction of an influx of inflammatory cells into the lung, and that these inflammatory cells are not responsible for the increase in airway hyperresponsiveness caused by O_3 exposure.

The primate has been used infrequently as a model for asthma. Yet, the monkey is an ideal model for these studies because both the lung morphology and the immune system are more like that of the human than are other animal models. Using a cynomolgus monkey model, in 1986 Biagini showed that exposure to 1 ppm O_3 enhanced development of allergy to inhaled platinum (Biagini et al. 1986). In this study airway response to allergen and methacholine challenge as well as skin test reactivity were found to be significantly affected in the combined allergen and O_3 group. The importance of this study is that it is the first primate study to show both functional and immunological enhancement of allergic responsiveness to allergen by O_3.

More recently, a Rhesus monkey model of asthma is being used to examine the influence of O_3 exposure on development of both immunological and structural attributes of asthma (Schelegle et al., 2001). The combined exposure to epizootic O_3 and house dust mite allergen has been shown to alter lung development (Evans et al. 2003), and to alter the immune response (Miller et al. 2003). These studies are ongoing and are expected to provide important mechanistic information regarding the interplay of O_3 and allergen in the developing infant. This model is particularly relevant because it uses an allergen that is particularly common for asthmatic human subjects (*Dermatophygoides farinae*, the house dust mite). These studies are also the first to provide morphologic, functional,

and immunological data supporting the enhancement effect of O_3 on allergic asthma. In one study infant rhesus monkeys were exposed, starting at 30 days of age, to 11 episodes of either filtered air and house dust mite allergen aerosol, or O_3 (0.5 ppm) and house dust mite allergen aerosol. Each cycle consisted of 5 days followed by 9 days of filtered air only. Monkeys that were either not sensitized with allergen or sensitized monkeys in filtered air had only mild airway effects. However, the monkeys that received the combined O_3 and allergen had a marked increase in serum IgE, histamine, and airways eosinophilia (Schelegle et al. 2003). These studies use an epizootic and cyclic O_3 and allergen exposure that is intended to replicate natural conditions. Moreover, studies using infant monkeys serve as a model for the developing human child (Miller et al. 2003).

11.4.13.2.6 Interactions of Ozone With Other Air Pollutants

Ambient air contains a complex mixture of pollutants. It is therefore not unexpected that a combination of pollutants could have a synergistic effect. Only in the research environment would we expect to see humans or animals exposed to single pollutants. Thus, studies have been performed in humans and in animal models using multiple pollutants.

Studies on human subjects showed that neither NO_2 nor SO_2 alone appear to "prime" an asthmatic response to allergen, but when inhaled together these gases can increase sensitivity to subsequently inhaled allergen (Peden et al. 1997).

Matsumura (1970b) used the guinea pig model to show that, like O_3, NO_2 and SO_2 exposure also enhances allergic reactivity. His data showed that singly a 30 minute exposure to either 5 ppm O_3, 70 ppm NO_2 or 330 ppm SO_2 cause enhancement of anaphylactic sensitization when antigen is presented by the respiratory route (Matsumura 1970a).

Several studies have demonstrated that SO_2 alone can influence allergic disease. Using the guinea pig model, Riedel et al. (1988) showed that exposure to lower levels of SO_2 (0.1 ppm to 16.6 ppm) for up to 12 weeks increased the antibody levels in serum and BAL fluid and enhanced bronchoconstriction in response to antigen exposure. In another study with guinea pigs (Park et al., 2001), results indicated that repeated exposure to low levels of SO_2 may enhance the development of ovalbumin-induced asthmatic reactions in guinea pigs.

Gilmour (1995) showed that Brown Norway rats sensitized to house dust mite allergen by aerosol exposure and subsequently exposed to 5 ppm NO_2 for 3 hours after allergen inhalation challenge had enhanced levels of serum IgE and mucosal IgA than air exposed allergen sensitized control rats.

The importance of these studies is that they demonstrate the likelihood that enhancement of allergic reactivity, and thus asthma, that is occurring in recent years may be influenced by synergism of multiple air pollutants interacting with environmental allergens.

11.4.13.2.7 Conclusions

Understanding the reasons for the increasing prevalence of asthma in industrialized countries requires consideration of multiple factors. Among these are changes in population hygiene and the absence of many infectious diseases common to previous generations, the occurrence of viral respiratory infections (such as respiratory syncytial virus) at an early age, exposure to indoor pollutants (such as side stream tobacco smoke) and oxidant air pollutants and increased levels of diesel exhaust particles. The effect of these factors and their interaction with the underlying genetics of the human subjects involved are possible contributing factors to the increase in allergic/atopic asthma. Epidemiological studies and animal models have supplied varied and significant information that should be useful in validation of the causal effects of the "asthma epidemic".

That the environmental factors cited above may be involved in the increase in asthma in the human population has been well demonstrated. However, the mechanisms involved are less clear. For example, the role of O_3 in sensitization to allergen versus its role in exacerbation of the disease remains somewhat ambiguous. Some studies in the mouse model have shown a role for O_3 in sensitization to inhaled allergen. Exactly how O_3 mediates this effect at the cellular and molecular level, particularly in humans, will require additional study.

11.4.14 Summary of the Interactive Effects Between Ozone and Other Ambient Co-pollutants

There is only limited information on interactive effects between O_3 and other ambient co-pollutants. As mentioned above, the reasons for the relative paucity of joint exposure studies include the difficulty of adequately controlling the concentrations of multiple pollutants simultaneously. The large number of pollutants in ambient air are subject to a practically infinite number of combinations by location and time, making selection of a simplified but still representative exposure atmosphere problematic. In addition, the complexity of atmospheric chemistry increases the difficulty of adequately assessing the exposure mixture as the number of pollutants increases. As opportunities for chemical reactions among various atmospheric chemical species increase, the possibility that observed effects may be related to unknown reaction products increases as well.

The available evidence suggests that O_3 is responsible for the observed effects in subjects exposed to mixtures of O_3 and other common air pollutants, including NO_2, PAN, H_2SO_4, HNO_3 or SO_2, and several common nitrate and sulfate aerosols, at least within the range of ambient concentrations. However, the sequential exposure studies by Aris et al. (1991), suggested that HNO_3 and H_2O fog exposure mitigated pulmonary function effects of a subsequent O_3 exposure. Also, Koenig et al. (1990) report that preexposure to O_3 induced significant pulmonary function decrements from a subsequent "subthreshold" SO_2 exposure suggests that the initial O_3 exposure may have induced airway hyperresponsiveness that may have potentiated the response to SO_2. Together, these studies suggest that some sequential exposures may predispose

susceptible individuals to responses that would not have occurred without the initial exposure. Given the dearth of controlled exposure data on pollutant interactions with O_3, there are still many unresolved issues, including the extent to which the results of the studies reviewed in this section apply to endpoints other than lung function.

Data investigating the possibility of interaction between allergens and O_3 suggest that O_3, at least under some conditions, may exacerbate allergic responses. Animal models also support this conclusion, although the biological mechanisms of these responses are unclear at this time.

11.5 Summary of the Findings of Controlled Exposure Studies

11.5.1.1 Pulmonary Function

Collectively, the available literature exploring the responses of human subjects exposed to controlled concentrations of O_3 indicates that one to three hour exposures to O_3 concentrations as low as 0.12 ppm with moderate to heavy exercise can induce decrements in pulmonary function and increases in respiratory symptoms for some individual subjects. Statistically significant group mean decrements have not been reported at O_3 concentrations less than 0.12 ppm. There are no studies of one to three hour exposures to O_3 concentrations lower than 0.08 ppm.

Interest in longer averaging times led to studies in healthy adults who performed a protocol simulating a day of active outdoor work or play. These studies demonstrate that statistically significant group mean decrements in FEV1 occur at O_3 concentrations as low as 0.08 ppm. O_3 concentrations between 0.04 and 0.08 ppm have not been investigated with multi-hour exposure protocols. Evaluation of the responses of the individual subjects who participated in these studies (decrements as high as -48%) demonstrates that significant decrements in FEV1 can occur in more responsive individuals when they undergo multi-hour exposure to O_3 exposure at levels at or below the current federal 8-hour standard, and only marginally above nonanthropogenic O_3 background levels.

11.5.1.2 Symptoms

Significantly increased symptoms of respiratory irritation, both in number and severity, have been reported with 1 to 3 hour exposures with heavy exercise at O_3 concentrations as low as 0.12 ppm in healthy adults. Symptoms, such as pain on deep breath, shortness of breath and lower respiratory discomfort, have been reported with 1 to 3 hour exposures to 0.16 to 0.18 ppm O_3 with moderate exercise. Increased respiratory symptoms have been observed following 6.6 hour exposures with moderate exercise at 0.08, 0.10 and 0.12 ppm O_3.

11.5.1.3 Nonspecific Airway Responsiveness

Increased nonspecific airway responsiveness has been reported with one to 3 hour exposures to 0.40, but not 0.20 ppm O_3 at rest. The lowest O_3 concentration at which an increase in nonspecific airway responsiveness has been reported in exercising subjects is 0.18 ppm, but there was no change at

0.12 ppm compared to FA exposure. Available data indicate that exposures to O_3 concentrations as low as 0.08 ppm for 6.6 hour can increase non-specific airway hyperresponsiveness.

11.5.1.4 Airway Inflammation

Increased levels of neutrophils and various proteins indicative of airway inflammation have been observed following 1 to 3 hour exposures of healthy adults to 0.20, 0.30 and 0.40 ppm O_3 with heavy exercise. There are no studies that have investigated airway inflammation after 1 to 3 hour exposures at O_3 concentrations lower than 0.20 ppm. Analysis of BALF after 6.6 hour exposures with moderate exercise to 0.08 and 0.10 ppm O_3 has demonstrated both cellular and biochemical evidence for airway inflammation. O_3 concentrations lower than 0.08 ppm for 6.6 hour or longer exposures have not been investigated.

Exposure to 0.08 ppm O_3 for 6.6 hour decreases the ability of alveolar macrophages to phagocytize microorganisms via the complement receptor. The data also suggest that O_3 exposures that induce airway inflammation could lead to fibrotic changes in the lung tissues, based on the increased fibronectin and protein recovered following 6.6 hour exposure to 0.10 ppm O_3. There was a considerable range in response magnitude between individual subjects in the changes in the cellular and biochemical markers measured, suggesting that there is a fraction of the population that is very sensitive to the inflammatory effects of O_3.

11.5.1.5 Population Groups Most at Risk

Responses to O_3 exposure are related primarily to the O_3 concentration, and secondarily to the breathing rate and duration of exposure. O_3 concentrations are highest outdoors, there being few indoor sources of O_3, and low to moderate penetration to indoor environments in the absence of open windows or doors. Consequently, the people most at risk of experiencing adverse health consequences from O_3 exposure are those who spend prolonged periods of time outdoors while participating in some activity that increases the breathing rate. This group is comprised primarily of children, outdoor workers and recreational/amateur/professional athletes.

It should be kept in mind that the subjects who participated in most studies discussed in this document have been representative of healthy, young and middle-aged adults, although the range of individual responsiveness to O_3 in this population group is very broad. The range of responses to O_3 exposure in people with compromised health status is largely unknown, although there is a growing body of literature addressing the responses of mild to moderate asthmatics. The asthmatics studied have typically had changes in symptoms, lung function and inflammation in the same range as nonasthmatics, but larger increases in airway reactivity than healthy, nonasthmatic people. However, since asthmatic individuals often have lower lung function and chronic airway inflammation compared to nonasthmatics, at baseline, even relatively small additional reductions in function or increases in inflammation can be greater cause for concern than similar changes in healthy people. Because of ethical

considerations, there are few studies of individuals with cardiovascular disease or COPD, and those few studies involved subjects with relatively mild disease. Similarly, for ethical reasons there are no controlled studies of infants or young children available. However, since seriously impaired individuals are unlikely to spend significant periods of time outdoors working or exercising, the exposure of such individuals is likely to be considerably smaller than that of healthier, more active people, and consequently their overall risk is likely to be less. Therefore, the findings derived from the available literature are likely representative of people who are most at risk of adverse health effects from O_3 exposure: those who are physically able to perform moderate exertion for several hours, and by extension, are likely to experience the greatest O_3 exposures.

Overall, the published literature suggests that exercise performance can be reduced under conditions where O_3 inhalation has induced pulmonary function decrements and/or symptoms of respiratory discomfort. Significant reductions in exercise performance have been reported with 2 hour exposures at O_3 concentrations as low as 0.06 ppm.

It should also be noted that controlled human exposure studies have limited sample sizes and do not study people with moderate to severe asthma or other respiratory diseases. Hence, the epidemiological studies are useful in shedding further light on populations at risk.

11.5.1.6 Pollutant Mixtures

Although there are isolated findings to the contrary, the available data do not support the likelihood of clinically meaningful interactions in human subjects between O_3 and gaseous nitrogen-based air pollutants, SO_2 or H_2SO_4 aerosols at concentrations in the ambient range. Observed responses at the pollutant concentrations studied to date appear to be attributable to the O_3 in the mixture. Research also suggests that pre-exposure to fog (water or nitric acid) may mitigate the effects of subsequent O_3 exposure, although inhalation of nitric acid gas had no effect on responses to O_3. There is evidence that concurrent exposures to high concentrations of PAN and O_3 result in pulmonary function and symptom responses somewhat larger than those observed following exposure to the same concentration of O_3 alone. However, typical ambient PAN concentrations are considerably lower than those utilized in these studies. Consequently, even if O_3 and PAN do interact in their effects on pulmonary function at high concentrations, it is unlikely that PAN contributes significantly to adverse health effects in healthy young and older adults at concentrations in the ambient range. There have been few human exposure studies on mixtures of O_3 with particulate matter, with the exception of H_2SO_4 aerosol.

11.5.1.7 Effect Modifiers

It is unresolved at this time whether there is a difference in the responsiveness of males and females to O_3 exposure. The conclusion reached with available data varies depending on whether or how the inhaled doses of O_3 are normalized, and at present there is no basis to recommend one approach over another.

Data addressing the issue of age-related responsiveness to O_3 are limited to studies that investigated pulmonary function and symptoms. The few data available do not identify children or adolescents as being either more or less responsive than young adults who have undergone similar exposure protocols, although children tend to report fewer symptoms. The lack of symptoms reported by children suggests a lower level of somatic awareness of pain/discomfort among children, which might result in their failure to curtail exposure in real-life situations. In contrast, after about age 30 pulmonary function changes due to O_3 exposure become progressively smaller. Middle-aged and older adults also tend to report few symptoms, even with exposure to O_3 concentrations in excess of 0.4 ppm, while young adults can be quite symptomatic following exposures at that level. Although children and adolescents do not appear to experience greater adverse responses than adults who complete similar exposures, they are among those most likely to spend significant periods of time outdoors while engaged in exercise, putting them at increased risk of adverse responses.

There is no information available on other endpoints, such as airway inflammation or airway hyperreactivity, other than for young adults.

There are insufficient data available to draw a conclusion as to whether there is a difference in the O_3 responsiveness of various socioeconomic groups (one study) or African-Americans (one study) compared to Caucasians. There are no data available on other ethnic or racial groups.

Though a variety of factors have been examined to explain differences in responsiveness to acute O_3 exposure, only current smoking and increasing age have been convincingly shown to be linked with responsiveness, both in an inverse direction. This reduced responsiveness in smokers may wane after smoking cessation.

11.5.1.8 Effective Dose

Responses to inhaled O_3 are roughly proportional to the “effective dose” (ED) of inhaled O_3. ED is defined as the simple product of O_3 concentration, ventilation rate and duration of exposure. The concept has been refined to indicate that O_3 concentration is the most significant of the three factors, explaining the largest share of the variance in responses. Ventilation rate explained the second largest portion, followed by exposure duration. Subsequent investigations revealed that increased ventilation rate accentuated the observed pulmonary response at any given O_3 concentration, and lowered the minimum O_3 concentration at which significant pulmonary responses were evident. Further, there is a positive correlation between O_3 concentration and the rate at which adverse responses develop: the higher the O_3 concentration, the more rapidly adverse effects become apparent. Consequently, a large number of exposure scenarios, based on varied O_3 concentrations, ventilation rates, and durations, could be developed that are likely to induce adverse health effects.

11.5.1.9 Relationship between Short-Term Effects and Long-Term Outcomes

The results of controlled human exposure studies utilizing O_3 exposures up to about 8 hour have clearly established that O_3 induces acute responses that qualify as adverse and raise concern that residual effects from repeated acute exposures could accumulate over time and lead to chronic effects or disease. However, practical and logistic considerations are such that controlled human exposure studies are unable to shed light on the impact of long-term exposures to O_3. What is known about long-term exposures comes from results of both epidemiological and animal studies. There are limitations to both of these bodies of literature that cannot be fully overcome, but they do provide some guidance into evaluating the likelihood for chronic effects from O_3 exposure. Epidemiology studies may not always provide clear causal relationships because of the presence of confounding factors (i.e., heat, humidity, other pollutants, subject characteristics). However the results can provide associations that may suggest causal relationships or provide hypotheses that can be investigated in animal models, and sometimes in controlled human studies. Animal toxicology studies are limited by incomplete knowledge of species sensitivity and dosimetry patterns compared to humans, although they can offer controlled experimental conditions for chronic exposures, provide evidence of causal relationships, and also allow investigation of endpoints not possible to study in humans.

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12 Epidemiological Studies

12.1 Field Studies Addressing Acute Respiratory Effects of Ozone

12.1.1 Introduction

Numerous field studies carried out over the past decade have tested for, and in many cases observed, acute associations between measures of daily respiratory ill-health and ozone concentrations in small groups of subjects. These studies have often focused on subjects with asthma (Table 12-1). What distinguishes acute field studies from other acute epidemiologic study designs is that field studies recruit and collect data from individual human subjects, as opposed to gathering administrative data on aggregate health outcomes, as in studies of daily mortality, hospital admissions, or ED visits. Because of the logistical burden associated with direct data collection from individual subjects, field studies tend to be small in both numbers of subjects and in duration of follow up. As a result, statistical power often limits the conclusions that can be drawn from these studies.

The most common outcomes measured in acute field studies on the effects of air pollution exposure have been lung function, respiratory symptoms, and asthma medication use (Brauer et al. 1996; Chen et al. 1999; Delfino et al. 1996; Delfino et al. 1997a; Gent et al. 2003; Gielen et al. 1997; Gold et al. 1999; Hiltermann et al. 1998; Linn et al. 1996; Ostro et al. 2001; Neas et al. 1999a; Romieu et al. 1997; Romieu et al. 1996; Ross et al. 2002). Only two studies looked at acute ozone effects on other respiratory outcomes, including nasal inflammation (Kopp et al. 1999), and school absences (Gilliland et al. 2001). The former group of 14 studies provides varying degrees of evidence supporting the conclusion that elevated ozone levels can have negative impacts on lung function and symptoms, confirming and adding to the body of knowledge that existed in the mid 1990s. Areas in which the new studies contribute the greatest amount of new information include effects on respiratory symptoms (e.g., Gent et al. 2003), which had not been well documented in previous studies, and the independent role of ozone in the presence of particulate matter and other pollutants (Brauer et al. 1996; Chen et al. 1999; Gent et al. 2003; Gilliland et al. 2001; Gold et al. 1999; Hiltermann et al. 1998; Romieu et al. 1997; Romieu et al. 1996). The current body of evidence provides substantial support for effects of ozone on respiratory symptoms such as cough, wheeze and shortness of breath, and also for ozone effects which are independent of, and frequently more robust than, those associated with other pollutants.

12.1.2 Ozone and Lung Function

A large body of literature from past clinical and field studies has clearly demonstrated reversible decrements in lung function following ozone exposures (US EPA 1996). Studies published over the past decade provide few new insights regarding these effects, with the exception of a study in Fraser Valley, British Columbia that reported unusually large effects on lung function among

agricultural workers ages 10-69 yrs exposed over long work-shifts while working outdoors (Brauer et al. 1996). Nine of 11 newer studies that tested for effects of ozone on lung function reported significant associations, although in some cases there are inconsistencies. Four studies carried out in the US are reviewed here first (Delfino et al. 1997b; Neas et al. 1999a; Linn et al. 1996; Ross et al. 2002).

One relatively small study saw no significant effects on morning (AM) and evening (PM) peak expiratory flow rate (PEFR) among 22 asthmatics living in Alpine, California, and ranging in age from 9-46 years, even though daily 12-hr mid-day mean ozone concentrations ranged from 34-103 ppb (Delfino et al. 1997b). Unique to this study, personal ozone exposures were measured using 12 hr passive ozone samplers that were worn by the subjects. These negative findings may reflect the small number of subjects and imprecision in both the personal monitoring and PEFR data. In another study focusing specifically on asthmatics, 40 asthmatics between the ages of 5 and 49 provided morning and evening PEFR measurements between May and October 1994 in the East Moline Illinois area (Ross et al. 2002). Questions regarding the statistical analysis make the interpretation difficult. However, significant associations were reported between both AM and PM PEFR and ozone concentrations. No effects of PM10 were observed in this study. The PM, but not AM, effects were robust to inclusion of temperature and/or pollen in the model.

Using a similar repeated measures design involving twice daily PEFR Neas et al. (1999a) saw evidence for effects due to ambient O_3 exposure among 156 children attending two summer day-camps in the Philadelphia, PA area. Afternoon PEFR (recorded before leaving camp) was related to same-day ozone concentration, and morning PEFR (recorded upon arrival at camp) was related to previous-day ozone concentration; however, the only statistically significant finding was for PM PEFR regressed on five-day average ozone concentration.

Linn et al. (1996) studied repeated measures of spirometric lung function among 269 school children in three southern California communities (Rubidoux, Upland, and Torrance). Spirometry provides more extensive, precise and reproducible measures of lung function than are available from peak flow meters, including forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1). Lung function was measured over five consecutive days once in each of three seasons over two school years. However, statistical power was limited by the narrow range of exposures that were experienced within each week. Between-week variability was effectively removed from the analysis by seasonal terms in the model. Furthermore, the study was restricted to the school year, eliminating most of the “high” O_3 season from consideration. As a result, ozone levels were quite low, with a 24 hr mean of just 23 ppb, and a range of 3-53 ppb. These factors would tend to compromise the power to detect associations between ozone exposures and outcomes. In a subset of subjects, personal ozone monitoring using passive dosimeters was correlated with central site ozone (r=0.61). The difference between AM (recorded near the beginning of the school day) and PM (recorded following lunch) FEV1 was significantly associated with same day ozone concentration. Other associations (involving individual AM

or PM FVC and FEV1 time points) went in the plausible direction but were not statistically significant.

Among international studies, a large study measured spirometric lung function in 895 school children ages 8-13 yrs in three towns in Taiwan (Chen et al. 1999). Unfortunately, lung function was measured only once for each subject. As a result, between-person variability could not be distinguished from within-person variability. This study design results in limited statistical power, compared with a design in which individual subjects provide multiple days of measurements. The latter study design enables each subject to serve as his/her own control, and provides information about both between and within subject responses. Still, the authors reported statistically significant associations between diminished FVC and FEV1 and the one-day lag of ozone concentration. Effect sizes were typical of those observed in past studies, i.e., 0.5 to 1.0 mL drop in FVC per ppb increase in ozone concentration. Ozone was the only significant air pollutant in multi-pollutant models including SO_2, CO, PM10, and/or NO_2. The ozone associations became non-significant when days with ozone above 60 ppb were excluded from the analysis, implying a practical threshold of around 60 ppb for this individual study.

In Mexico City, two identical studies of asthmatic school children were carried out simultaneously in the northern (Romieu et al. 1996) and south-western sections of the city (Romieu et al. 1997). In the northern study, 71 mildly asthmatic school children, 5-7 years old, were followed over time for daily AM/PM PEFR. In single-pollutant models, ozone at lags 0, 1, and 2 was associated with diminished AM (before breakfast) and PM (bedtime) PEFR, but only the lag 0 AM effect was statistically significant. The ozone effect became non-significant when PM2.5 was added to the model. In the south-western study, 67 mildly asthmatic children 5-13 years old were followed in both summer and winter for daily AM/PM PEFR. Significant effects of same day and lag 1 day ozone were observed on PM PEFR, with effects larger for lag 1. Associations involving ozone were stronger than those involving PM2.5. Another study in south-western Mexico City analyzed AM/PM PEFR data collected from 40 school children ages 8-11 years (Gold et al. 1999). Subjects provided measurements upon arrival at, and before departing from, school each day. Diminished PEFR was associated with lag 1 ozone, but the only statistically significant findings were obtained for PEFR regressed on the 10-day moving average of ozone. This may imply a cumulative effect of ozone, or may reflect confounding by other time-varying factors. This is in accord with controlled human exposure studies that have shown a decline in baseline pulmonary function with repeated days of O_3 exposure (see section xxx), and with epidemiologic studies that have assessed lung function over the course of the ozone season (Brauer et al. 1996; Kinney and Lippmann 2000). A similar study carried out in Amsterdam involving 61 school children 7-13 years old, the majority with doctor-diagnosed asthma, reported effects of lag 2 ozone on AM PEFR (Gielen et al. 1997). However, the reported two-day lag effect on AM lung function, in the absence of a same day effect on PM function, seems implausible in light of findings of controlled human exposure studies (see Chapter 11).

Among the international studies looking at acute lung function, only one focused exclusively on asthmatics (Hiltermann et al. 1998). There, 60 non-smoking adults in Bilthoven, The Netherlands, ages 18-55 years were followed between July and October, 1995 with morning and afternoon PEFR measurements. Ozone was associated with declines in PEFR, but statistical significance was not achieved.

The most interesting and potentially useful data, with respect to ambient air quality standards, on ozone effects on lung function were reported by Brauer and colleagues (1996). The investigators measured spirometric lung function before and after outdoor summer work shifts on a group of 58 berry pickers in Fraser Valley, British Columbia. Subjects ranged form 10 to 69 years old (mean of 44 years). Work shifts averaged 11 hrs in duration. Exertion levels in that study were estimated using portable heart rate monitors carried by a representative subset of subjects for 16 workshift periods of four or more hours. Heart rates were essentially constant over the workshift, averaging 36% higher than resting levels. The authors estimated that minute ventilations may have averaged roughly 30 L/min during work. Post-shift FEV1 and FVC showed large decreases as a function of ozone concentration, and those effects remained significant when PM2.5 was added to the model. Lung function on the morning following exposure also was significantly lower. On a unit ozone basis, the effects observed in this study were larger than have been reported previously. For example, afternoon FVC was 5.4 mL lower per 1 ppb increase in ozone concentrations. Further, ozone effects remained significant when data were restricted to days with 1 hr maximum ozone concentrations under 40 ppb. These observations suggest that the extended exposure period outdoors, combined with elevated levels of exertion throughout exposure, enhanced response in this group. There is also a possibility of greater responsiveness related to generally less advantaged health status in the study population.

12.1.3 Respiratory Symptoms and Medication Use

While lung function effects of ozone have been well documented for many years, data on associations with respiratory symptoms have been much less extensively documented, especially in epidemiology studies. Twelve new studies published over the past decade address this question, and together represent a significant new body of data on symptom effects of ozone.

Respiratory symptoms are usually measured in the context of acute air pollution field studies using questionnaire forms, i.e., “daily diaries,” that are filled out by study subjects without direct supervision of research staff. Questions usually address daily experience of coughing, wheezing, shortness of breath (or difficulty breathing), production of phlegm, and others. While convenient and potentially useful in identifying acute episodes of morbidity, measurements of daily symptoms are prone to a variety of errors. These include misunderstanding of the meaning of symptoms, variability in individual interpretation of symptoms, inability to remember symptoms if not recorded soon after their occurrence, reporting bias if days of high air pollution levels are identifiable by subjects, and the possibility of falsified data. In spite of these potential problems, ease of data

collection has made daily symptom assessment a common feature of field studies. Many of the studies reviewed above for lung function results also included measurements of daily symptoms.

Among the 12 studies reporting results for daily symptoms, seven reported associations with ozone concentrations that appear fairly robust (Desqueyroux et al. 2002; Gent et al. 2003; Gold et al. 1999; Hiltermann et al. 1998; Romieu et al. 1997; Romieu et al. 1996; Ross et al. 2002). One of the largest and best-conducted studies was that of Gent and colleagues (2003), where 271 asthmatic children under age 12 living in southern New England were each followed over six months (April through September) for daily symptoms. The data were analyzed for two separate groups of subjects, 130 who used maintenance asthma medications during the follow-up period, and 141 who did not. The need for regular medication was considered a proxy for more severe asthma. Significant effects of lag 1 ozone were observed on a variety of respiratory symptoms in the medication user group only. Both daily maximum 1-hr and 8-hr concentrations were similarly related to symptoms, including chest tightness and shortness of breath. Effects of ozone, but not PM2.5, remained significant and even increased in magnitude, in two-pollutant models. Of particular importance, some of the significant associations were seen at 1 hr maximum ozone levels of 43.2 ppb or higher and at 8 hr average ozone levels of 63.3 ppb or higher. Given the large number of significant associations observed in the medication user subgroup, it is somewhat surprising that no effects were observed among asthmatics not using maintenance medication. It is not clear whether this stratification was determined a-priori or was chosen following preliminary analyses. In terms of person-days of follow-up, this is by far the largest of the currently available studies addressing symptom outcomes in relation to ozone, and provides the strongest evidence for effects of ozone independent of PM2.5.

Only one other US study reported significant effects of ozone on symptoms, that of Ross and colleagues, who studied 40 asthmatics ages 5-49 yrs in the East Moline, Illinois area (Ross et al., 2002). There, an undefined four-level symptom score was associated with daily 8-hr max ozone concentrations, controlling for pollen and mold spore levels.

Aside from these two studies, all other US studies carried out over the past decade have failed to detect symptom effects associated with ozone. Delfino and colleagues (1996) followed 12 asthmatic ages 9-18 yrs living in San Diego, CA for respiratory symptoms over a two month period, and saw no relationship with central site ambient ozone. Personal ozone exposures measured with passive diffusion monitors were associated with a composite symptom score, but the relationship disappeared when week-day/week-end differences were controlled in the statistical analysis. Study power was likely compromised by the small sample size. The observation of stronger effects based on personal monitoring is intriguing, and suggests that substantial gains in power may be achievable when exposure misclassification is reduced through use of measured personal exposure rather than central site O_3 concentration. However, a similar study of 22 asthmatics ages 9-46 yrs in Alpine, CA saw no effects of ozone on

symptoms when personal O_3 exposure was used as the exposure metric (Delfino et al., 1997). One final US study reported no associations between daily symptoms and ambient ozone concentrations in a cohort of 138 African American children ages 8-13 years old with asthma followed over three months (Aug-Oct) in Central Los Angeles and Pasadena, CA (Ostro et al. 2001). However, use of extra asthma medications was associated with lag 1 1-hr maximum ozone concentrations. Finally, the study by Linn and colleagues (1996) of 269 fourth and fifth grade school children in southern California reported no associations between symptoms and ozone, but as noted earlier, power to test ozone effects was limited due to the low and narrow range of ozone concentrations observed during the field sampling period.

Several non-US studies have reported significant symptom associations with ozone. Exacerbation of COPD symptoms was significantly associated with ozone concentrations in a group of 39 adult patients (mean age 67 years) followed over a 14 month period in Paris (Desqueyroux et al. 2002). There were no effects of PM10, SO_2, or NO_2 in that study. Interestingly, in contrast to controlled human studies (see Sections 11.1.1.6 and 11.4.6), the ozone effect appeared larger among subjects who smoked and those with more severe COPD. However, the very low O_3 concentrations experienced during that study (e.g., summer mean 8-hr max O_3 of 21 ppb) raise plausibility questions. In a study of 60 non-smoking asthmatic adults (ages 18-55) in Bilthoven, The Netherlands, Hiltermann and colleagues (1998) reported significant associations between ozone and daily symptoms of shortness of breath and pain upon deep inspiration. The ozone associations were stronger than those of PM10, NO_2, SO_2, and black smoke (BS). No differences in response were evident between subgroups of subjects defined on the basis of steroid use or airway hyper-responsiveness. No effects of ozone were evident with respect to daily use of bronchodilator or steroid inhalers.

Several non-US studies have examined associations between symptoms and ozone among children (Gielen et al. 1997; Gold et al. 1999; Romieu et al. 1997; Romieu et al. 1996). Three Mexico City studies reported significant associations between symptoms and O_3, including a study of 71 children ages 5-7 (Romieu et al., 1996), 67 children ages 5-13 (Romieu et al., 1997) and 40 children ages 8-11 (Gold et al., 1999). The symptoms most often associated with ozone in these studies were cough, phlegm, and difficulty breathing. Effects were most often seen at exposure lags of 0 through 2. Of these three studies, only one (Romieu et al., 1997) could clearly separate the ozone effects from other pollutants. A study of 61 mostly asthmatic children ages 7-13 in Amsterdam found only limited evidence for symptom increases associated with O_3 exposure (Gielen et al. 1997). Of 14 symptoms analyzed, only upper respiratory symptoms were associated with ozone concentrations. There were no associations between bronchodilator use and ozone in this study.

12.1.4 Other Acute Outcomes in Children: School Absences and Upper Airway Inflammation

Absence from school was associated with ozone concentrations in a study of 1,933 fourth grade students from 12 southern California communities participating in the Children's Health Study (Gilliland et al. 2001). All school absences that occurred over a six month period, January-June 1996, were followed up with phone calls to determine whether they were illness-related or not. For illness-related absences, further questions assessed whether the illness was respiratory or gastrointestinal, with respiratory including runny nose/sneeze, sore throat, cough, earache, wheezing, or asthma attack. Multiple pollutants were measured at a central site in each of the 12 communities. The statistical analysis controlled for temporal cycles, day of week, and temperature, and expressed exposure as a distributed lag out to 30 days. Some concern exists regarding the possibility of residual seasonal confounding given the six month time span of the follow-up period. Significant associations were found between the 30-day distributed lag of ozone (10am to 6pm average) and all absence categories. Bigger effects were seen for respiratory (83% increase) than for non-respiratory (37% increase) causes per 20 ppb rise in ozone 10am –6pm O_3 concentrations. Among the respiratory absences, larger effects were seen for lower respiratory diseases with wet cough (174% increase) than for upper respiratory diseases (45% increase) per 20 ppb rise in average ozone concentration for the time period of 10 AM to 6 PM. All effects were summed over the 30 day distributed lag. PM10 was only associated with upper respiratory disease absences. Due to its size and comprehensive characterization of exposures and health outcomes, this study is especially valuable in assessing adverse acute health effects of ozone in children. The study spanned a period, January through June, when O_3 levels increase markedly. Thus, a wide range of exposures were captured while staying mostly below the highest levels observed in the summer season.

Acute airway inflammation has been shown to occur among adults exposed to 80 ppb ozone over 6.6 hrs with exercise in controlled chamber studies (Devlin et al. 1991). Kopp and colleagues (1999) attempted to document inflammation of the upper airways in response to summer season ozone exposures by following a group of 170 second and third grade school children ages 8-10 yrs in two towns in the German Black Forest from March to October of 1994. To assess inflammation, the investigators collected nasal lavage (NL) samples at 11 time points spanning the follow up period. NL samples were analyzed for markers of inflammation, including eosinophil cationic protein (ECP), albumin, and leukocyte counts. Subjects were excluded who were sensitized to inhaled allergens. When analyzed across the entire follow up period, no statistical association was detectable between upper airway inflammation and ozone concentrations. More detailed analysis showed that the first significant ozone episode of the summer was indeed followed by a rise in ECP levels; however, subsequent and even higher ozone episodes did not affect ECP. These findings suggest an adaptive response in terms of inflammation in the nasal airways that is consistent with controlled human studies (see Section 11.4.9), but do not preclude the possibility

that other, unmeasured effects including cell damage or lower airway effects, may occur with ongoing summer season exposures. In fact, a study of joggers repeatedly exposed to ozone while exercising over the summer in New York City suggested that cell damage may occur in the absence of ongoing inflammation (Kinney et al., 1996).

Table 12-1: Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Brauer et al. 1996 Fraser Valley, British Columbia Jun-Aug 1993	58 berry pickers ages 10-69 had lung function measured before and after a series of outdoor workshifts (avg duration=11 hrs) over 59 days. Pooled regression with subject-specific intercepts, with and without temperature control.	1-hr max O_3: Mean 40.3 ppb SD 15.2 Workshift O_3: Mean 26.0 ppb SD 11.8	PM2.5 and other PM measures	Endshift FEV1 and FVC significantly diminished in relation to O_3 levels. PM2.5 also related to lung function declines, but O_3 remained significant in 2-pollutant models. Next morning lung function remained diminished following high ozone days. O_3 effects still evident at or below 40 ppb. There was an overall decline of lung function of roughly 10% over course of study, suggesting subchronic effect. Levels of other pollutants low during study.	Regressions on mean O_3 over work shift: Endshift lung function: FEV1: -3.8 mL/ppb (SE 0.4) FVC: -5.4 mL/ppb (SE 0.6) Next morning function: FEV1: -4.5 mL/ppb (SE 0.6) FVC: -5.2 mL/ppb (SE 0.7)
Chen et al. 1999 3 Taiwan towns: Sanchun, Taihsi, Linyuan May 1995-Jan 1996	Valid lung function data obtained once on each of 895 children in three towns. Examined relation between lung function and pollution concentrations on same day and over previous 1, 2 and 7 days. Multi-pollutant models examined.	1-hr max O_3: range: 19.7-110.3 ppb	SO_2, CO, PM10, NO_2	FEV1 and FVC significantly associated with 1-day lag O_3. FVC also related to NO_2, SO_2, and CO. Lag 1 O_3 was sole significant pollutant in multi-pollutant models. No PM10 effects. O_3 effect eliminated when data truncated above 60 ppb.	1-hr max O_3 lag 1: FEV1 -0.64 mL/ppb (SE 0.34) FVC: -.79 mL/ppb (SE 0.32) O_3 with NO_2 in model: FEV1 -0.85 mL/ppb (SE 0.34) FVC: -0.91 mL/ppb (SE 0.37)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Delfino et al. 1996 San Diego, CA September-October 1993	12 well-characterized moderate asthmatics ages 9-18 (7 male/5 female) followed over 6 weeks for medication use and respiratory symptoms. Allergy measured at baseline with skin prick tests. Personal O_3 measured with passive badge. Analysis with GLM mixed model.	1-hr max O_3: Mean 68 ppb SD 30 12-hr O_3: Mean 43 ppb SD 17 12-hr personal O_3: Mean 11.6 ppb SD 11.2	PM2.5, SO_4, H+, HNO_3, pollen, fungi	No effect of ambient O_3 on symptom score. Personal O_3 significant for symptoms, but effect disappeared when confounding day of week effect was controlled with weekend dummy variable. B2 inhaler used among 7 subjects was significantly related to personal O_3. Results of this small study suggest the value of personal exposure data in providing more accurate estimates of exposures. However, nearly 50% of personal O_3 measurements were below limits of detection, diminishing value of these data.	B2 agonist inhaler use in relation to personal O_3 exposures in 7 subjects: Slope estimate: 0.0152 (SE 0.0075) p=0.04 (units not given, but assume puffs/day/ppb)
Delfino et al. 1997b Alpine, CA May 9, 1994 to July 3, 1994	22 asthmatics ages 9-46 followed for respiratory symptoms, AM-PM peak flow rate (PEFR), and B2 agonist inhaler use. Personal O_3 measured for 12 hrs/day using passive monitors. GLM mixed model.	1-hr max O_3: Mean 88 SD (25) 12-hr O_3: Mean 64 ppb SD 17 Personal O_3: Mean 18 ppb SD 14	PM10, pollen, fungi	No O_3 effects observed.	No quantitative results for O_3.

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Desqueyroux et al. 2002 Paris Oct 1995-Nov 1996	39 adult patients with severe COPD (mean age 67 yrs) followed over 14 months by physicians for exacerbations. Logistic regression with GEE, examining exposures lags 0-5 days.	8-hr 10-6 O_3: Mean (SD) Summer: 21 (9) ppb Winter: 6 (5) ppb	PM10, SO_2, NO_2	50 COPD exacerbations observed over follow up period. 1, 2, and 3-day lag 8-hr O_3 significantly related to exacerbations. No other pollutants significant. Very low O_3 levels raise plausibility and confounding concerns.	Odds ratio per 5 ppb change in lag 1 O_3 8-hr mean per: 1.56 (1.05-2.32) Effects appeared larger among smokers and those with worse gas exchange lung function.
Gent et al. 2003 Southern New England April-September 2001	271 children (<12 yrs) with active, doctor-diagnosed asthma followed over 183 days for respiratory symptoms. For analysis, cohort split into two groups: 130 who used maintenance medication during follow up and 141 who did not, on assumption that medication users had more severe asthma.	1-hr max O_3: Mean (SD) 58.6 (19.0) ppb 8-hr max O_3: 51.3 (15.5) ppb	PM2.5	Correlation between 1-hr max O_3 and daily PM2.5 was 0.77 during this warm-season study. Large numbers of statistical tests performed. Significant associations between symptoms and O_3 seen only in medication-user subgroup. PM2.5 significant for some symptoms, but not in two-pollutant models. O_3 effects generally robust to PM2.5.	For 130 regular med. users, Odds ratios for lag 1 50 ppb change in O_3 levels: Chest tightness: 1-hr max O_3: 1.26 (1.00-1.48) 8-hr max O_3: 1.33 (1.09-1.62) 1-hr max O_3 w/PM2.5 in model: 1.47 (1.18-1.84) Shortness of breath: 1-hr max O_3: 1.22 (1.02-1.45) 8-hr max O_3: 1.30 (1.05-1.61)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Gielen et al. 1997 Amsterdam April 26-July, 1995	61 children (age 7-13) from two schools for sick children, followed for twice daily PEFR, symptoms, and medication usage. Majority of cohort had doctor-diagnosed asthma.	8-hr max O_3: Mean (SD) 34 (8) ppb 1-hr max O_3: 39 (8) ppb	PM10, BS, pollen	AM PEFR significantly associated with lag 2 8-hr O_3. BS also associated with PEFR. Among 14 symptom models tested, only one yielded a significant O_3 finding (for upper respiratory symptoms). This is likely spurious. PM10 and BS, but not O_3, were related to B2 agonist inhaler use.	Percent change in PEFR for 42.3 ppb change in lag 2 8-hr max O_3: AM data: -1.85% (-0.14 to -3.58), $p<.05$ PM data: -1.88% (0.18 to -3.94), $p<.10$
Gilliland et al. 2001 12 southern California communities January-June 1996	1,933 4th grad children followed for school absences. Each absence classified as illness-related or not. Among former, further classified into respiratory or gastro-intestinal. Respiratory absences further classified into upper or lower. Pollution measured in central site in each town. Analysis of distributed lag effects controlling for time, day of week, temperature in a Poisson model.	Levels not reported here.	PM10, NO_2	O_3 strongly associated with illness-related and respiratory absences. PM10 only associated with upper respiratory absences. Long distributed lag effects for O_3 raise questions about adequacy of control for seasonal changes.	Percent change in absences associated with a 20 ppb rise in 8-hr 10-6 O_3: Absences due to: All illness: 62.9% (18.4-124.1) Non-respiratory illnesses: 37.3% (5.7-78.3) Respiratory illnesses: 82.9% (3.9-222.0) Upper respiratory: 45.1% (21.3-73.7) Lower respiratory w/wet cough: 173.9% (91.3-292.3)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Gold et al. 1999 SW Mexico City 1991	40 school children ages 8-11 in polluted community followed for twice daily PEFR and respiratory symptoms. PEFR deviations in AM/PM from child-spec means analyzed in relation to pollution, temperature, season, and time trend. AM symptoms analyzed by Poission regression.	24-hr avg O_3: Mean 52.0 ppb IQR 25	PM2.5, PM10	Reported significant declines in PEFR in relation to 24-hr avg O_3 levels. Effects did not vary by baseline symptom history. Lags chosen to maximize effects and varied by outcome. O_3 generally robust to PM2.5. Morning phlegm significantly related to lag 1 O_3.	Change in AM PEFR (L/min) and SE per ppb 24-hr avg O_3: Lag 1: -0.09 (0.05) 10-d moving avg: -0.65 (0.25) 10-d poly lag: -0.74 (0.20) Change in PM PEFR (L/min) and SE per ppb 24-hr avg O_3: Lag 1: -0.08 (0.05) 10-d mov avg: -0.58 (0.17) 10-d poly lag: -0.62 (0.17) % change in PEFR for 25 ppb change in 24-hr avg O_3: AM: 10-d lag: -3.8% (-5.8,-1.8) PM: 9-d lag: -4.6% (-7.0,-2.1) AM phlegm per 25 ppb O_3: 1.1% (1.0-1.3)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Hiltermann et al., 1998 Bilthoven, The Netherlands July 3-October 6, 1995	60 adult non-smoking intermittent to severe asthmatics (ages 18-55) followed over 96 days. Measured AM/PM PEFR, respiratory symptoms, and medication use. Analysis controlled for time trends, aeroallergens, environ tobacco smoke exposures, DOW, temp. Lags 0-2 examined.	8-hr max O_3: Mean 41 ppb Range 6-94	PM10, NO_2, SO_2, BS	O_3 had strongest association with symptoms of any pollutant analyzed. PEFR lower with O_3 but not statistically significant. No effect on medication use. No effect modification by steroid use or hyperresponsiveness.	Odds ratios for symptoms per 51 ppb change in 8-hr max O_3: Shortness of breath: 1.18 (1.02-1.36) Sleep disturbed by breathing: 1.14 (0.90-1.45) Pain on deep inspiration: 1.44 (1.10-1.88) Cough of phlegm: 0.94 (0.83-1.07) Bronchodilator use: 1.05 (0.94-1.19)
Kopp et al., 1999 Two towns in Black Forest of Germany March-October 1994	170 school children followed over 11 time points with nasal lavage (NL) sampling. Subjects were not sensitive to inhaled allergens. NL samples analyzed for eosinophil cationic protein (ECP), albumen, and leukocytes.	½-hr max O_3 mean, 5%, and 95%: Villingen: 33, 1, 71 Freudenstadt: 53, 23, 91	PM10, NO_2, SO_2, TSP	ECP levels peaked soon after first major O_3 episode of summer, but did not show response to later, even higher, O_3 episodes. Overall analysis found no significant effect of summer O_3 on outcomes. These observations are consistent with an adaptive response in terms of nasal inflammation.	No quantitative results.

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Linn et al. 1996 3 California Towns: Rubidoux, Upland, and Torrance Fall-Spring for 1992-1993 and 1993-1994 school years	269 school children (age unspecified), each followed for AM/PM lung function and symptoms for one week in Fall, Winter, and Spring over two school years. Personal exposure monitoring in a subset. Analyzed PM symptoms vs. same-day pollution and AM symptoms vs. lag 1 pollution.	24-hr avg O_3: Mean (SD) 23 (12) ppb	PM2.5, NO_2	Central site O_3 correlated with personal exposures at 0.61. O_3 effects observed on lung function but only significant for FEV1 in one analysis. No effects on symptoms. O_3 effects were not robust to NO_2 or PM2.5. Power may have been limited by short followup within seasons (limiting both person-days and variability in exposures).	Change in lung function (mL) per ppb 24-hr avg O_3 (SE). FVC AM: -0.21 (0.22) FVC PM -0.20 (0.29) FVC AM/PM difference: -0.25 (0.25) FEV1 AM: -0.26 (0.25) FEV1 PM: -0.18 (0.26) FEV1 AM/PM difference: -0.58 (0.23)* * $p<0.05$
Neas et al. 1999b Philadelphia, PA July 12-September 3, 1993	156 children (age unspecified) at two summer camps followed for twice daily PEFR. Study mainly focused on detecting PM effects.	12-hr 9a-9p O_3: SW Camp: Mean 57.5 ppb IQR 19.8 NE Camp: Mean 55.9 ppb IQR 21.9	H+, SO_4, PM2.5, PM10, PM10-2.5	Some O_3 effects detected as well as PM effects. PM effects strongest for SO_4. O_3 effects not robust to SO_4 in two-pollutant models, whereas SO_4 relatively robust to O_3.	Change in PEFR (L/min) for 10 ppb increase in 24-hr 9a-9p avg O_3: PM PEFR: -1.10 (-2.83, 0.64) Next AM PEFR: -1.77 (-3.70, 0.17) PM PEFR in relation to 5-day mean O_3: -2.58 (-4.82,-0.35)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Ostro et al., 2001 Central Los Angeles and Pasadena, CA August-October 1993	138 African American children ages 8-13 with doctor diagnosed asthma requiring medications in past year followed for daily respiratory symptoms and medication use. Lags of 0-3 days examined.	1-hr max O_3 mean (SD): LA: 59.5 (31.4) ppb Pasadena: 95.8 (49.0) ppb	PM10, NO_2, pollen, mold	PM10 / O_3 correlation = 0.35. Significant O_3 effect seen for extra medication use (above normal use). No O_3 effect on symptoms in expected direction observed. Inverse association seen for cough. PM10 effects seen at lag 3. Time factors not explicitly controlled in analysis; may have led to confounding of O_3 effects.	Odds ratio for extra medication use per 40 ppb 1-hr max O_3: 1.15 (1.12-11.19) Odds ratios for respiratory symptoms per 40 ppb lag 3 1-hr max O_3: Shortness of breath: 1.01 (0.92-1.10) Wheeze: 0.94 (0.88-1.00) Cough: 0.93 (0.87-0.99)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Romieu et al. 1996 N. Mexico City April 24-July 7, 1991 and November 1, 1991-February 28, 1992	71 mildly asthmatic children followed for PEFR and respiratory symptoms. Lower respiratory symptoms (LRI) included cough, phlegm, wheeze and/or difficulty breathing.	1-hr max O_3 mean (SD): 190 (80) ppb	PM2.5, PM10, NO_2, SO_2	O_3 effects observed on both PEFR and symptoms. Symptom, but not PEFR, effects robust to PM2.5 in two-pollutant models. Symptoms related to O_3 included cough, difficulty breathing, and LRI.	PEFR change for 50 ppb increase in 1-hr max O_3: AM PEFR (L/min): Lag 0: -2.44 (-4.4,-0.49) Lag 1: -0.23 (-0.41,1.62) Lag 2; -1.49 (-3.80,0.80) PM PEFR (L/min): Lag 0: -0.56 (-2.7,1.6) Lag 1: -1.27 (-3.2,0.62) Lag 2: -1.92 (-4.5,0.66) LRI symptoms odds ratio for 50 ppb increase in 1-hr max O_3: Lag 0: 1.09 (1.03-1.15) Lag 1: 1.10 (1.04-1.17) Lag 2: 1.04 (0.97-1.12)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Romieu et al. 1997 SW. Mexico City April 24-July 7, 1991 and November 1, 1991-February 28, 1992	Same period as Romieu et al., 1996, but in different section of city. 67 mildly asthmatic children age 5-13 followed for twice daily PEFR, and respiratory symptoms. Up to 2 months follow up per child. Analysis included temperature and looked at lags 0-2. No time controls. Lower respiratory symptoms (LRI) included cough, phlegm, wheeze and/or difficulty breathing.	1-hr max O_3 mean (SD): 196 (78) ppb	PM10	O_3 had significant effects on PEFR and symptoms, with largest effects at lags 0 and 1. Symptoms related to O_3 included cough, phlegm, and LRI. O_3 effects stronger than those for PM10.	PEFR change for 50 ppb increase in 1-hr max O_3: AM PEFR (L/min): Lag 0: -1.32 (-3.21,0.57) Lag 1: -0.39 (-2.24,1.47) Lag 2; -0.97 (-2.94,0.99) PM PEFR (L/min): Lag 0: -1.81 (-3.60,-0.01) Lag 1: -2.32 (-4.17,-0.47) Lag 2: -0.21 (-2.44,2.02) LRI symptoms odds ratio for 50 ppb increase in 1-hr max O_3: Lag 0: 1.11 (1.05-1.19) Lag 1: 1.08 (1.01-1.15) Lag 2: 1.07 (1.02-1.13)

Table 12-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean O_3 levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Ross et al. 2002 East Moline, IL and nearby communities May-October 1994	59 asthmatics ages 5-49 recruited. 19 lost to follow-up, yielding study population of 40. Assessment of PEFR and respiratory symptoms. Analytical methods unclear in terms of control for time factors.	8-hr max O_3 mean (SD): 41.5 (14.2) ppb IQR 20 ppb	PM10, SO_2, NO_2, pollen, fungi.	Saw significant associations between O_3 and both PEFR declines and symptom increases. Most but not all effects remained after controlling for temperature, pollen and fungi. No PM10 effects observed.	PEFR change for 10 ppb increase in 8-hr max O_3: AM PEFR (L/min): -2.29 (-4.26,-0.33) (AM PEFR effect disappeared when temperature added to model) PM PEFR (L/min): -2.58 (-4.26,-0.89) Symptom score (on scale of 0-3) change per 20 ppb increase in 8-hr max O_3: AM: 0.08 (0.03, 0.13) PM: 0.08 (0.04, 0.12)

12.2 Effects of Ozone on Daily Hospital Admissions and Emergency Department Visits

12.2.1 Introduction: Modeling Issues

The relationship between daily numbers of hospital admissions and emergency department visits (ED) and levels of ozone and related environmental factors has been analyzed in a wide variety of locales over the past decade, yielding insights into the possible effects of ozone on acute exacerbations of respiratory and cardiovascular diseases. These daily time series studies exploit the high degree of day to day variability in ambient air pollution concentrations to develop quantitative estimates of impacts on daily health outcomes. The basic analytical approach used to estimate the effects of ozone in this type of study is multiple regression. Because a given location is followed over time, many factors that might confound a multi-city "cross sectional" study do not affect time series studies. Cross sectional confounders include cigarette smoking, diet, occupation, and other risk factors that may vary across cities in a way that correlates with variations in air pollution levels. In contrast, in a daily time series study these factors are unlikely to vary over time in a way that correlates with day-to-day variations in air pollution. Longer-term secular time trends, such as changes in morbidity due to improved clinical management of disease, generally do not present a confounder problem in time series studies because these trends are removed analytically. Other advantages of the daily time series study design include the relatively large sample sizes in terms of person-days, and the ready availability of data, making such studies convenient and economical to conduct in a wide variety of locations.

However, several challenges present themselves with respect to designing and interpreting time series studies of hospital admissions and emergency department visits. The principal challenge facing the analyst in the daily time series context is avoiding bias due to confounding by short-term temporal factors operating over time scales from days to seasons. On a seasonal scale, the analysis must remove the influence of the strong seasonal cycles that usually exist in both health outcomes (usually higher in the cold season) and ozone (usually higher in the warm season). On a daily scale, weather factors and other air pollutants may also confound the association of interest.

Inadequate control for seasonal patterns in time series analyses leads to biased effect estimates. In the case of ozone, inadequate seasonal pattern control generally yields statistically significant inverse associations between ozone and health outcomes. Several examples of this phenomenon exist in the recent literature reviewed below (Anderson et al. 1998; Burnett et al. 2001; Prescott et al. 1998; Thompson et al. 2001). For winter-peaking pollutants such as CO and NO_2, the bias is in the direction of overly positive effect estimates. Several statistical approaches are available to control for this seasonal confounding, including weighted moving average filters, sinusoidal functions of time, and LOESS and Spline functions. All involve fitting smoothing functions to the time series of daily outcome counts which ideally removes seasonal variability from

the data and leaves only short term health variations to be explained by air pollution and weather variables. Another point worth noting is that temporal cycles in daily hospital admissions or emergency department visits are often considerably more episodic and variable than is usually the case for daily mortality. As a result, smoothing functions that have been developed and tuned for analyses of daily mortality data may not work as well at removing cyclic patterns from morbidity counts.

Three methods are commonly used for season adjustment, and an important distinction exists in the manner in which these seasonal adjustments are applied in the analysis. Pre-adjustment involves applying the adjustment to both outcome and air pollution variables prior to the regression analysis. In this case, the regression is done on the residuals following subtraction of smooth functions for each variable. Co-adjustment involves applying the adjustment as part of the regression analysis, by fitting a function of time while simultaneously fitting the regression effect of air pollution and weather factors. Until recently, these two approaches have been viewed as largely interchangeable. However, it has been demonstrated recently that the co-adjustment approach can lead to biased air pollution effect estimates in cases where both outcome and pollution variables exhibit strong seasonal cycles (Burnett et al. 2001). This was demonstrated using a 15 year time series of daily hospital admissions for acute respiratory diseases in children under age 2 in Toronto, Canada. Pre-adjustment followed by regression analysis yielded a statistically significant estimate of 16.1% <u>increase</u> in admissions per 45.2 ppb 1 hr maximum ozone from January to December. However, when the co-adjustment method was applied, there was a statistically significant 8.1 % <u>decrease</u> in admissions per 45.2 ppb ozone. The authors suggested that the co-adjustment method allows ozone to compete with the smoothing variable to explain some of the seasonal variability in the outcome, whereas pre-adjustment eliminates the seasonal variability prior to analysis of ozone effects. Interestingly, when the authors limited the analysis to the warm season (May-August), both methods yielded similar results (approximately a 33% increase in admissions, even after controlling for other pollutants, including SO_2), implying that seasonal stratification can remove a significant amount of the confounding seasonality. These findings are important to consider in reviewing the acute ozone morbidity literature since the vast majority of studies published over the past decade have used the co-adjustment method. It is also important to note that in some situations, prefiltering might remove some of the variation in the health endpoint that could be attributed to the air pollutant of concern, leading to an underestimate of the effect of air pollution.

The third method of adjusting for season involves stratification of the full time-series by warm and cold weather months. This technique has been used more for time-series studies of mortality (see Section 12.4) than morbidity. However, several of the studies of emergency room visits focus their analysis on the warm season. As a result, these studies may provide a clearer assessment of the effects of O_3.

A related issue is the potential biases that may exist in studies that employed the Generalized Additive Model (GAM) method in the S-plus software package to adjust for time and/or weather factors. It has been reported that the default S-plus GAM function can lead to suboptimal regression estimates for particulate matter and an underestimate of the standard error of that effect estimate (Dominici et al. 2002). Little attention has been paid to the influence of the GAM function on effect estimates for ozone. However, because ozone co-varies more strongly with both weather and time factors than does PM, the problem may be more acute for ozone than for PM. Few of the studies reviewed here used the GAM approach, and for those that did, no consistent pattern of results can be discerned.

Potential confounding by daily variations in co-pollutants and weather is another analytical issue to be considered. With respect to co-pollutants, daily variations in ozone tend not to correlate highly with most other criteria pollutants (e.g., CO, NO_2, SO_2, PM10), but may be more correlated with secondary fine particulate matter (e.g., PM2.5) measured during the summer months. Assessing the independent health effects of two pollutants that are somewhat correlated over time is problematic. However, much can be learned from the classic approach of first estimating the effects of each pollutant individually, and then estimating their effects in a two-pollutant model. If a pollutant-specific effect estimate does not change markedly in a two-pollutant model compared to a single-pollutant model, this is taken as evidence for independent effects of that pollutant. In that case, the pollutant-specific effect may be termed 'robust.' In the case of weather factors such as temperature and relative humidity, much of the rationale for considering these as potential confounders is based on the well-known effects of heat stress on risk of premature mortality. There is relatively little known about the effects of heat and humidity on morbidity risk. Still, to be conservative, most studies include one or more weather variables as covariates. This conservative approach carries a risk however of removing a portion of a real air pollution effect if the weather variables are highly correlated with pollution. In the case of ozone, in contrast to PM, this risk may be substantial given the high correlation between ozone and temperature.

Finally, one additional issue that relates to interpretation of these studies is that of exposure misclassification. As indicated above, O_3 does not penetrate well into the indoor environment; estimates suggest that 20-80% of ambient O_3 penetrates indoors. However, for houses with closed windows and operating air conditioners, penetration may drop to below 20%. Once indoors, O_3 reacts with wall surfaces and materials. Sarnat et al. (2001) demonstrated a very low and statistically non-significant association between personal exposure to O_3 and ambient O_3 in Baltimore. Thus, the signal of ambient O_3 may be significantly weakened, and personal exposures will vary greatly based on personal behaviors, such as time outdoors, exercise, opening of doors and windows, and use of air conditioning.

12.2.2 Hospital Admissions for Respiratory Diseases

Hospital admissions represent a medical response to a serious degree of morbidity for a particular disease. Hospitalizations are sometimes scheduled in advance when a particular clinical treatment is needed. However, unscheduled admissions are ones that occur in response to unanticipated disease exacerbations and are more likely to be affected by environmental factors, such as air pollution. As such, most of the hospital admissions studies reviewed here focused specifically on unscheduled admissions. Study details and results from 26 hospital admissions studies published over the past decade are summarized in Table 12-2.

The most robust and informative results on the effects of ozone on respiratory hospital admissions are those from studies carried out using a consistent analytical methodology across a broad geographic area (Burnett et al. 1997a; Anderson et al. 1997). Both studies reported significant ozone effects on respiratory hospital admissions. Cardiovascular admissions were not studied here. The largest such study to-date was carried out using data on all-age respiratory hospital admissions from 16 Canadian cities with populations exceeding 100,000 covering the period 1981-1991 (Burnett et al. 1997a). In addition to ozone, the authors evaluated health effects of SO_2, NO_2, CO, and COH (coefficient of haze, a surrogate for black carbon particle concentrations). The statistical analysis involved co-adjustment for seasonal cycles using a 19-day moving average filter, with stratification by seasons. Pooling the 16 cities, a significant positive association was observed between respiratory hospital admissions and lag 1 daily 1-hr maximum ozone concentrations in spring and summer. The results for fall were also positive, though of smaller magnitude. There was no evidence for an ozone effect in the winter season. The results for the under 65 yr age group and the over 65 yr age group were similar. Control outcomes related to blood, nervous system, digestive system, and genitourinary system disorders were not associated with ozone. Positive associations were also observed for COH and CO. In secondary analyses pooling the spring, summer and fall data, ozone effect estimates were very consistent across cities, and were robust to inclusion of co-pollutants. Inclusion of dewpoint temperature led to a 25% attenuation of the ozone effect, but it remained very significant when data were pooled across cities. There is a possibility of overcontrol given the uncertain biological basis for effects of dewpoint temperature on respiratory health. Other ozone metrics that also were evaluated included the 24-hr mean, the 8am-8pm mean, and the daily 8-hr maximum; however the 1-hr max had the strongest associations with admissions.

In a previous study focused mainly on evaluating health impacts of sulfate particles, Burnett and colleagues (1995) reported results from a time series analysis of all-age respiratory hospital admissions to 168 hospitals in Ontario, Canada over the six-year period 1983-1988. The outcome data were pre-filtered to remove seasonal variations using a weighted 19-day moving average. The authors reported that ozone was associated (significance not given) with

respiratory hospital admissions; however no quantitative results for ozone were presented.

Results from an analysis of six European cities indicated strong and consistent ozone effects on unscheduled hospital admissions for chronic obstructive pulmonary disease (Anderson et al. 1997). The six cities included – Amsterdam, Barcelona, London, Milan, Paris and Rotterdam – were among those included in the multi-city APHEA study. The number of years of available data varied from 5 to 13 years among the cities. In addition to ozone, the study considered health impacts of SO_2, NO_2, TSP, and BS (black smoke). Control for seasonality involved co-adjustment for sinusoidal functions of time. Statistical modeling for each city was carried out separately by a city-specific analysis team, which chose which lags to use for each pollutant, as well as other model details. The city- and pollutant-specific effect estimates were then pooled across cities using weighted means. Significant effects were seen for ozone, BS, TSP, and NO_2. Ozone effects were statistically significant in full year analyses, and appeared larger in the warm (April-September) half of the year compared to the cool (October-March) half. The authors reported that, "the most consistent and significant findings were for ozone, and there was no significant heterogeneity between the cities." No two-pollutant models were reported.

Several additional studies carried out in one or two cities over a span of five or more years provide substantial additional evidence regarding ozone effects on respiratory hospital admissions (Burnett et al. 1999, 2001; Lin et al. 2003; Moolgavkar et al. 1997; Petroeschevsky et al. 2001; Schouten et al. 1996; Sheppard et al. 1999). Many, but not all, reported significant ozone effects.

Two separate analyses of a large dataset from Toronto, Canada spanning the years 1980-1994 reported significant ozone effects on respiratory hospitalizations for all ages (Burnett et al. 1999) and for persons under age 2 (Burnett et al. 2001). Ozone was not associated with cardiac outcomes in Burnett et al. (1999). Although there was a high correlation between ozone and sulfate in these Canadian studies, both of these studies demonstrated ozone effects that were robust when PM and SO_2 measures were added to the regression. In contrast, PM effects from univariate regressions were markedly attenuated when ozone was added to regression. In the 2001 report, the authors compared all-year to warm-season results for ozone using either the co-adjustment or pre-adjustment method for controlling seasonal patterns. As noted above, application of the co-adjustment method to full-year data yielded ozone results that appeared to be confounded by seasonal patterns. In contrast, the pre-adjustment method applied to the full-year data, or either adjustment method applied only to warm season data, yielded plausible and apparently unbiased effect estimates for ozone. The authors cautioned that the co-adjustment method may be prone to residual seasonal confounding when applied in full-year analyses. This raises concerns regarding confounding bias that may be present in some of the studies reviewed below, as noted. It is important to note that Burnett et al. (1999) used the GAM LOESS function in a co-adjustment setting to control seasons and weather factors and did not carry out seasonally-stratified analyses. Thus,

quantitative results from that study should be interpreted with caution. In addition, the pollution data from southeastern Canada often exhibits a high correlation between O_3 and fine particle sulfate (r ~ 0.4), making it more difficult to associate the health outcome with a single pollutant.

Using a different analytical approach (case-crossover analysis), Lin and colleagues (2003) found no evidence for ozone effects on asthma admissions in 6-12 year olds over the period 1981-1993 in Toronto. There have been few studies utilizing the case-crossover methodology in the air pollution epidemiology literature, making it difficult to interpret these findings. Moolgavkar and colleagues (1997) reported significant and robust ozone effects on respiratory hospital admissions in adults 65 and older in Minneapolis-St. Paul MN, but not in Birmingham, AL. One may speculate that the absence of effects in the southern city may reflect less penetration of ozone into the indoor environment due to greater use of air conditioning, and thus less correlation between central site ozone monitoring and actual exposures of the urban populace. In Brisbane Australia over the years 1987-1994, significant ozone effects on all-age and age-stratified asthma and total respiratory hospital admissions were observed (Petroeschevsky et al., 2001). The ozone effects were robust to inclusion of PM (based on light scattering) and SO_2 in co-pollutant regressions. Effect sizes appeared consistent in the warm and cool seasons, possibly reflecting the relatively small degree of seasonal variation in ozone levels observed in Brisbane. Significant ozone (and other pollutant) effects on asthma hospitalizations for persons under age 65 were reported for Seattle by Sheppard et al. (1999). Anderson reported significant ozone effects in London during the warm season (1998). Less consistent effects of ozone and other pollutants were seen in Amsterdam and Rotterdam in a study made difficult to interpret due to the large number of statistical tests performed (Schouten et al. 1996). While some inconsistencies are noted across studies, this body of evidence from single or pairs of cities studied over five more years supports the findings of the multi-city analyses, of significant and robust effects of ozone on various respiratory disease hospitalization outcomes.

A third set of studies have examined associations between ozone and respiratory and/or cardiac hospitalizations in single cities over shorter (< 5 year) time spans. Positive and significant ozone effects were reported for both respiratory and cardiac admissions in Toronto, Canada (Burnett et al. 1997b), and for respiratory admissions in Buffalo, NY (Gwynn et al. 2000), Spokane, WA (Schwartz 1996); Cleveland (Schwartz et al. 1996), Northern New Jersey (Weisel et al. 2002), Sao Paolo, Brazil (Gouveia and Fletcher 2000b), London (Ponce de Leon et al. 1996), and Helsinki, Finland (Ponka and Virtanen 1996). However, no association between ozone and respiratory or cardiac hospital admissions were seen in Los Angeles (Linn et al. 2000), or for ischemic heart disease in the South Coast Air Quality Management District (Mann et al. 2002), or cardiac admissions in Valencia, Spain (Ballester et al. 2001). No significant associations with asthma admissions were seen in Central Los Angeles (Nauenberg and Basu 1999), with total respiratory admissions in Drammen, Sweden (Hagen et al. 2000), nor with respiratory and cardiac admissions in Edinburgh, Scotland (Prescott et al. 1998).

The co-adjustment approach for controlling temporal confounding was used in most of these studies and may have led to residual confounding of the ozone effects.

In one study in Belfast, Northern Ireland, authors reported significant protective 'effect' of ozone (Thompson et al. 2001). The Helsinki study reported significant effects of ozone on both asthma and on digestive disorders in a setting of very low ozone concentrations (Ponka and Virtanen 1996), which raises questions of plausibility. Several of the studies reporting non-significant ozone effects were carried out in locations with very low ozone levels, suggestive of a non-linear exposure-response relationship (Ballester et al. 2001; Hagen et al. 2000; Prescott et al. 1998; Thompson et al. 2001). Inadequate control of seasonal confounding may also underlie some of the negative (and especially protective) findings. An additional factor likely contributing the variability of results is the relatively small sample sizes, in terms of numbers of days, included in many of these studies. The negative findings in the LA basin are surprising given the elevated ozone concentrations observed there (Linn et al. 2000; Mann et al. 2002). This may reflect uncontrolled confounding by seasonal factors.

Table 12-2 Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Anderson et al., 1996 6 European Cities: (Amsterdam, Barcelona, London, Milan, Paris, Rotterdam) Periods vary	Emergency COPD admissions for all ages. Each city analyzed previously by individual teams. Results combined here via meta-analysis.	Range across cities: 1-hr max O_3: Med 18-39 ppb 8-hr max O_3: Med 10-35 ppb	TSP, SO_2, NO_2, BS	0-5, depending on city.	Poisson GLM using APHEA methodology. Results stratified by season. O_3 most consistent and significant predictor of admissions. Warm season effect larger.	Weighted-mean effects across 6 cities: O_3 1-hr max (per 25 ppb): 1.029 (1.011-1.047) O_3 8-hr max (per 25 ppb): 1.043 (1.022-1.065)
Anderson et al. 1998 London 1987-1992	Admissions for asthma in ages 0-14, 15-64, and 65+ age groups	8-hr max O_3: Mean 15.5 IQR 13 1-hr max O_3: Mean 20.6 IQR 16	SO_2, NO_2, BS, pollens	0-5 explored	Poisson GLM using APHEA method; co-adjustment. O_3 significantly associated with asthma admissions in the warm for all ages and for the 15-64 age group. Warm season O_3 effect robust in 2-pollut models. Inverse associations observed in the cool season for some age groups.	Single pollutant models for lag 1 O_3 per 10 ppb for all ages: Whole year: 1.007(0.099-1.010) Warm season: 1.021(1.006-1.038) Cool season: 0.928 (0.946-0.992)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Ballester et al., 2001 Valencia, Spain 1994-1996	Emergency total cardiac admissions for all ages.	8-hr max O_3: Mean 23 ppb Range 5-64	SO_2, NO_2, CO, BS	0-5 explored	Poisson GLM using APHEA methodology. Results stratified by season. No O_3 effects.	O_3 8-hr max (per 5 ppb): All cardiac (lag 2): 0.9905 (0.9710-1.0104)
Burnett et al. 1995 168 Hospitals in Ontario, Canada 1983-1988	Cardiac and respiratory admissions for all ages and within age strata. Study focused mainly on testing for sulfate effects.	1-hr max O_3: Mean 36.3 ppb		Lag 1 O_3	GLM with pre-adjustment of outcome variables. Results stratified by season. Authors report that O_3 associated with respiratory admission in warm season only.	No quantitative results presented for O_3.
Burnett et al. 1997a 16 Canadian Cities 1981-1991	Total respiratory admissions for persons <65 and >64.	For all cities, 1-hr max O_3: Mean 31 ppb 95th %tile 60 Apr-Dec only: Mean 33 ppb IQR 11 95%tile 64	SO_2, NO_2, CO, COH	Lags 0-2 explored. Lag 1 chosen as most significant	Poisson GLM with co-adjustment. Results stratified by season. Significant O_3 effect observed in warm season only. No 0_3 effects on control outcomes. Results consistent across cities.	Lag 1 O_3 1-hr max (per 30 ppb): Jan-Mar: 0.994 (0.964-1.025) Apr-Jun: 1.042 (1.012-1.073) Jul-Sep: 1.050 (1.026-1.074) Oct-Dec: 1.028 (0.998-1.059)
Burnett et al., 1997b Toronto, Ontario 1992-1994 Summers only	Unscheduled cardiac and respiratory admissions for all ages.	1-hr max O_3: Mean 41.2 IQR 22	PM2.5, PM10, H+, SO_4, SO_2, NO_2, CO, COH	Lags 0-4 explored. The three day avg. lagged 1 or 2 days chosen as most significant.	Poisson GLM with co-adjustment. Results stratified by season. O_3 and COH strongest predictors of outcomes. O_3 effects on both outcomes were robust to PM. PM effects were not robust to O_3.	O_3 1-hr max (per 11.5 ppb), controlling for temp and dewpoint: Respiratory (lag 1, 3-d avg): 1.064 (1.039-1.090) Cardiac: (lag 2, 3-d avg): 1.074 (1.035-1.115)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Burnett et al. 1999 Toronto, Ontario 1980-1994	Cause-specific admissions for all ages. Cause categories included asthma, COPD, respiratory infections, heart failure, ischemic heart disease, and cerebrovascular disease.	24-hr avg O_3: Mean 19.5 ppb IQR 19	Estimated PM2.5, PM10, PM10-2.5, CO, NO_2, SO_2	Single and multi-day averages from 0-2 days explored. Best fit picked for each outcome.	Poisson GAM pre-adjustment. O_3 effects seen for respiratory outcomes only. O_3 effect robust to PM; not visa versa. No seasonal stratification.	Single-pollutant models for O_3 24-hr average (per 19.5 ppb): Asthma (lag 1, 3-d avg): 1.063 (1.036-1.091) COPD (lag 2, 3-d avg): 1.073 (1.038-1.107) Respiratory Infection (lag 1, 2-d avg): 1.044 (1.024-1.065)
Burnett et al., 2001 Toronto, Ontario 1980-1994	Acute respiratory disease admissions for ages <2 and >18.	1-hr max O_3: Mean 45.2 IQR 25	Estimated PM2.5, PM10, PM10-2.5, CO, NO_2, SO_2	Single and multi-day averages from 0-5 days explored. Best fit picked.	Poisson GLM with pre-adjustment. Sensitivity analyses using co-adjustment. Results stratified by season. O_3 effects significant only in warm season. O_3 effect robust to PM; not visa versa.	Single-pollutant models for O_3 1-hr max (per 45.2 ppb): Acute respire disease age <2 (lag 0, 5-d avg): 1.348 (1.193-1.523)
Gouveia and Fletcher 2000a Sao Paulo, Brazil Nov 1992-Sep 1994	Total respiratory, pneumonia, and asthma admissions for ages <5.	1-hr max O_3: Mean 32 ppb IQR 25	PM10, SO_2, NO_2, CO	0-2 explored. Best fit picked.	Poisson GLM with co-adjustment using sine/cosine waves. Significant O_3 effects on total respiratory and pneumonia admissions. O_3 effects fairly robust to NO_2 and PM10.	Single-pollutant models for O_3 1-hr max (per 60.9 ppb): Total respiratory (lag 0): 1.054 (1.003-1.107) Pneumonia (lag 0): 1.076 (1.014-1.142) Asthma (lag 2): 1.011 (0.899-1.136)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Gwynn et al. 2000 Buffalo, NY May 1988-Oct 1990	Total respiratory admissions for all ages.	24-hr avg O_3: Mean 26 ppb IQR 14.8	PM10, SO4, H+, COH, CO, NO_2, SO_2	0-3 explored. Best fit picked.	Poisson GAM control of temperature; moving average control for time. O_3 significant predictor of outcome. No 2-pollutant models reported.	O_3 24-hr avg (per 14.8 ppb): Total respiratory (lag 1): 1.029 (1.013-1.045)
Hagen et al. 2000 Dramen, Sweden Nov 1994-Dec 1997	Total and respiratory admissions for all ages.	24-hr avg O_3: Mean 22.6 ppb IQR 13.4	PM10, NO_2, SO_2, benzene, toluene, HCHO	0	Poisson GAM co-adjustment. Single and multi-pollutant models evaluated. No O_3 effects. O_3 levels very low and cycles may not have been adequately controlled.	Single-pollutant model for O_3 24-hr avg (per 13.4 ppb): Total respiratory (lag 0): 0.964 (0.899-1.033)
Lin et al. 2003 Toronto, Ontario 1981-1993	Asthma admission for 6-12 year olds. Case-crossover design.	1-hr max O_3: Mean 30 ppb IQR 20	CO, SO_2, NO_2	Explored 4-7 day averages ending on admit day	Case-crossover analysis. No O_3 effects observed.	No significant effects.
Linn et al. 2000 Los Angeles, CA 1992-1995	Total respiratory and total cardiac admissions for age >64.	24-hr avg O_3: Mean (SD): Winter 14 (7) Spring 32 (10) Summer 36 (8) Fall 15 (9)	PM10, CO, NO_2	0	Poisson GLM; co-adjustment. Only significant O_3 effects observed were inverse associations with total cardiac admission in full-year and winter season, suggesting residual confounding. No significant effects of O_3 on respiratory disease admits.	No significant effects.

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Mann et al. 2002 Los Angeles basin 1988-1995	Ischemic heart disease admissions for adults 40 and over.	8-hr max O_3: Mean 50.3 ppb IQR 39.6	PM10, CO, NO_2	0-5 single and multi-day averages explored.	Poisson GAM with co-adjustment. No significant O_3 effects observed overall or in warm season. CO and NO_2 significant in full-year analyses. P	No significant effects.
Moolgavkar et al. 1997 Minneapolis/St. Paul, MN and Birmingham, AL 1986-1991	Pneumonia and COPD admissions for adults >64.	Metric not specified: Mean ppb(IQR): MN 26 (15) AL 25 (13)	PM10, SO_2, NO_2	Explored lags 0-3. Best fit picked.	Poisson GLM with co-adjustment. Both O_3 and PM10 significant in MN; not in AL. O_3, but not PM10, effects were robust to NO_2 and SO_2.	Single-pollutant models (20 df smoother) for O_3 (per 15 ppb) in MN: Pneumonia (lag 1): 1.066 (1.034-1.098) COPD (lag 0): 1.045 (0.995-1.067)
Nauenberg and Basu, 1999 Los Angeles, CA 1991-1994	Unscheduled asthma admissions for all ages.	14-hr average O_3: Mean 19.9 ppb SD 11.1	PM10	0	Poisson GLM with pre-adjustment. No significant effects of O_3. No warm season results presented.	No significant effects.
Petroeschevsky et al. 2001 Brisbane, Australia 1987-1994	Unscheduled asthma, total respiratory and total cardiac admissions in several age strata: 0-4, 5-14, 15-64, 65+, all.	1-hr max O_3: Mean 25.3 ppb Range 2.5-107.3 8-hr 10-6 O_3: Mean 19 ppb Range 1.7-64.7	B_{scat}, SO_2, NO_2	0-4 single and multi-day averages.	Poisson GLM using APHEA co-adjustment methodology. Results stratified by season. O_3 significantly related to asthma and total respiratory admissions, not for cardiac. Effects varied by age group. O_3 effects robust to co-pollutants	Single-pollutant models for O_3 8-hr 10am-6pm (per 10 ppb): All-age respiratory (lag 2): 1.023 (1.003-1.043 All-age asthma (5-d avg): 1.084 (1.037-1.133)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Ponce de Leon et al. 1996 London Apr 1987-Feb 1992	Total respiratory admissions in several age strata: 0-14, 15-64, 65+, all.	8-hr 9am-5pm: Mean 15.6 ppb IQR 14	BS, SO_2, NO_2	0-3 single and multi-day averages.	Poisson GLM using APHEA co-adjustment methodology. O_3 significant predictor overall. Effect larger and more significant in warm season. Effect robust to co-pollutants. Effects varied by age.	All-age single-pollutant models for O_3 8-hr 9am-5pm at lag 1: All year (per 26 ppb): 1.0293 (1.0113-1.0477) Warm season (per 29 ppb): 1.0483 (1.0246-1.0726) Cool season (per 20 ppb): 0.9960 (0.9717-1.0208)
Ponka et al., 1996 Helsinki, Finland 1987-1989	Asthma admissions for persons 0-14, and 15-64 years.	8-hr max O_3: Seasonal means ranged from 7.6-15 ppb.	TSP, SO_2, NO_2	0-5 single day lags explored.	Poisson GLM using APHEA methodology. Reported significant O_3 effect for 0-14 age group only, but also for control (digestive disease) conditions. O_3 levels very low.	Not quantitatively useful.
Prescott et al. 1998 Edinburgh, 1992-1995.	Total cardiac, and total respiratory admissions by age (< 65 and 65 +).	24-hr avg O_3: Mean 14.5 ppb Range 1-37	BS, PM10, NO_2, SO_2, CO; also 2-poll. model	Lag 0, 3-day averages.	Poisson GLM, month dummy variables; co-adjustment. No O_3 or other pollution effects on respiratory admissions. Significant inverse association of O_3 with cardiac admissions in older age group. Very low O_3 concentrations.	Single-pollutant models for O_3 24-hr avg (per 10 ppb): Cardiac (lag 0, 3-d avg): Age 65+: 0.941(.886-.999) Age <65: 1.041(.946-1.144) Respiratory (lag 0, 3-d avg): Age 65+: 1.009(.916-1.111) Age <65: 0.971(.885-1.068)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Schouten et al. 1996 Amsterdam and Rotterdam 1977-1989	Unscheduled asthma, COPD, and total respiratory admissions in all ages.	1-hr max O_3: Amsterdam: Mean 40 ppb 95th%tile 77 Rotterdam: Mean 39 ppb 95th%tile 83	SO_2, NO_2, BS	0-5 single and multi-day averages.	Poisson GLM using APHEA methodology; co-adjustment. No consistent O_3 effects. Concern regarding multiple comparisons.	Not quantitatively useful.
Schwartz 1996 Spokane, WA Apr-Oct, 1988-1990	Total respiratory admissions in persons 65 and older.	1-hr max O_3: Mean 40 ppb IQR 12 24-hr avg O_3: Mean 29 ppb IQR 9	PM10	2	Poisson GAM; co-adjustment. Results available only for warm season. O_3 and PM10 both significant predictors of outcome. No 2-pollutant models reported. O_3 effects robust to more extensive temperature specification.	Single-pollut models for 25.45 ppb O_3 at lag 2: 24-hr avg O_3: 1.284 (0.926-1.778) 1-hr max O_3: 1.244 (1.002-1.544)
Schwartz et al. 1996 Cleveland, OH Apr-Oct 1988-1990	Total respiratory admissions in persons 65 and older.	1-hr max O_3: Mean 56 ppb IQR 28	PM10, SO_2	0-7 days explored.	Poisson GLM with sinusoids; co-adjustment. Results available only for warm season. O_3 and PM10 both significant predictors of outcome. No 2-pollutant models reported.	Single-pollutant model for O_3 1-hr max per 100 ug/m3 (51 ppb) avg of lags 0 and 1: 1.09 (1.02-1.16)
Sheppard et al., 1999 Seattle, WA 1987-1994	Asthma admissions in persons under age 65.	8-hr max O_3: Mean 30.4 IQR 20	PM2.5, PM10, PM10-2.5, SO_2, CO	2 day	Poisson GLM. Results stratified by season. O_3 significant predictor of outcome. No 2-pollutant models reported for O_3.	Single-pollutant model for lag 2 O_3 8-hr max per 20 ppb: 1.06 (1.02-1.11)

Table 12-2 (cont.): Effects of Ozone on Daily Hopsital Admissions

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Thompson et al. 2001 Belfast, N. Ireland 1993-1995	Asthma admissions in children (age not specified)	24-hr avg O_3: Warm season: Mean 18.7 IQR 9 Cold season: Mean 17.1 IQR 12	PM10, SO_2, NO_2, CO, benzene	Averages of lags 0-3 explored.	GLM with sinusoids. Pre-adjustment. Significant inverse O_3 associations in full-year and cold-season models. No O_3 effect in warm season. Very low O_3 levels.	Results not quantitatively useful.
Weisel et al. 2002 New Jersey Summer 1995	Total respiratory admissions for all ages.	1-hr, 5-hr, and 8-hr daily max O_3 analyzed. Levels not reported.	none	1-3 days explored.	No control for time, but authors report no auto-correlation, which alleviates concerns about lack of control. Significant O_3 effects reported. No other pollutants included.	Reports only regression slopes and p values. Cannot calculate RRs without mean admission counts.
Wong et al. 1999 Hong Kong Jan 1995-Jun 1997	Total and cause-specific cardiac admissions in all ages.	1-hr max O_3. Levels not reported.	NO_2, SO_2, respirable PM	0-5 day averages.	GLM with sinusoids; co-adjustment. O_3 significantly associated with outcome in cool season only. Brief report hinders interpretation.	Two pollutant models for O_3 1-hr max per 25.45 ppb for mean of lags 0 and 1: Total cardiac: All year: 1.03 (1.00-1.07) Warm: 1.01 (0.95-1.07) Cool: 1.08 (1.02-1.14)

12.2.3 Emergency Department Visits for Respiratory Diseases

Emergency department visits represent another important acute outcome that may be affected by ozone exposures. Morbidities that result in ED visits are closely related to, but are generally less severe than, those that result in unscheduled hospital admissions. In many cases, acute health problems are successfully treated in the ED; a subset of more severe cases that present initially to the ED may require admission to the hospital. If ozone exposure increases the likelihood of hospital admissions, as suggested above, then it is likely that ED visits would also be affected, and to a greater degree.

Several studies have been published in the past decade examining the temporal associations between ED visits for asthma or other respiratory diseases and ozone exposures (Table 12-3). As a group, these ED studies tend to be smaller in terms of geographic and temporal coverage than those reviewed above for hospital admissions. Results regarding associations between ED visits and ozone levels also tend to be somewhat less consistent, with effects apparent only in selected sub-groups and at certain lags. Among 20 studies with adequate controls for seasonal patterns, 16 reported at least one significant positive association involving ozone: Cassino et al. (1999) for asthma, Delfino et al. (1998, 1997b) for respiratory complaints, Hernandez-Guarduno (1997) for respiratory complaints, Jaffe et al. (2003) for asthma, Jones et al. (1995) for respiratory complaints, Lin et al. (1999) for respiratory complaints, Martins et al. (2002) for chronic lower-respiratory complaints, Stieb et al. (1996) for asthma, Tenias et al. (1998, 2002) for asthma and COPD, Tobias et al. (1999) for asthma, Tolbert et al. (2000) for asthma, White et al. (1994) for asthma, Romieu et al. (1995) for asthma, and Weisel et al. (1995) for asthma.

One of the longest running asthma ED studies was carried out during the months of May through September from 1984-1992 in St. John, New Brunswick, Canada (Stieb et al. 1996). Effects were examined separately among children aged 0-15 and in persons older than 15. A significant effect of ozone on ED visits in the over 15 age group was reported, with evidence of a heightened increase in risk when 1 hr maximum O_3 exceeded 75 ppb. Effects on children were elevated at concentrations above 75 ppb, but not statistically significant. A five-year record of June-August asthma ED data for Medicaid recipients aged 5-34 years from three Ohio cities was analyzed by Jaffe et al. (2002). A statistically significant ozone effect was observed in a three-city pooled analysis. In another relatively large study, Tolbert and colleagues (2000) analyzed pediatric asthma ED visits over three summers in Atlanta, GA. Significant effects of both ozone and PM10 were observed in univariate regressions; both, however, became non-significant in two-pollutant regressions, reflecting the high correlation between the two pollutants (r=0.75). Two studies in Sao Paulo, Brazil reported significant ozone effects on respiratory ED visits among children (Lin et al. 1999) and persons 65 and older (Martins et al. 2002) based on a two-year data record. Ozone results were robust in models that included PM10. ED visits for asthma and COPD among persons over 14 years old were robustly associated with relatively low ozone levels in Valencia, Spain in data from 1994 and 1995 (Tenias et al. 2002;

Tenias et al. 1998). The associations with asthma were larger in the warm season than in winter (Tenias et al., 1998). Associations of COPD with ozone were only significant at lags 4 and 5, with effect sizes that did not vary markedly across summer vs. winter, both of which findings seem somewhat implausible (Tenias et al., 2002).

Negative findings for ozone were reported by Castellsague and colleagues (1995) based on analysis of a five year record of adult asthma ED visits in Barcelona, Spain. The authors controlled extensively for weather factors. Hajat and colleagues (2002) found no evidence for effects of ozone on general practitioner consultations for upper respiratory disease complaints in London, England. This outcome is likely to involve a greater degree of scheduling than is the case for ED visits. The authors utilized the GAM method for co-adjustment of seasonal and weather covariates. Chew and colleagues (1999) saw no evidence for ozone effects on childhood asthma exacerbations in Singapore. Finally, Schwartz et al. (1993) reported no association between O_3 and emergency room visits for asthma in Seattle.

For many of the remaining 'positive' studies, inconsistencies mar an interpretation of likely causal effects in many cases. For example, effects on adult ED visits for asthma in NYC were apparent only among a subgroup of 285 heavy smokers, and not among 552 non-smokers or 278 light smokers (Cassino et al. 1999). In Montreal, ozone effects on asthma ED visits were seen in a short record from the summer of 1993 but not in a similar record from the summer of 1992 (Delfino et al. 1997a). The significant 1993 results were seen only for persons older than 65 years, in spite of greater asthma prevalence among children. A very similar analysis of an additional two summers (1989 and 1990) revealed an ozone association only for 1989 and again only in the over 65 age group (Delfino et al. 1998). These inconsistent results from small, self-selected study periods do not provide quantitatively useful results. An analysis of data on respiratory ED visits from June-August of 1990 in Baton Rouge, LA reported ozone effects in adults, but not in children or among the elderly (Jones et al. 1995). Hernandez-Garduno and colleagues (1997) reported significant positive effects of ozone on respiratory visits to general practitioner outpatient clinics in Mexico City, but effects were seen only for lags 0 and 5. Tobias and colleagues (1999) showed that regression results for asthma ED visits could be quite sensitive to methods used to control for asthma epidemics in Barcelona, Spain. Ozone was associated with the outcome variable in only one of eight models tested.

Several other ED studies looking at ozone are not interpretable due to inadequate control for seasonal patterns, very low ozone levels, or because no quantitative results were shown for ozone (Buchdahl et al. 1996; Buchdahl et al. 2000; Lierl and Hornung 2003; Lipsett et al. 1997; Garty et al. 1998; Holmen et al. 1997; Nutman et al. 1998).

Several of the studies on emergency room visits for asthma examined the shape of the concentration-response function for possible non-linearities and thresholds. This, coupled with information on the O_3 concentrations in negative

studies provides useful information on the concentrations that may be of particular concern. For example, as reported above, Stieb et al. (1996) found an increased risk for emergency room visits among adults exposed to concentrations above 75 ppb (with a maximum concentration of 160 ppb). The analysis of emergency room visits for childhood asthma in summertime Atlanta by White et al. (1994) indicated a significantly elevated risk when 1 hr O_3 exceeded 110 ppb (maximum 160 ppb). Weisel et al. (1995) analyzed the relationship between summertime O_3 and emergency room visits for asthma. They reported statistically significant effects for 1 hr O_3 concentrations above 60 ppb (maximum 100 ppb). Romieu et al. (1995) reported an association between emergency room visits for childhood asthma and O_3 in Mexico City. Particularly large risks were observed after consecutive days when the 1 hr maximum O_3 concentration exceeded 110 ppb. As reported above, Tolbert et al. (2001) reported an association between pediatric emergency room visits for asthma and O_3 in Atlanta. Elevated risks were apparent at concentrations greater than 8 hr averages of 70 ppb, with effects becoming statistically significant when 8 hr average O_3 concentration was between 100 and 113 ppb. Although a similar analysis was not reported for 1 hr O_3 concentrations, the related concentrations for elevated risk would be approximately 980 ppb, with statistical significance at approximately 115-130 ppb.

Among the negative studies in North America, Schwartz et al. (1993) reported no association between emergency room visits for asthma and low levels of O_3 (actual level not reported). Finally, in a correlational analysis, Bates et al. (1990) found no association between emergency room visits for asthma in Vancouver among those less than age 15, age 15 to 60, and 61 and above. Again, the 1 hr maximum O_3 concentration was low, with only three days exceeding 80 ppb.

12.2.4 Which Diseases Have Been Most Consistently Associated with Ozone?

The vast majority of hospitalization and ED studies conducted over the past decade have looked at effects of ozone on either total respiratory diseases and/or on asthma. Significant associations with ozone have been seen with both outcomes in many cases. Total respiratory causes may include asthma, pneumonia, bronchitis, emphysema, cancers, other upper and lower respiratory infections such as influenza, and a few other minor categories, with asthma typically dominating the daily event counts. Chronic bronchitis and emphysema are often combined, with or without asthma, to define chronic obstructive pulmonary disease (COPD), which is a prominent diagnosis among older adults with lung disease. In the recent literature reviewed here, no clear pattern is evident regarding associations of ozone with specific respiratory disease outcomes. Large multi-city studies of hospital admissions have reported significant ozone associations with total respiratory hospitalizations (Burnett et al. 1997a) and COPD (Anderson et al. 1997). Many of the individual city studies have reported associations with total respiratory admissions and a few with asthma. In the case of ED studies, asthma has been studied most often, with variable results.

Among the subset of the hospital admissions studies that have examined associations of ozone with cardiovascular outcomes, most have found no consistent positive associations (Burnett et al. 1995; Burnett et al. 1999; Linn et al. 2000; Mann et al., 2002; Ballester et al. 2001; Petroeschevsky et al. 2001; Prescott et al. 1998). The exceptions are one study in Toronto which reported robust associations with both total respiratory and cardiovascular hospital admissions (Burnett et al. 1997b), and one in Hong Kong, in which circulatory, ischemic heart, and heart failure were all significantly associated with ozone in the cool but not the warm seasons (Wong et al. 1999). The authors of the latter report speculated that differing activity patterns and home ventilation factors may have been responsible for the seasonal differences. That is, ozone may penetrate indoor environments more readily during the cool season when less air conditioning is used, resulting in higher population exposures even with lower ambient concentrations. Based on this small set of studies, current evidence does not support a conclusion that ozone has independent effects on cardiovascular hospitalizations.

12.2.5 Other Issues: Age, Averaging Times, Thresholds, Co-Pollutants

With respect to age-specificity of associations between ozone and acute respiratory hospitalizations or ED visits, no clear pattern emerges from recent studies. Significant associations have been reported for all-ages (Anderson et al., 1997; Burnett et al., 1995, 1997b, 1999; Gwynn et al., 2000; Weisel et al., 2002), adults or elderly (Burnett et al., 1997a; Delfino et al., 1997, 1998; Moolgavkar et al., 1997; Schwartz, 1996; Schwartz et al., 1996), and children (Burnett et al., 2001; Gouveia et al., 2000; Lin et al., 1999; Ponka et al., 1996; Tolbert et al., 2000). Interestingly, studies that have examined effects in multiple age strata have often seen effects only in non-pediatric strata (Delfino et al., 1997, 1998; Steib et al., 1996; Jones et al., 1995). Several studies that focused on children did not report significant ozone effects, though in some cases these studies are limited by small size, inadequate control of seasonal patterns, or very low ozone levels (Hajat et al., 2002; Lierl et al., 2003; Lin et al., 2003; Thompson 2001). If ozone is causally related to exacerbations of respiratory diseases leading to hospital usage, it would be surprising not to see effects most prominently among children, for whom asthma is most prevalent and exposures may be greater.

A variety of ozone exposure metrics have been used in the studies reviewed here, including the daily 1-hr maximum, the daily 8-hr running maximum, the 8-hr mid-day mean, and the 24-hr mean. Because all of these metrics are highly correlated with one another, it is not surprising that similar qualitative results are seen regardless of the metric used. In theory it should be possible to examine statistics related to model fit to gain insights into the relative impacts of alternative averaging times. Such data are currently lacking however. It is also important to note that, because concentrations are lower and less variable for the longer averaging times, relative risks of adverse health outcomes for a specific given concentration range are not directly comparable across metrics, unless the exposure increment is a relative measure, e.g., based on the interquartile range

of concentrations. Although results from the epidemiologic studies reviewed here do not make it possible to choose an optimal exposure metric, when taken together with information from controlled chamber exposure studies, it would appear that the daily 8-hr maximum ozone concentration represents an optimal exposure metric for health risk assessment.

An important consideration in determining whether a safe level of ozone can be identified is whether the concentration-response (C-R) relationship is linear across the full concentration range or instead shows evidence of a threshold. In the case of ozone and acute hospital usage, those studies, which have examined the shape of the C-R function, have often seen evidence of an effect threshold. In a study of all-age respiratory hospital admissions in Toronto, effects of ozone appeared to become apparent only above about 30 ppb daily 1-hr maximum (Burnett et al. 1997b). Similar findings were reported for respiratory admissions in Buffalo (Gwynn et al. 2000). In a study of ED visits for asthma in St. John, New Brunswick, effects observed in the over 15 year age group were apparent only when data above the 95th percentile (75 ppb daily 1-hr max) were included (Stieb et al. 1996). Supporting evidence for an effect threshold is provided by the numerous studies where ozone effects were seen only in the warm months when ozone levels are higher and more variable (for example, (Burnett et al. 1995; Burnett et al., 2001; Ponce de Leon et al. 1996; Anderson et al. 1998). On the other hand, several studies that examined the shape of the C-R function did not find evidence for a threshold (Burnett et al. 1997a; Petroeschevsky et al. 2001; Tenias et al. 1998).

Another important consideration is whether the ozone effects observed in studies of hospital admissions and ED visits appear to be related specifically to ozone as opposed to co-pollutants such as PM. On this issue, the evidence is fairly supportive of independent effects for ozone. Numerous studies have reported ozone effects which are robust to inclusion of co-pollutants in the analytical model, including PM (Burnett et al. 1997b; Burnett et al. 1999; Burnett et al. 2001; Moolgavkar et al. 1997; Schwartz 1996; Schwartz et al. 1996; Tenias et al. 2002; Tenias et al. 1998).

Table 12-3 Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Castellsague et al. 1995 Barcelona 1985-1999	Daily ED visits for asthma in persons > 14 yrs.	1-hr max O_3: Summer: Mean 43 IQR 22 Winter: Mean 29 IQR 16	BS, SO_2, NO_2	0-5	Poisson regression with year and month dummy variables and extensive control for weather factors (min, max, mean temperature, relative humidity, dewpoint temperature; continuous and categorical parameterizations)	O_3 1-hr max (per 12.7 ppb) Lag not specified Summer 0.991 (0.939-1.045) Winter 1.055 (0.998-1.116)
Cassino et al. 1999) New York City July 1992-December 1995 continuous	Daily time series study of ED visits in a cohort of 1115 adult asthmatics ages 18-84, stratified into 552 never-smokers, 278 light smokers, and 285 heavy smokers. Ethnicity: Hispanic 58%; African-American 26%; White 11%; other 5%.	24-hr avg O_3: mean: 17.5 ppb; IQR: 9-23 1-hr max O_3: mean: 37.2 ppb; IQR: 20-48	CO, NO_2, SO_2	0,1,2 and 3	Used Poisson regression. Loess with GAM used to control for temporal (periods greater than 3 months) and weather factors. No warm-season results presented. Significant O_3 effects seen only at lag 2 among heavy-smokers. Co-pollutants did not have effects. Concerns regarding possible GAM-related biases. Short-term cycles and episodic variations in asthma may not have been controlled adequately with 3-mo period loess. Multiple tests performed, and inconsistent results across smoking strata and lags, raise possibility of chance findings. No PM included.	O_3 24-hr avg (per 14 ppb): Heavy Smoker Subgroup: Lag 0: 0.87 (0.75-1.02) Lag 1: 1.07 (0.93-1.24) Lag 2: 1.26 (1.1-1.44) Lag 3: 0.96 (0.83-1.1)

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Chew et al. 1999 Singapore Jan 1990-Dec 1994	Emergency department visits for asthma among persons 3-21 years.	1-hr max O_3: Mean 23 ppb SD 15	TSP, PM10, SO_2, NO_2	0, 1, and 2	Simplistic but probably adequate control for time by including lag 1 outcome as covariate. In models that included covariates, O_3 had no significant effect.	No results presented.
Delfino et al. 1997a Montreal June 15-September 20, 1992 and 1993	Daily time series ecologic study of ED visits for respiratory complaints within 5 age strata (<2, 2-18, 19-34, 35-64, >64).	8-hr max O_3: 1992 mean: 28.8 ppb SD: 11.3 1993 mean: 30.7 ppb SD: 11.5 1-hr max O_3: 1992 mean: 33.2 ppb SD: 12.6 1993 mean: 36.2 ppb SD: 13.8	PM10, PM2.5, SO_4, H+	0,1 and 2	Used ordinary least squares, with 19-day weighted moving average pre-filter to control cycles; weather also controlled. Significant effects reported for lag 1 O_3 in 1993 only for the >64 age group. This O_3 effect reported to be robust in two-pollutant models. Low ozone levels raise plausibility concerns. Short data series, multiple tests performed, and inconsistent results across years and age groups raise possibility of chance findings. Lag not specified a-priori.	1993 , >64 yrs: O_3 8-hr max (per 30.7 ppb): Lag 1: 1.22 (1.09-1.35) O_3 1-hr max (per 36.2 ppb): Lag 1: 1.21 (1.08-1.34)

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Delfino et al. 1998 Montreal June-August, 1989 and 1990	Daily time series ecologic study of ED visits for respiratory complaints across all ages and within 4 age strata (<2, 2-34, 35-64, >64).	8-hr max O_3: 1989 mean: 37.5 ppb SD: 15.5 1990 mean: 29.9 ppb SD: 11.2 1-hr max O_3: 1989 mean: 44.1 ppb SD: 18.3 1990 mean: 35.4 ppb SD: 12.9	Estimated PM2.5	0,1, and 2	Same analytical approach used in Delfino et al., 1997. Results presented only for 1989. Significant effects reported for lag 1 O_3 in 1989 only for the >64 age group. This O_3 effect reported to be robust in two-pollutant models.	1989, >64 yrs: 8-hr max O_3 (per 30 ppb): Lag 1: 16.1% (2.1-30.1) 1-hr max O_3 (per 40 ppb): Lag 1: 12.5% (-3.1-28.1) No significant O_3 effects in other age groups or for 1990.
Hajat et al. 2002 London 1992-1994	Daily doctor consults for upper respiratory diseases for ages 0-14, 15-64, and >64.	8-hr max O_3: Warm season: Mean 22.7 ppb SD 12.2 Cold season: Mean 12.1 SD 7.6 All year: Mean 17.5 SD 11.5	BS, SO_2, NO_2, CO, PM10, pollen	0-3	Poisson regression with GAM control of time and weather; co-adjustment. All year and seasonal models run. 1 and 2 pollutant models run. Significant negative effects for O_3. This may reflect residual confounding by seasonal factors.	Not quantitatively useful.

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Hernandez-Garduno et al, 1997 Mexico City May 1992-January 1993	Visits to clinics for respiratory diseases among persons 1mo to 92 yrs.	1-hr max O_3: Levels not reported.	SO_2, NO_2, CO	0-5	GLM with pre-adjustment. O_3 at lags 0 and 5 significantly associated with daily visits for all ages, 14 and under, and 15 and over ages. Neither O_3 nor NO_2 significant in 2-pollutant model.	RRs cannot be computed from available results.
Jaffe et al. 2003 Cincinnati, Cleveland, and Columbus, OH June-August, 1991-1996	Daily time series study of ED visits for asthma among Medicaid recipients aged 5-34 years.	8-hr max O_3: Cincinnati mean: 60 ppb; SD: 20 Cleveland mean: 50 ppb; SD: 17 Columbus mean 57 ppb; SD: 16	PM10, NO_2, SO_2	1,2, and 3	Poisson regression with control for city, day of week, week, year, min temperature, overall trend, and a dispersion parameter. No specific effort to control cycles, but regression residuals were uncorrelated, presumably due to seasonal restriction, and the week and min temperature controls. Results shown for individual cities and overall. PM10 available only every 6th day. Positive relationships between ED visits for asthma and max 8-hr O_3 levels lagged 2-3 days. Results of borderline statistical significance. Other pollutants also related to asthma ED visits in single-pollutant models.	8-hr max O_3 (PER 30 ppb) for all ages: Cincinnati Lag 2: 1.16 (1.00-1.37) Cleveland Lag 2: 1.03 (0.92-1.16) Columbus Lag 3: 1.16 (0.98-1.37) Three cities: 1.09 (1.00-1.19)

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Jones et al., 1995 Baton Rouge, LA June-August 1990	Daily ED visits for respiratory complaints over a three-month period in pediatric (0-17), adult (18-60), and geriatric (>60) subgroups.	24-hr avg O_3: Mean 28.2 ppb; SD 11.7 1-hr max O_3: Mean 69.1 ppb; SD 28.7	No co-pollutants. Mold pollen data included	None specified	Relatively simple statistical approach using ordinary least squares regression to model effects of O_3 by itself and of O_3 along with pollen counts, mold counts, temperature, and relative humidity. No direct control of cycles but authors reported non-significant auto-correlations among model residuals. Data restriction to 3-month period may have reduced any cyclic behavior. Significant associations between respiratory ED visits and O_3 observed for adult age group only in multiple regression models.	24-hr avg O_3 (per 20 ppb): Pediatric:0.87 (0.65 to 1.09) Adult: 1.20 (1.01 to 1.39) Geriatric: 1.27 (0.93 to 1.61)
Lin et al. 1999 Sao Paulo, Brazil May 1991-April 1993	Daily pediatric (no age range specified) respiratory ED visits	1-hr max O_3: Mean 34 ppb SD 22	SO_2, CO, PM10, NO_2	0-6 examined; settled on 1-5 day moving avg.	Seasonal control using month dummy variables. Also controlled day of week, temperature. Both O_3 and PM10 associated with outcome alone and together.	1-hr max O3 (per 5 ppb): Respiratory ED visits with O3 alone: 1-5 d MA: 1.022 (1.016-1.028) With PM10 in model: 1-5 d MA: 1.015 (1.009-1.021)

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Martins et al. 2002 Sao Paulo, Brazil May 1996-September 1998	Daily ED visits for chronic lower respiratory diseases among persons over age 64.	1-hr max O_3: Mean 34 ppb SD 21 IQR 21	CO, NO_2, SO_2, PM10	2-7 examined; settled on 0-3 day moving avg.	Poisson analysis with GAM control for time and weather. Only O_3 and SO_2 significant in one-variable models. O_3 effect remained significant when SO_2 included in 2-pollutant model.	1-hr max O_3 (per 18.26 ppb): 0-3 d MA: 1.14 (1.04-1.23)
Stieb et al. 1996 Saint John, New Brunswick, Canada May-September 1984-1992	Daily ED visits for asthma	1-hr max O_3: Mean 41.6 95th %tile 75	SO_2, NO_2, SO_4, TSP	0-3 examined; lag 2 reported	Poisson analysis with control of time based on 19-day moving average filter. Also controlled day of week and weather variables. O_3 only pollutant consistently associated with ED visits for asthma, but effect appeared non-linear, with health impacts evident only above 75 ppb O_3.	Asthma ED visits increased 33% when daily 1-hr max O_3 exceeded 75 ppb.

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Tenias et al. 1998 Tenias et al. 2002 Valencia, Spain 1993-1995	Daily ED visits for asthma and COPD among persons > 14 yrs.	1-hr max O_3: Median 32 ppb 95th %tile 52	BS, NO_2, SO_2, CO	0-5 examined; only lag 5 reported	Poisson analysis using APHEA methodology. Compared warm and cold season effects. GAM explored in sensitivity analysis. For asthma, both O_3 and NO_2 significant 1 and 2 pollutant models, and O_3 effect larger in warm season. For COPD, both O_3 and CO significant in both 1 and 2 pollutant models and no difference in O_3 effects by season.	1-hr max O_3 (per 5 ppb): Asthma lag 5: Warm seas. 1.08 (1.02-1.05) Cold seas. 1.04 (0.97-1.11) All year 1.06 (1.01-1.11) COPD lag 5: All year1.06 (1.02-1.10)
Tobias et al. 1999 Barcelona 1986-1989	Daily asthma ED visits analysed in relation to O_3 and other air pollutants with aim of testing sensitivity of results to 4 alternative methods for controlling asthma epidemics.	Levels not reported	BS, NO_2, SO_2	Not specified	Poisson analysis using APHEA methodology. Asthma epidemics either not controlled, or controlled with one, six, or individual epidemic dummy variables.	O_3 results were sensitive to method of asthma epidemics, with regression coefficients ranging over 5-fold depending on the model. Only 1 of 8 models reported had a significant O_3 effect.

Table 12-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Tolbert et al., 2000 Atlanta GA June-August, 1993-1995	Pediatric (ages 0-16) asthma ED visits over three summers in Atlanta. A unique feature of the study was assignment of ozone exposures to zip-code centroids based on spatial interpolation from 9 ozone monitoring stations.	8-hr max O_3: Mean 59.3 ppb; SD 19.1 1-hr max O_3: Mean 68.8 ppb; SD 21.1	PM10, NO_2 Mold and pollen data included	1	Sound statistical approach. Of particular note was a-prior specification of model selection, including lag 1 for all pollutants and met variables. Secondary analysis using logistic regression of the probability of daily case (asthma) visits, referenced to total visits (asthma + non-asthma). Examined evidence for non-linearity of O_3 associations in logistic analysis. Significant association with O_3 and PM10 in one-pollutant models, but not in two-pollutant models (correlation between O_3 and PM10: r=0.75). Secondary analysis indicated non-linearity, with effects clearly significant only for days >=100 ppb vs. days <50 ppb.	8-hr max O_3 (per 20 ppb) in one-pollutant Poisson regression model: Lag 1: 1.040 (1.008-1.074) in one-pollutant logistic regression model: Lag 1: 1.04 (1.02-1.07) in logistic regression model with PM10 as covariate: Lag 1: 1.024 (0.982-1.069)

12.3 Studies Addressing Respiratory Effects of Long-Term Ozone Exposures

12.3.1 Introduction

In addition to the growing literature examining the acute impacts of O_3 on mortality risk, the area of epidemiologic investigation that has yielded the most important new information on O_3 effects in the past decade has been the study of health impacts of long-term O_3 exposures. Controlled exposure studies have lead the way in characterizing the acute effects of O_3, which include transient decreases in lung function and increases in pulmonary inflammation and symptoms of respiratory irritation. However, epidemiology has a key role to play in addressing long-term impacts in humans since it is impractical to study these effects using controlled human exposure studies.

Epidemiologic interest in investigating long term effects has been motivated by several considerations. Animal toxicology studies carried out since the late 1980's have demonstrated that long-term exposures can result in permanent changes in the small airways of the lung, including remodeling of the airway architecture and deposition of collagen. In addition, controlled human exposure studies have demonstrated acute inflammation in the lung at ambient exposure levels following single O_3 exposures. These observations stimulated interest in determining whether repeated exposures over multiple episode periods and/or multiple years would lead to persistent inflammation and damage to the human lung, especially in the small, terminal bronchiolar regions where vulnerability is greatest. Much of the epidemiologic literature addressing long-term O_3 exposure thus focuses on evaluating whether there is evidence for these effects in free-living individuals.

While this issue is critically important, the challenges to addressing it epidemiologically are formidable, and this is reflected in the small and relatively limited literature that exists in this area. Long-term O_3 concentrations tend to correlate with long-term concentrations of other pollutants, making attribution to specific pollutants difficult. Subtle pulmonary effects require health outcome measures that are sensitive, and must usually be directly collected from individual human subjects, rather than from administrative data bases. This tends to make studies difficult and expensive. Thus, as a group, these studies tend to be smaller and less statistically powerful than ecologic studies such as time series mortality or hospital admissions studies.

Epidemiologic efforts to test for long-term O_3 effects have been underway for at least 20 years (Hodgkin et al. 1984; Detels et al. 1987; Detels et al. 1991; Schwartz 1989; Stern et al. 1989; Stern et al. 1994; Mullahy and Portney 1990; Zwick et al. 1991; Calderon-Garciduenas et al. 1992; Euler et al. 1988; Abbey et al. 1993; Schmitzberger et al. 1993. However, an extensive review of the relevant literature through 1996 (U.S. EPA 1996) concluded that the available literature provided "only suggestive evidence for health effects of chronic O_3 exposure." Most of the then-available studies were limited by inadequate

exposure assessment and inability to isolate O_3 effects from those of other pollutants, especially particulate matter (U.S. EPA 1996).

Here we review studies published from 1996 onward in which health effects were tested in relation to O_3 exposures extending from several weeks to many years (Table 12-4). The available literature falls in five general categories: studies addressing respiratory inflammation in high vs. low O_3 exposure groups or time periods; studies looking at seasonal changes in lung function and respiratory symptoms as a function of seasonal average O_3 exposures; studies examining long-term mortality risks; studies addressing growth or decline of lung function over many years in relation to long-term O_3 exposures; and finally, studies addressing cross-sectional associations between asthma prevalence and long-term O_3 exposures.

12.3.2 Respiratory Inflammation

As noted (see Section 11.4.3.4 and Tables 11-5, 11-6, 11-8, and 11-12), human chamber studies have clearly demonstrated that acute (2-7.6 hr) O_3 exposures, with light to moderate exercise, result in inflammation in the lung, including the alveolar region where gas exchange takes place. This acute effect is potentially important for chronic effects because it is established that repeated inflammation can result in the release of substances from inflammatory cells that can damage the sensitive cells lining the lung. Over extended periods, this could lead to permanent damage to and re-structuring of the small airways and alveoli. In addition, since inflammation is a fundamental feature of asthma there has been concern that O_3-induced inflammation could exacerbate existing asthma or perhaps promote the development of asthma among genetically pre-disposed individuals.

In a group of 19 adult joggers living and working on an island in New York harbor, there was little evidence for acute inflammation in bronchoalveolar lavage (BAL) fluids collected during the summer of 1992 compared with BAL fluids collected from the same subjects in winter (Kinney et al. 1996). However, there was evidence of greater levels of cell damage, measured by lactate dehydrogenase (LDH) in the fluids. The three-month mean of the daily 1-hr O_3 maxima was 58 ppb in the summer and 32 ppb in winter. PM10 and NO_2 concentrations were similar across the two seasons. These results are consistent with attenuation of acute inflammatory effects (which has been demonstrated in multi-day chamber exposures, see Section 11.4.9.8), but ongoing cellular damage due to repeated O_3 exposures over the course of a summer.

A series of interesting studies of children living in Mexico City and a rural area of Mexico have demonstrated inflammation of and genetic damage to cells in the nasal passages of the urban compared to the rural children (Calderon-Garciduenas et al. 1997; Calderon-Garciduenas et al. 1995; Calderon-Garciduenas et al. 1999). Pollution effects in the nose can be viewed as surrogates for effects that might occur in the lungs, although doses to nasal tissues are usually higher for a given ambient pollutant concentration. In the

1997 study, 129 children living in Mexico City were compared to 19 children living in a clean coastal town. Children ranged in age from 6 to 12 years, had no history of smoking or exposure to environmental tobacco smoke, and were not taking asthma medications. Cells collected from the lining of the nose had significantly higher amounts of DNA damage in the urban vs. non-urban children. Among urban children, DNA damage was greater with increasing age, suggesting an accumulation of damage over time with ongoing pollution exposures. A follow-up study of 86 urban and 12 non-urban children reported similar findings, in addition to increased levels of specific DNA mutations (Calderon-Garciduenas et al. 1999). It is not know what if any functional significance these mutations may have; however, the observation of any mutations in free-living humans exposed to ambient air pollution is some cause for concern. They also noted elevated respiratory symptom prevalence in the urban children; 46% of urban children reported cough or chest discomfort whereas no rural children reported these symptoms. Specific attribution of these effects to O_3 is difficult given the complex mixture of pollutants present in Mexico City's air. In particular, the DNA effects seem more plausibly related to other components of urban air, such as semi-volatile organics. However, the inflammatory changes such as increased neutrophil levels observed in the earlier studies would be consistent with known effects of O_3.

12.3.3 Seasonal Changes in Lung Function

While it has been well documented in both chamber and field studies that daily, multi-hr exposures to O_3 result in transient declines in lung function, much less is known about the effects of repeated exposures to O_3 on lung function (see Section 11.4.9 for discussion of controlled studies on this topic). Two studies reported over the past decade have examined lung function changes over seasonal time periods with differing levels of O_3 exposures (Frischer et al. 1999; Kinney and Lippmann 2000). In the larger of the two studies, Frischer and colleagues (1999) collected repeated lung function measurements on 1,150 Austrian school children (mean age 7.8 years) from nine towns that differed in mean O_3 levels. Lung function was measured in Spring and Fall over a three year period, yielding six measurements per child. The seasonal change in lung function was analyzed in relation to seasonal mean O_3 exposures, controlling for baseline function, atopy (assessed by skin prick testing), sex, study site, ETS, season, temperature, and height change. Mean O_3 exposures in each town were significantly associated with decrements in lung function. FVC declined by between 10 and 33 mL for each ppb increase in mean seasonal 24 hr ozone concentrations. FEV_1 declined by between 24 and 34 mL. Other pollutants (PM10, SO_2, and NO_2) had less consistent associations with lung function changes. These results suggest that seasonal O_3 exposures result in decrements in lung function. Left unanswered was whether these effects would resolve following exposure to lower O_3 exposures over the winter months.

In a pilot study (Kinney and Lippmann 2000), 72 non-smoking young adults (mean age 20 yrs) from the second year class of students at the US Military Academy at West Point, NY provided two lung function measurements, one

before and one after the summer. During the summer, all students traveled to one of four US locations to undergo intensive training over a five-week period. The authors hypothesized that lung function would decline over the summer and that these functional declines would be greater among students who trained in locations with higher O_3 levels. There was a significant mean decline of 44 mL in FEV1 over the summer for all subjects, with a larger decline (78 mL) among the Ft. Dix students than among the remaining students (31 mL). Ozone levels at Ft. Dix averaged 71 ppb over the summer training (mean of daily 1-hr maxima), vs. a range of 55 to 62 ppb at the other locations. The significant decline in lung function among all students may have reflected the consistently high levels of self-reported exposures to vehicle exhaust, dust, and ETS, at all four locations. Though conclusions are limited by the small size of the study, results are consistent with a seasonal decline in lung function that may be due, in part, to O_3 exposures.

12.3.4 Mortality Risk

There is inconsistent and inconclusive evidence for a relationship between long-term O_3 exposure and increased mortality risk (Abbey et al. 1999; Pope et al. 2002). A long-term prospective cohort study of 6,338 non-smoking, non-Hispanic white subjects living in California found a significant association between long-term O_3 exposure and increased lung cancer risk among males only (Abbey et al. 1999). Lung cancer mortality risk was 4.1 times greater (95% CI: 1.8-9.7) for males exposed to an interquartile change of 551.1 hours per year with O_3 levels above 100 ppb. A nearly significant association was observed with long term average daily 24-hr mean O_3 concentrations in males, as well. A particular strength of this study was the extensive effort devoted to assessing long-term air pollution exposures, including interpolation to residential and work locations from monitoring sites over time and space. However, the observation of a lung cancer effect but no effect on cardiopulmonary mortality raises questions of plausibility. In addition, there is concern that the lung cancer findings in males are spurious given the large number (sixteen) of analytical permutations tested. No association was observed between long-term concentrations of O_3 and either all-cause or cardiopulmonary mortality.

No statistically significant effect of long-term O_3 concentrations on mortality risk was observed in a larger prospective cohort study of approximately 500,000 US adults (Pope et al. 2002). However, the association of July-September 1 hr maximum O_3 concentrations with cardiopulmonary mortality was positive and nearly significant (relative risk for change of 60 ppb O_3 was approximately 1.08). Strong and consistent effects of PM2.5 were observed in that study for both lung cancer and cardiopulmonary mortality. The O_3 effect needs to be interpreted with caution since this pollutant has not undergone the sensitivity analysis necessary to allow for inferences to be made. Since O_3 will penetrate at different rates depending on localized behaviors, regional differences and spatial autocorrelation need to be carefully evaluated. However, this study reinforces the possibility that longer-term exposure to O_3, due to its inflammatory properties, could be associated with significant adverse outcomes.

12.3.5 Long-term Effects on Lung Function

Lung capacity grows during childhood and adolescence as body size increases, reaches a maximum in the decade of the 20s, and then begins declining steadily and progressively with age. There has long been concern that long-term exposure to O_3 or other air pollutants might lead to slower growth in lung capacity, diminished maximally attained capacity, and/or more rapid decline in capacity with age. In the case of cigarette smoking, it has been well documented that lung function declines more rapidly with age in a dose-dependent way among adults who smoke cigarettes. Interestingly, adults who stop smoking return to a normal rate of decline in capacity, although there is no evidence that they regain the capacity previously lost due to smoke exposure. Because O_3 is a strong respiratory irritant, and is known to result in acute lung function declines as well as inflammation, it seems plausible that there might be a negative impact of long-term O_3 exposures on lung function growth and decline. It may be speculated that effects which operate on the upslope of function during childhood might carry greater long-term risks. Thus, studies of effects on diminished rate of lung function growth in children are especially important.

Several studies published over the past decade have examined the relationship between lung function and long-term O_3 exposure. The most extensive and robust recent study of respiratory effects, including lung function, in relation to long-term air pollution exposures among children has been the Children's Health Study carried out in 12 communities of southern California starting in 1993 (Gauderman et al. 2000; Peters et al. 1999a). An initial report examined the relationship between lung function at enrollment and levels of air pollution in the community (Peters et al. 1999a), and found evidence for declines in FVC, FEV_1, PEFR and FEF25-75% (the latter two being statistically significant) among females in relation to elevated annual mean daily 1 hr maximum O_3 levels. For male children, there were declines in all four lung function variables only in a subset of subjects who reported spending more time outdoors; these associations were significant only for FVC and FEV1. There were no significant associations between long-term O_3 exposures and self-reports of respiratory symptoms or asthma (Peters et al., 1999b). In a longitudinal analysis of lung function growth in the fourth grade cohort, decrements in lung function growth were associated with particulate matter and NO_2, but not with O_3 (Gauderman et al. 2000).

Evidence for a relationship between long-term O_3 exposures and decrements in maximally attained lung function was observed in a nationwide cohort of 520 first year students at Yale College in New Haven, CT (Galizia and Kinney 1999; Kinney et al. 1998). Each student performed one lung function test in the Spring of their first year at college. Ozone exposures were estimated by linking 10-year mean summer-season 1-hr maximum O_3 levels at the nearest monitoring station to the residential locations reported each year from birth to the time of measurement. Students who had lived four or more years in areas with long-term summer O_3 levels above 80 ppb had significantly lower FEV_1 (-3.0 percent; 95% CI -0.22 to -5.92 percent) and FEF25-75% (-8.11 percent; 95% CI -2.32 to -

13.90 percent) compared to their classmates with lower long-term exposures, controlling for age, sex, race, height, parental education and maternal smoking. Stratified by sex, males had larger effect estimates than females, which might reflect higher outdoor activity levels during childhood. A similar study of 130 first year college freshmen at the University of California at Berkeley also reported significant effects of lifetime mean of daily 10 AM to 6 PM O_3 averages on lung function (Kunzli et al. 1997; Tager et al. 1998). Enrollment was limited to students from either the San Francisco or Los Angeles metropolitan areas. After controlling for city of origin, FEF25-75% and FEF75% were significantly lower in relation to long-term exposures. No effects were seen for PM10 and NO_2. In another California-based study, there was no relationship between acute responsiveness to 400 ppb O_3 over two hours, measured with exercise in a controlled chamber environment, and long-term changes in lung function in a group of 45 adults (Gong et al. 1998) drawn from a longitudinal cohort study that had previously reported faster lung function decline in persons living in high O_3 communities (Detels et al. 1987).

12.3.6 Risk of Asthma Development

Two recent reports from longitudinal cohort studies have reported associations between the onset of asthma and long-term O_3 exposures (McDonnell et al. 1999; McConnell et al. 2002). Longitudinal studies provide the strongest evidence on the question of asthma development because new onset of asthma is observed prospectively. An inherently weaker approach is to examine cross-sectional relationships between measures of asthma or atopy prevalence and O_3 exposures (Charpin et al. 1999; Kuo et al. 2002; Ramadour et al. 2000).

Significant associations between new cases of asthma among adult males and long-term O_3 exposure estimates were reported in a cohort of 3091 non-smoking adults followed through 1992 who were 25 years or older when enrolled in 1977 (McDonnell et al. 1999). This is the same California adult cohort described earlier (Abbey et al. 1999). To be eligible for the study, subjects had to have lived 10 or more years within 5 miles of their current residence in 1977. Residences from 1977 onward were followed and linked in time and space to interpolated concentrations of O_3, PM10, SO_2, and NO_2. New asthma cases were defined as a self-report at either the 1987 or 1992 questionnaire follow-up of a doctor telling the subject they had asthma among those who had answered “no” to this question upon enrollment in 1977. Only 32 incident cases were seen among 972 eligible males; there were 79 incident cases among 1786 eligible females. For males, there was about a two-fold increase in new asthma for a 27 ppb change in long-term mean 24 hr average O_3 exposure. For females, there was no evidence of any relationship between asthma risk and O_3 exposure. While one can speculate about possible reasons for the gender differential, the complete lack of association among females, where more new cases were seen, casts some doubt on these results.

A similar sub-study of incident asthma cases in relation to long-term O_3 exposure was carried out on the Children’s Health Study cohort (McConnell et al. 2002; Peters et al. 1999c). Here, annual surveys of 3535 initially non-asthmatic

children (ages 9-16 at enrollment) enabled identification of new-onset asthma cases through 1998. Asthma risk was not higher for residents of the six high O_3 communities (four year mean of January-December 10am-6pm ozone was 60 ppb, May to September mean of 8 hr maximum O_3 concentrations was 84 ppb) vs. residents of the six low O_3 communities (4-year January-December mean of 10am-6pm ozone concentrations was 40 ppb, May to September mean of 8 hr maximum O_3 concentrations was 49 ppb). However, within the six high O_3 communities, asthma risk was elevated by 3.3 fold for children who played three or more sports as compared with children who played no sports. This association was absent in the low- O_3 communities. No associations with asthma were seen when communities were stratified by PM10, PM2.5, NO_2, or inorganic acid vapors. These results are intriguing in their suggestion of effect modification, by physical activity, of the impacts of O_3 on asthma risk. Replication of these findings in other cohorts would lend greater weight to a causal interpretation.

Recent cross-sectional surveys have detected no associations between asthma prevalence, asthma-related symptoms, or allergy to common aeroallergens and long-term O_3 exposures among children after controlling for covariates (Charpin et al. 1999; Kuo et al. 2002; Ramadour et al. 2000). However, in all cases, reported O_3 levels were quite low. Thus, the question remains unsettled as to whether long-term O_3 is a risk factor for allergy or asthma based on the cross-sectional survey approach.

Table 12-4: Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Abbey et al. 1999 California 1977-1992 24-hr avg O_3: mean: 26.1 ppb; IQR: 12.0 hrs/yr >100 ppb: mean: 330; IQR: 551	Prospective cohort study of 6,338 non-smoking non-Hispanic white adult members of 7th-Day Adventist church. Participants were aged 27-95 years at enrollment in 1977. Mortality events followed through 1992. Extensive exposure assessment, with assignment of individual long-term exposures to O_3, PM10, sulfate particles, and SO_2, is a unique strength. All results were stratified by sex. Used Cox proportional hazards analysis, with control for age at enrollment, past smoking, environmental tobacco smoke exposures, education, occupation, and body mass index. Analyzed mortality from all natural causes, cardiopulmonary, nonmalignant respiratory, and lung cancer. O_3 results were presented for both metrics noted in first column.	Of 16 regressions involving O_3 exposures (2 sexes; 4 causes; 2 O_3 metrics), eight were positive and one was statistically significant, for lung cancer in males for O_3 > 100 ppb. Lung cancer mortality relative risks in males (95% CI): 24-hr avg O_3 (12.03 ppb): 2.10 (0.99, 4.44) O_3 hrs/year > 100 ppb (551.1): 4.10 (1.81, 9.69) Inconsistency across metrics, outcomes and genders raises possibility of spurious finding. The lack of cardiopulmonary effects raises plausibility concerns.
Calderon-Garciduenas et al., 1997 W Mexico City (urban) and a coastal town (control) Sep-Nov, 1995 O_3 hrs/mo>120 ppb: mean: 82	129 urban and 19 control children 6-12 years old with no history of smoking or ETS and no current medication use for atopy or asthma. Three nasal biopsies obtained at 4-week intervals, and analyzed for DNA damage based on the presence of DNA fragments.	Urban children had significantly more DNA fragments than did control children ($p<0.0001$). Percentage of damaged cells was 82% (SE: 6.4) in urban children and 17% (SE 6.1) in control children. Among urban children, more DNA damage was seen with increasing age. Subjects exposed to complex urban pollution mix, making it difficult to attribute effects to O_3 specifically. However, authors note that O_3 was the pollutant with most exceedences of air quality standard.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Calderon-Garciduenas et al. 1999 SW Mexico City May and June, 1996 Urban Location: O_3 hrs/mo>80 ppb; max hr: May: 161; 232 ppb June: 98; 261 ppb Control Location: May: mean O_3 < 10 ppb	86 urban and 12 control children 6-13 years old with no history of smoking or ETS and no use of medication for atopy or asthma. Urban children stratified into five groups: first through fifth grades. Nasal epithelial biopsies obtained from inferior nasal turbinates, and analyzed for single strand DNA breaks (SSB) and for 8-OHdG (8-hydroxy-2'-deoxyguanosine), a mutagenic lesion produced by G->T mutations. These outcomes relate to possible carcinogenic effects of air pollution exposures. Multiple air pollutants monitored in South West Mexico City within 3 miles of urban subject residences. PM10 levels during study (mean): May: 53 ug/m3 June: 61 ug/m3	No respiratory symptoms reported by control children; urban children reported multiple nasal and lung symptoms, including cough and chest discomfort among 46% of urban children, with higher rates for fifth vs. first graders. 8-OHdG was approximately 3-fold higher in biopsies from urban children; however, no differences by school grade. SSBs were more common in urban vs. control children, with age-dependent increase for the urban children. These results suggest that DNA damage is present in the nasal epithelial cells of children living in highly polluted SW Mexico City, and may reflect enhanced risk of cancer later in life. Though O_3 represents an important component of the pollution mix, it is not possible to attribute effects solely to O_3.
Charpin et al. 1999 Seven towns in SE France January and February, 1993 O_3 8-hr max: Mean: 15-26 ppb O_3 24-hr mean: Mean: 10-20 ppb	2073 10 and 11 year olds from seven towns tested for atopy based on skin prick testing (house dust mite, cat dander, grass pollen, cypress pollen, and Alternaria). Towns represented a range of ambient ozone and other pollutant (NO_2 and SO_2) levels. Tested hypothesis that atopy is greater in towns with higher photochemical pollution levels. To be eligible, subjects must have resided in current town for at least 3 years. Authors stated that Jan-Feb pollution levels correlated with levels observed throughout the year, though no data given to support this..	No differences in atopy levels were seen across the seven towns. Authors concluded that long-term exposures to oxidant pollution do not favor increased allergy to common allergens. The very low winter O_3 levels monitored, and lack of long-term exposure data, make it impossible to reach this conclusion in a definitive manner.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Chen et al. 2002 Washoe County, Nevada 1991-1999 O_3 8-hr max: Mean: 27 ppb Range: 3-62 ppb	Birth weight for 39,338 single births analyzed in relation to mean PM10, O_3 and CO levels in trimesters 1, 2, and 3.	PM10 only was associated with decreased birth weights. Ozone levels quite low throughout study.
Frischer et al. 1999 9 communities in Austria 1994-1996 O_3 24-hr mean (SD): Summers: 34.8 ppb (8.7) Winters: 23.1 ppb (7.7)	Communities chosen to represent a broad range of O_3 concentrations; a two-fold range in mean levels was observed. 1,150 children (mean age 7.8 years; 52% male) from grades 1 and 2 measured for spirometric in Spring and once in Fall over three years (total of six measurements per child), to determine if seasonal exposure to ozone would be associated with diminished lung function growth, especially over the Summer seasons. O_3 levels were low during lung function testing periods. Participation rates high. At baseline, collected respiratory histories and tested for allergy by skin prick. Tested for associations between change in lung function (FVC, FEV1, and MEF50) over each season and O_3 concentrations, controlling for baseline function, atopy, sex, site, ETS exposure, season, and change in height. Other pollutants studied included PM10, SO_2, and NO_2.	Seasonal mean O_3 exposures were associated with decrements in all three lung function measures. Inconsistent results seen for other pollutants. Summer season lung function decrements per unit O_3 were larger when data restricted to children who spent whole summer in their community. No evidence for non-linear O_3 effect. No confounding of O_3 effect by temperature, ETS, or acute respiratory illnesses. Slopes (SEs) (mL/day/ppb): FVC: Summer O_3: -0.018 (0.005) Winter O_3: -0.010 (0.006) Summer O_3 (restricted to subjects who stayed in community): -0.033 (0.007) FEV1: Summer O_3: -0.029 (0.005) Winter O_3: -0.024 (0.006) Summer O_3 (restricted to subjects who stayed in community): -0.034 (0.009)

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Galizia and Kinney 1999 Kinney et al. 1998 Nationwide sample 1995 10-year mean of June-August daily 1-hr max: Mean (SD): 61.2 ppb (15.5) Min: 13 ppb 25th %tile: 53 ppb Median: 61 ppb 75th %tile: 68 ppb Max: 185 ppb	Nationwide sample of 520 young adults.. Subjects were non-smokers, 17-21 years old, and 262/258 female/males. Each subject provided one spirometric lung function measurement in the Spring of their first year at Yale College in New Haven, CT, and completed a questionnaire addressing residential history, respiratory diseases, and activity patterns. Long-term O_3 exposure treated as dichotomous variable: subjects who lived for 4+ years in counties with 10-year summer mean O_3 greater than 80 ppb vs. those who did not. Four lung function variables (FVC, FEV1, FEF25-75%; FEF75) regressed on O_3 exposure, controlling for age, height, height squared, sex, race, parental education, and maternal smoking history. Respiratory symptom histories (cough, phlegm, wheeze apart from colds, and composite index for any of the three symptoms) logistically regressed on O_3 exposure, controlling for sex, race, parental education, and maternal smoking.	Significant decrements in FEV1 and FEF25-75% in relation to O_3 exposure were observed for all subjects and for males alone, but not for females alone. Similar patterns observed for FVC and FEF75, but not with statistical significance. Lung function percent differences (95% CI) for high vs. low O_3 exposure groups: FEV1: All subjects: -3.07% (-0.22 to -5.92) Females: -0.26% (3.79 to -4.31) Males: -4.71% (-0.66 to -8.76) FEF25-75%: All subjects: -8.11% (-2.32 to -13.90) Females: -1.96% (6.39 to -10.30) Males: -13.02% (-4.87 to -21.17) Wheeze and 3-symptom index (RSI) were significantly elevated for high O_3 exposure group. Odds ratios (95% CI): Wheeze: 1.97 (1.06-3.66) RSI: 2.00 (1.15-3.46)
Gauderman et al. 2000 12 southern California communities (see also Peters et al., 1999a,b)	Analysis of longitudinal lung function change in relation to long-term air pollution levels in the Children's Health Study.	In the fourth grade cohort, decreased lung growth was associated with exposure to particulate matter and NO_2, but not with O_3.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Gong et al. 1998 Glendora, CA Longitudinal cohort study with follow up at three time points: 1977-1978, 1982-1983, and 1986-1987. Annual means of daily 1-hr O_3 maxima ranged from 118 to 134 ppb from 1983 to 1986	164 adults (mean age 45 years; 56 male/108 female) from a high O_3 community underwent lung function testing in 1986/87 (time 3). Subjects were recruited from a cohort of 208 non-smoking adults who had previously been measured on two previous occasions: 1977/78 (time 1) and 1982/83 (time 2). Analyzed change in lung function between times 1, 2, and 3. Subjects were also asked to undergo controlled exposures to 0.40 ppm ozone over 2 hours with intermittent exercise. 45 subjects agreed to participate. Investigators hypothesized that acutely responsive subjects would show more rapid declines in function between times 1, 2 and 3.	Trends in lung function: Mean FVC and FEV1 increased from time 2 to time 3 (though not significantly), whereas an earlier analysis of the time 1 to time 2 change had found a significant decline in function (Detels et al., Chest 92:594-603, 1987). There was evidence for 'regression to the mean,' in that subject with larger declines in function from time 1 to 2 tended to have larger increases in function from time 2 to 3. Power was limited by the small population available for follow up. Acute responsiveness vs. trends: There was no correlation between acute responsiveness to 0.4 ppm O3 (FVC or FEV1) and the trends over time in lung function at times 1, 2, and/or 3.
Kinney et al., 1996 New York, NY 1992 and 1993 Mean of daily 1-hr O_3 maxima over summer (July-September) and winter (January-March) (ppb): Summer 1: 58 Winter: 32 Summer 2: 69	19 healthy adult joggers (18 male; ages 23-38) from the Governors Island US Coast Guard facility in New York harbor underwent a series of two bronchoalveolar lavages (BAL), first in summer 1 and then again in winter. Six subjects underwent a third BAL in Summer 2 when O_3 levels were higher than Summer 1. Study tested whether inflammatory markers in BAL fluid were elevated during the summer O_3 season among adults who regularly exercised outdoors. Outcomes included cell differentials, release of IL-8, TNF-? and reactive oxygen species by macrophages, and concentrations of protein, LDH, IL-8, fibronectin, a-1-AT, C3a, and PGE_2 in BAL fluids.	There was no evidence of acute inflammation in Summer 1 compared to winter; i.e., neutrophil differentials, IL-8 and TNF-a showed no significant differences. However, a measure of cell damage, LDH, was elevated in the summer, suggesting possible O_3-mediated damage to the lung epithelium with repeated exposures to O_3 while exercising. Summer 1 O_3 levels were atypically low for NYC. Among six subjects who agreed to undergo a third BAL test in Summer 2, LDH was again elevated compared to Winter. In addition, IL-8 was elevated in Summer 2, suggesting acute inflammation.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Kinney and Lippmann 2000 West Point, NY Spring-Fall, 1990 Mean of daily 1-hr O_3 maxima over 5-week summer training (ppb): Ft Dix, NJ: 71.3 Ft Benning, GA: 55.6 Ft Leonard Wood, MO: 55.4 Ft Sill, OK: 61.7	72 non-smoking students at the U.S. Military Academy at West Point, NY measured for lung function and respiratory symptoms before (April) and after (Aug-Sep) taking part in intensive, largely outdoor, summer training over five weeks (7/11-8/15) at four U.S. military bases. O_3 levels in the Ft Dix, NJ area were consistently higher than at the three remaining three locations. Analysis assessed change in lung function and respiratory symptoms measured before and soon after the summer training, and examined whether adverse trends would be more pronounced in students exposed to higher O_3 levels.	Mean FEV1 declined significantly by 44 mL (SE 21 mL) over the two measurement points for all subjects combined. This may reflect combined effects of O_3 along with frequent exposures to dust, vehicle exhaust, and ETS, reported by subjects from all four locations in a post-summer questionnaire. A larger mean decline was seen at the higher O_3, Ft Dix, site (-78 mL) than at the remaining three sites (-31 mL), suggesting an influence of O_3 exposures. Also, a larger decline was observed in 61 subjects with post-summer measurements in the first two weeks after returning from training than among subjects measured in the 3rd and 4th weeks after returning, which is consistent with the lung function effects being somewhat transient. These results are consistent with subchronic effects of both particulate matter and ozone on lung function declines over seasons.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Kunzli et al. 1997 Two California Cities Range of lifetime mean of daily 10am-6pm O_3 averages: San Francisco: 16-33 ppb Los Angeles: 25-74 ppb	In a pilot study, 130 freshman students at the University of California at Berkeley measured for lung function and histories of residential locations and indoor/outdoor activity patterns and levels. By design, students had resided in one of two metropolitan areas prior to attending that differed greatly in O_3 concentrations, San Francisco or Los Angeles. A key goal was to test whether measures of small airways function (e.g., N_2 washout, FEF 25-75, FEF75%) were sensitive measures of long-term O_3 impacts. Lifetime exposures to O_3, PM10 and NO_2 assigned by interpolation to sequence of residence locations from available monitoring stations. Multiple exposure measures were derived with varying degrees of incorporation of time-activity information, going from ecological concentration to individual time-activity weighted exposure. Performed linear regression analysis of lung function on O_3 exposures, controlling for height, ethnicity, sex, region and sex*region.	Decreased FEF25-75% and FEF75% were significantly related to long-term O_3 exposures. Results were similar whether O_3 exposure was purely ecologic or incorporated time-activity information. FVC, FEV1, and N_2 washout were generally not associated with O_3 levels. No evidence for PM10 or NO_2 main effects or confounding of O_3. Similar patterns of O_3 results using hours > 60 ppb as exposure metric instead of daily 10-6pm means. Surprisingly, region was a major negative confounder; i.e., lung function was lower on average among students who had lived in the cleaner city, San Francisco, than among those who had lived in Los Angeles. O_3 exposures were significant predictors only after control of the regional effect. For a 20 ppb increase in the lifetime average daily 10-6 ppm O_3, FEF75% decreased 334 mL/sec (95% CI: 11 to 657 mL/sec), a 14% decline.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Kuo et al. 2002 Central Taiwan Annual mean O_3 ranged from 18.6 to 27.3 ppb across 7/8 study communities with data	Respiratory questionnaire administered to 12,926 children ages 13-16 at 8 junior high schools in central Taiwan, to determine asthma prevalence. The association between asthma prevalence and air pollution exposure analyzed by simple Pearson correlations of prevalence with annual mean air pollution levels (for O_3, SO_2, PM10, and NO_2), and by multiple logistic regression, controlling for gender, age, residential area, parental education, ETS, incense, and activities. The 775 asthmatics who were identified then provided follow-up data on symptoms and exacerbations over a 1 year period. Simple Pearson correlations computed between monthly hospital admissions and air pollution levels, not controlling for covariates such as season or weather.	Asthma prevalence ranged from 5.5% to 14.5% across the 8 schools. Based on simple Pearson's correlations, mean O_3 and NO_2 levels were correlated with variations in asthma prevalence. Only NO_2 remained significant in multiple logistic regression analyses. Monthly correlations of hospital admissions for asthmatics yielded variable results, all of which would be confounded by temporal factors. Study suggests no association between long-term O_3 concentrations and asthma prevalence in children after controlling for NO_2. Longitudinal hospital admissions results are inconclusive due to analytical limitations.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
McConnell et al. 2002 12 southern California communities 1994-1997 four-year mean (SD) concentrations: 6 Low Pollution Communities: Max 1-hr: 50.1 (11.0) Mean 10am-6pm: 40.0 (7.9) Mean 24-hr: 25.1 (3.1) 6 High Pollution Communities Max 1-hr: 75.4 (6.8) Mean 10am-6pm: 59.6 (5.3) Mean 24-hr: 38.5 (11.0)	3535 non-asthmatic children aged 9-16 years recruited in 1993 and 1996 and followed with annual surveys through 1998 to determine incidence of new onset asthma. Participation in sports assessed at baseline. Co-pollutants included PM10, PM2.5, NO_2, and inorganic acid vapors. Asthma incidence was examined as a function of number of sports played in high and low pollution communities, controlling for age, sex, and ethnic origin.	Asthma incidence was not higher in the high pollution communities as compared with the low pollution communities, regardless of the pollutant used to define high/low. In fact, the high O_3 communities had generally lower asthma incidence; this was significant only for max 1-hr O_3. However, in high O_3 communities, there was a 3.3 fold increased risk of asthma in children playing 3 or more sports vs. those playing no sports; no such increase was observed in the low O_3 communities. No other pollutant showed this association. These results suggest that high levels of physical activity is associated with risk of new asthma development for children living in communities with high O_3 levels. These findings require replication.

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
McDonnell et al. 1999 California 1973-1992 20 year mean (SD) of 9am-5pm daily mean O_3: 46.5 (15.3)	3091 non-smoking adults aged 25+ years at enrollment in 1977 completed questionnaires at three time points: 1977, 1987, and 1992. To be eligible, subjects had to have lived 10 or more years within 5 miles of current residence. Residential histories used to interpolate air pollution levels to zip centroids over the period 1973-1992, yielding 20-year mean exposure estimates for O_3, PM10, SO_2, and NO_2. New asthma cases defined as answering yes to doctor diagnosed asthma at either 1987 or 1992 follow up among those answering no at enrollment in 1977. Multiple logistic regression used to test for associations between new-onset asthma and long-term exposures to air pollution, controlling for age, education, pneumonia or bronchitis before age 16, and ever smoking. All models run separately for males and females.	There were 32 incident cases of asthma among 972 males and 79 incident cases among 1786 females. In logistic regression analyses, long-term O_3 exposures were associated with increased risk of incident asthma among males but not females. Other pollutants were neither associated with asthma incidence nor were confounders of the O_3 association in males. RR (95% CI) for 27 ppb change in long term O_3 exposure: Males: 2.09 (1.03 to 4.16) Females: 0.86 (0.58 to 1.26)

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Peters et al., 1999a,b 12 southern California communities 1993-1994 1994 mean of daily 1-hr maxima: 12-community mean: 64.5 Range: 35.5-97.5	3676 children ages 9-16 enrolled in 1993 into the first cohort of the Children's Health Study provided questionnaire data on respiratory disease histories and covariates. 3293 subjects also underwent pulmonary function testing, although 2781 were used in air pollution regressions for reasons not made clear. Air pollution data collected in 1994 for O_3, PM10, PM2.5, NO_2, and inorganic acid vapors. For analysis of respiratory diseases, individual pollutants tested for associations with ever asthma, current asthma, bronchitis, cough, and wheeze after controlling for covariates. For analysis of lung function, individual pollutants and pairs of pollutants were regressed with FVC, FEV1, FEF25-75%, and PEFR, controlling for usual demographic and anthropometric covariates.	Acids and NO_2, but not O_3, were associated with prevalence of wheeze. No associations of O_3 with any of the respiratory diseases or symptoms. Decreased lung function was associated with multiple pollutants among females but not males. Associations were stronger when 1994 air monitoring data were used in the regressions rather than 1986-1990 agency data collected prior to the study. For O_3 exposure in females, all four lung function variables declined with increasing exposure; only PEFR and FEF25-75% were significant, as follows: For all females: Slopes (SE) for 40 ppb increase in annual mean daily 1-hr maximum O_3: PEFR: -250.9 mL/sec (69.9) FEF25-75%: -124.7 mL/sec (44.0) In males who spent more time outdoors, all four lung function parameters declined with higher exposure to O_3; this was significant only for FVC and FEV1. For males more outdoors: Slopes (SE) for 40 ppb increase in annual mean daily 1-hr maximum O_3: FVC: -128.6 L (56.0) FEV1: -136.3 L (51.3)

Table 12-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

Reference/Citation, Location, Duration, Ozone Index/Concentration	Study Description:	Results and Comments
Pope et al. 2002 Nationwide Cohort 1982-1998 17-year mean of daily 1-hr maximum O_3 (SD): 59.7 (12.8) ppb	Approximately 500,000 members of ACS cohort enrolled in 1982 and followed through 1998 for all-cause, cardiopulmonary, lung cancer, and all non-cardiopulmonary/non-lung cancer mortality. Air pollution concentrations in urban area of residence at time of enrollment assessed from 1982 through 1998.	No significant effect of ozone on mortality risk, though the association of third quarter ozone concentrations with cardiopulmonary mortality was positive and nearly significant. Residential location was known only at enrollment to study in 1982. Thus, exposure misclassification is likely to be high.
Ramadour et al. 2000 (see also Charpin et al. 1999) Seven towns in SE France January-February 1993 Range of town-specific means over 2-month study period (8-hr O_3): 15-26 ppb	2445 children (ages 13 and 14 and who had lived at current residence for at least 3 years) from schools in seven towns in SE France completed ISAAC survey of asthma and respiratory symptoms. This area has highest O_3 levels in France. In addition to O_3 measurements, also collected data on SO_2 and NO_2. Analyzed relationships between asthma and other respiratory conditions with mean air pollution levels across the seven towns using logistic regression, controlling for family history of asthma, personal history of early-life respiratory diseases, and SES. Also did simple univariate linear regressions.	In logistic regressions, no significant associations seen between O_3 and 12 month history of wheezing, history of asthma attack, exercise induced asthma and/or dry cough in last 12 months. In simple bivariate scatterplots of respiratory outcomes (12-month wheezing or asthma) vs. mean O_3 for the 8 towns, there appeared to be strong positive relationships. No data on slope estimates given. Concerns about potential confounding across town limits the interpretation of this study.
Ritz et al. 2000 Southern California 1989-1993 Mean (SD) O_3 9am to 5pm over 6 weeks before birth: 36.9 ppb (19.4)	Data on 97,159 singleton births over period 1989-1993 linked to air monitoring data during different periods of pregnancy to determine associations between pollution exposures and preterm birth. Besides O_3, pollutants of interest included PM10, NO_2, and CO. Multiple regression analysis used, controlling for maternal age, race, education, parity, and other factors.	Both PM10 and CO during early or late pregnancy were associated with increased risk for preterm birth. No associations observed with O_3.

12.4 O_3 and Acute Mortality

12.4.1 Introduction

Until recent years, the role of O_3 as a risk factor for premature death has received relatively little attention. One early study reported significant effects of lag 1 oxidants on daily deaths in a 10 year record from Los Angeles, controlling for temperature and other pollutants, including particulate matter (Kinney and Ozkaynak 1991). Two other early studies, one for Detroit, MI data (Schwartz 1991) and the other for St. Louis, MO, and Kingston-Harriman, TN, (Dockery et al. 1992) reported that particulate matter (PM), but not O_3, was significantly associated with mortality. This small and inconsistent literature made it difficult to draw a firm conclusion one way or the other as to whether O_3 was a risk factor for acute mortality (e.g., USEPA 1996).

Over the past five years, however, there has been a tremendous increase in the number of studies addressing the acute relationship between daily mortality and O_3. Results are now available from several multi-city analyses, including the NMMAPS reanalysis of 90 US cities (Dominici et al. 2003), as well as a large number of individual city analyses. While interpretation is made difficult by several issues unique to the analysis of acute O_3 effects, there is now sufficient evidence to reach the preliminary conclusion that summer season O_3 is likely to be an independent risk factor for premature mortality.

12.4.2 Interpretational Issues

To a substantial extent, the growing literature on acute O_3 effects can be viewed as an artifact of the exploding interest in studying acute PM effects. Unlike many of the earlier PM-mortality studies, recent studies have tended to include multiple co-pollutants, including O_3. For example, of the 84 time-series mortality studies published since 1995, 35 studies examined PM but not O_3; 47 studies examined both PM and O_3; and only two studies examined O_3 but not PM. In many of the multi-pollutant studies, O_3 is treated primarily as a potential confounder of the PM effects under study. As a result, many of these studies lack specific hypotheses regarding mortality effects of O_3, and fail to provide the range and depth of analyses, including sensitivity analyses, that would be most useful in judging whether O_3 is an independent risk factor for acute mortality. This is in contrast to the morbidity studies where hypotheses regarding O_3 effects on respiratory symptoms, lung function, and ED visits, etc. have been tested, with O_3 treated as a key pollutant.

It is also worth noting that for O_3 we lack an historical reference point, such as the December 1952 London episode, that so clearly demonstrated the mortality impacts of a PM and SO_2 mixture. Instead, the early recognition that summer oxidant air pollution has adverse health effects, came mainly from studies in Los Angeles and other major cities with high density of automobiles, and was based on respiratory symptoms. Thus, the foci of PM epidemiology and that of O_3 epidemiology have been historically different. However, as noted above, the increased attention to PM-mortality associations in the 1990's led to an increase

in studies that also examined O_3, most often as a potential confounder of PM effects.

To avoid confounding, time-series mortality studies include (1) adjustment for seasonal and other "long-wave" temporal trends; and, (2) adjustment for weather effects. O_3's correlation with these confounding terms tends to be higher than that for PM or other gaseous pollutants. PM in the U.S. generally does not show as strong seasonal cycles as O_3, because PM tends to reflect both primary emissions (throughout the year, but often higher in winter in most U.S. cities) and secondary aerosols in summer. Thus, model specifications that may be appropriate for PM may not necessarily be adequate for O_3. As a result, O_3 effect estimates are more sensitive to the methods used to control for these factors. Few studies to date have thought through these issues thoroughly in the context of analyzing O_3 effects, primarily because, the driving force has been to fit PM effects, and because the sensitivity of the model specifications is generally much less. Of particular importance is the strong seasonal cycle for O_3, high in summer and low in winter, which is opposite to the usual cycle in daily mortality, high in winter and low in summer. Most studies have used a smooth function of mortality (e.g., LOESS or Spline functions) to coadjust for seasonality and other temporal trends while simultaneously fitting the O_3 effect. However, recent evidence suggests that this approach may not fully remove the confounding influence of seasonal cycles when dealing with a pollutant like O_3 which itself has a strong seasonal cycle (Burnett et al., 2001). To be assured of avoiding this problem in locations where O_3 is highly seasonal, time series analyses of year round O_3 data must pre-adjust for time trends and seasonality. Alternatively, analysis can be restricted to the warm season, thereby reducing the degree of seasonality in the exposure data.

A related, more technical, issue is the choice of degrees of freedom for the smoothing function of time. By using more degrees of freedom, a tighter fit to the temporal variations in mortality can be achieved. The question is, how much is enough but not too much? Table 12-5 lists some examples of the models used to adjust for temporal trends and weather effects in recent studies that examined O_3. There appears to be no consensus as to what number of degrees of freedom (per year) is appropriate in adjusting for seasonal trends. Statistical diagnostics such as AIC or residual autocorrelation or dispersion of the regression model are often used to choose the degrees of freedom for temporal trend, but these diagnostics do not provide epidemiological justification for, or interpretation of the fitted model. Clearly, using more degrees of freedom in temporal trend fitting means ascribing more details (i.e., shorter temporal fluctuations) of daily mortality to unmeasured potential confounders and possibly taking away the weather and air pollution effects. Therefore, it is not surprising that we often observe smaller pollution effect estimates when more degrees of freedom are used to adjust for temporal trends. More work is needed in this area to reduce the uncertainty involved in the interpretation of O_3 mortality effect estimates.

Table 12-5 Examples of model specifications used in recent studies to adjust fortemporal trends and weather in recent morality studies that analyzed O_3.

Study	Adjustment for temporal trend	Adjustment for weather
Moolgavkar et al., 1995 Philadelphia, PA	Data were analyzed for each of 4 seasons.	Quintiles of temperature.
Anderson et al., 1996 London, England	Sine/cosine terms, linear trend, influenza epidemics	Three linear terms: (1) < 5 °C; (2) 5-20 °C; and (3) > 20 °C.
Borja-Aburto et al. 1997a Borja-Aburto et al. 1997b. Mexico City, 1990-1992	Sine/cosine, spline functions were examined but minimum temperature alone as seasonal trend variable was chosen as the best specification. Data were also analyzed for rainy (May-October) and dry (November-April).	Minimum temperature.
Touloumi et al. 1997 APHEA 1	Sine/cosine terms, linear trend	Temperature and humidity terms were fitted in each of the four cities using parametric terms (linear and quadratic) and/or indicator variables.
Chock et al., 2000 Pittsburgh, PA	bs (days, df=4/yr to 13/yr); season specific analysis also conducted.	V-shape function with the minimum at 25°C.
Samet et al. 2000 Dominici et al. 2003 90 largest U.S. cities	ns (days, df=7/yr); season specific analysis also conducted.	ns (temp, df=6) + ns (lag1-3temp, df=6) + ns (dewp, df=3) + ns (lag1-3dewp, df=3)
Hoek et al., 2000 The Netherlands	ns (days, df=10/yr) + influenza-of-past-3 wks	Warm: 0 for temp <= 14°C; temp for temp > 14 °C. Cold: 0 for temp >= 14°C; temp for temp < 14 °C.
Fairley D 2003 Santa Clara County, CA	ns (days, df=7 for 7yrs) + ns (day-of-year, df=12)	ns (min temp, df=3) + ns (max. temp, df=2)
Lippmann et al, 2000 Ito K 2003 Detroit, MI	ns (days, df=12/yr)	ns (temp, df=2) + lo (lag1-3temp, df=2) + hot-and-humid indicator

Note: bs: B-splines; ns:natural splines; lo: LOESS.

As shown in Table 12-5, the weather models used in recent mortality time-series analyses can be classified as using: (1) quantile (e.g., quartile, quintile, etc.) indicators of temperature; (2) a parametric functional form, such as V- or U-shape functions, of temperature; and, (3) parametric (e.g., natural splines) or non-parametric (e.g., LOESS) smoothing function of temperature. More recent studies tend to use the smoothing function of temperature variables. While these methods provide flexible ways to fit mortality as a function of temperature and other weather variables, there are two major issues that need to be resolved before mortality effects of O_3 can be meaningfully interpreted.

The first issue relates to the range of temperatures over which adjustment occurs. Most researchers agree that there are mortality effects of extreme temperature (i.e., heat waves or cold spells). However, the fitted functions of temperature used in model adjustment include the "mild" temperature range in which we do not expect mortality effects of temperature. Because daily fluctuations of air pollution, and especially O_3, are strongly influenced by weather conditions including temperature, ascribing the association between temperature and mortality to all temperature effects may under-estimate the effects of air pollution. The prevailing smoothing approaches will fit the data flexibly, but the epidemiological interpretation of the fitted slope of temperature/mortality relationship is rarely, if at all, given.

As can be seen in Table 12-5, the use of (parametric or non-parametric) smoothers has become popular in recent years, and is the currently the most commonly used approach to adjust for temporal trends in mortality time-series studies. However, this flexible fitting approach still cannot adjust for the relationships among variables that change across seasons. This is particularly important for O_3, which appears to change its relationship with temperature (and with other pollutants) across seasons. In some urban locations during the winter, O_3 comes mainly from the free troposphere and can be considered a tracer for relatively clean air. The clean air during the winter is associated with high-pressure systems (i.e., cold, clean air coming down from upper atmosphere). Thus, sunny clear winter days in the urban environment are the days when O_3 levels may be relatively high (compared with other winter days) but when air pollution levels from primary emissions (e.g., NO, which also "quenches" O_3), SO_2 and PM from local sources) are low. This leads to negative correlation between O_3 and the primary pollutants.

This changing relationship between O_3 and temperature (and other pollutants) across seasons and its potential implications to health effects modeling appear to be under-appreciated or unrecognized in the mortality time-series literature. Even the flexible smoother-based adjustments for seasonal trend and other unmeasured time-varying variables cannot adjust or take into account these complex relationships among variables that change across seasons. One obvious way to alleviate or avoid this complication is to analyze data stratified by season. An alternative approach is to include separate O_3 concentration variables for each season (by multiplying O_3 concentrations with season indicator

variables) as was done by Chock et al. (2000) in his analysis of Pittsburgh, PA data.

Several studies have examined season-specific effects of ozone. Moolgavkar et al. (1995) examined the relationship between daily mortality and air pollution (TSP, SO_2, and O_3) by season in Philadelphia, PA for the period 1973-1988. The Poisson regression (GLM) model also included quintile of temperature. While both TSP and SO_2 showed positive and significant associations with mortality in all four seasons in single pollutant models, O_3 showed positive and significant associations only in summer. The O_3-mortality association was negative (though not significantly) in winter. Additions of TSP or SO_2 in the regression did not attenuate the O_3 coefficient in the summer, and in the three-pollutant model in summer, only O_3 remained significant. In another Philadelphia data analysis by (Moolgavkar et al. 1996), in which GAM was used (only one smoothing term was included) to adjust for temperature, O_3 was positively and significantly associated with mortality in summer only, and negatively and significantly associated with mortality in winter in single pollutant models. With all the pollutants (TSP, SO_2, NO_2, and O_3) in the model, the O_3 effect remained significant in summer. These results illustrate the importance of season-specific analyses of O_3 effects on mortality.

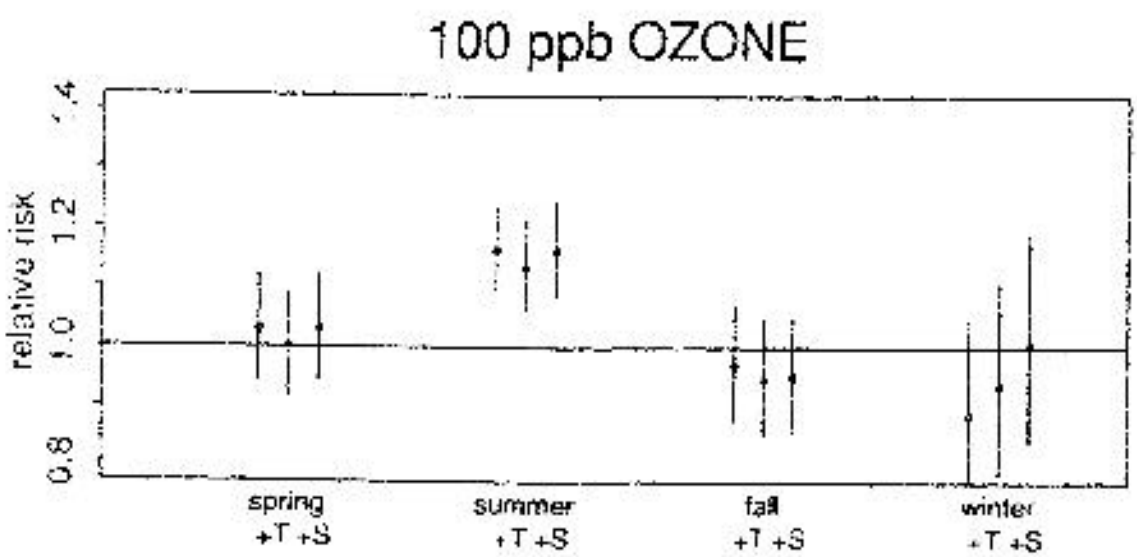

Figure 12-1 Estimated total (non-accidental) mortality relative risk per 100 ppb 24-hr average daily O_3 for each seasonal data set (Moolgavkar et al. 1995). For each season, the left-hand estimate is for O_3 alone; the estimate in the middle with "+T" is with TSP, the right-hand estimate with "+S" is with both TSP and SO_2 in the model.

Anderson et al. (1996) examined the relationship between air pollution (O_3, NO_2, black smoke, and SO_2) and daily mortality (all cause, cardiovascular, and respiratory) in London, England for study period 1987-1992 using the Poisson GLM model. They examined the associations for all seasons, as well as the warm season (April to September) and the cool season (October to March) separately. Their results indicate that the estimated O_3 relative risks were larger for the warm season than for the cool season for all cause mortality. For example, the percent excess risk estimated per 10th-to-90th percentile ppb increment of 8-hr (9am to 5pm) average O_3 was 3.48% (1.73, 5.26) for the warm season (7-36 ppb), 0.77% (-0.88, 2.44) for the cool season (2-22 ppb), and 2.43% (95%CI: 1.11, 3.76) for the full year (3-29 ppb). A similar pattern was seen for cardiovascular mortality, but the estimated risk was negative (not significantly) for the cool season. For respiratory mortality, the estimated excess risks were similar between the cool and warm seasons.

The most robust data on O_3 effects on mortality are those from the National Mortality and Morbidity Air Pollution Study (NMMAPS) reported originally by Samet and colleagues (2000) and reanalyzed using non-GAM methods by Dominici et al. (2003). This study tested acute air pollution effects on mortality in the largest 90 cities in the US. The focus of the study was PM10, but O_3 and other gaseous pollutants were also analyzed in single and multi-pollutant models. In the original analysis (using default GAM convergence criteria), the combined excess risk estimate for O_3 at lag 0 day was essentially zero for all seasons combined, but positive for summer (June, July, and August) and negative for winter (December, January, and February) in the same magnitude (~0.5% and ~-0.5% excess mortality per 10ppb 24-hr average O_3, respectively). In Dominici et al.'s re-analysis, the O_3 effect estimate was positive and significant for summer (0.4% excess mortality per 10 ppb 24-hr average O_3), and negative and significant for winter (-0.5%). These results are consistent with the results reported by Moolgavkar and co-investigators (1995), and raise a serious concern regarding the interpretation of O_3 mortality risk estimates from analyses of year-round data. It would be of interest to examine the effects of O_3 in multiple cities that, for example, have very low correlation between O_3 and particulate matter, for which significant effects on mortality have been demonstrated.

There are other studies that also reported larger excess mortality risks in the warm (or summer) season than in the cool (or winter) season. These include the analyses of: the Netherlands data (Hoek et al. 2000; re-analysis by Hoek G 2003), Brisbane, Australia (Simpson et al. 1997), Montreal, Canada (Goldberg and Burnett 2003), and Vancouver, Canada (Vedal et al. 2003). These studies showed either smaller than summer or slightly negative (but not significant) risk estimates for the cool season. Of the studies that analyzed data by season, only one study in Pittsburgh, PA (Chock et al. 2000) showed negative risk estimates in summer. The results that show more positive and significant associations between O_3 and mortality in warm seasons are consistent with the expectation that O_3, if harmful, should have a positive association with health outcomes in summer when the levels are substantially higher than during the winter. However, the negative O_3-mortality associations seen for winter in the 90 cities

study and Philadelphia data suggest that further examination of this issue is required. Specifically, if O_3 in winter at low levels is shown to be negatively associated with the factors (e.g., PM) that are positively associated with mortality, then these potentially spurious negative O_3-mortality associations can be explained. In other words, if days with very low winter O_3 levels often have elevated PM concentrations, then elevated mortality due to PM might be spuriously associated with low O_3. Alternatively, the negative winter findings may reflect uncontrolled confounding by seasonal cycles. Thus, O_3-mortality effect estimates computed for year-round data need to be interpreted with caution.

Multi-pollutant models have often been used to examine the role of other air pollutants as potential confounders of O_3 effects. The results from the NMMAPS reanalysis study appear to suggest that the addition of PM10 in the model did not substantially reduce summer season O_3 mortality risk estimates, at least for the largest risk estimate at lag 0 day (Dominici et al. 2003), as shown in Figure 12-2. There are many single-city studies that estimated O_3 mortality risk with co-pollutants in the model for year-round data. In general, O_3 risk estimates in these studies were not substantially affected by inclusion of other air pollutants in the models.

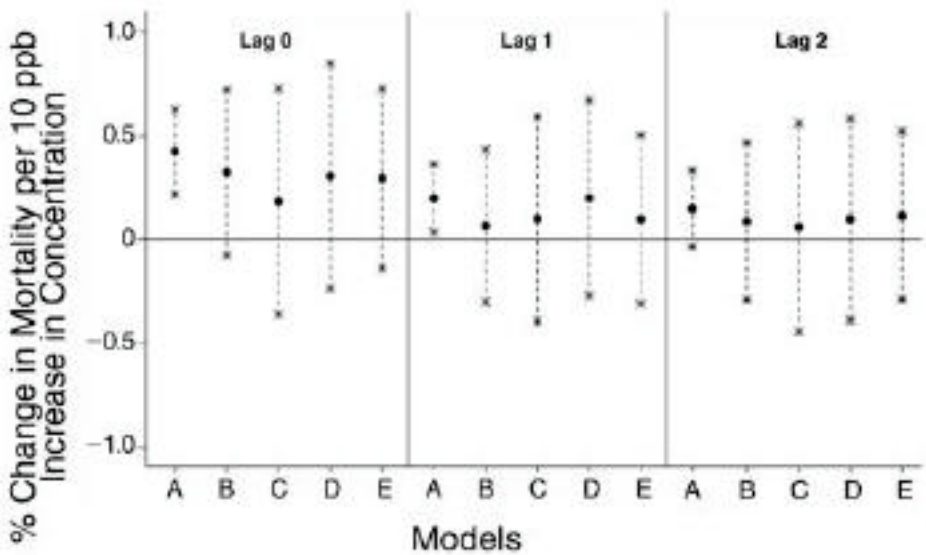

Figure 12-2 From Dominici et al. (2003). Posterior means and 95% posterior intervals of national average estimate of O_3 effect on total mortality from non-external causes at lags 0, 1, and 2 within sets of the 90 cities with pollutant data available. Models A = O_3 alone; B = O_3 + PM10; C = O_3 + PM10 + NO_2; D = O_3 + PM10 + SO_2; E = O_3 + PM10 + CO.

Other studies that estimated warm season O_3 mortality risks with co-pollutants in the model include Moolgavkar et al.'s (1995) analysis of Philadelphia data, Anderson et al.'s (1996) analysis of London, England data, Chock et al.'s (2000) analysis of Pittsburgh, PA data, and Vedal et al.'s Vedal et al.'s (2003) analysis of Vancouver, Canada data. In these studies, except Chock et al.'s study, O_3 mortality risk estimates in summer were significantly positive and were not substantially reduced by addition of co-pollutants. In Chock et al.'s analysis, O_3 risk estimates for age 75 and over were negative (though not significantly) for all four seasons (least negative in summer) even in single pollutant models, and the co-pollutant models resulted in significantly negative O_3 risk estimates in some cases. Unfortunately, none of the aforementioned four studies that reported O_3 risk estimates with co-pollutants by season included PM2.5 (TSP in Philadelphia; BS in London; PM10 in Vancouver and Pittsburgh). The studies that did have PM2.5 data, including Santa Clara County, CA (Fairley 1999; Fairley D 2003), Philadelphia, PA (Lipfert et al. 2000), Detroit, MI (Lippmann et al. 2000; Ito K 2003), examined co-pollutant models for year-round data only, but O_3 risk estimates were not substantially affected by the addition of PM2.5. Only the Lipfert et al. (2000) and Burnett et al. (2000) studies examined O_3 and sulfate simultaneously. Thus, there remains some uncertainty regarding the potential confounding between O_3 and summer-haze PM. In summary, in most studies, O_3 mortality risk estimates were not sensitive to additions of co-pollutants, either for year-round data or for seasonal data, but uncertainty remains as to the potential confounding by summer haze PM, such as sulfate aerosols.

12.4.3 Acute Mortality Risk Estimates for O_3

This section reviews available O_3-mortality excess risk estimates in the current literature. For simplicity, this review excludes the (20+) studies that used GAM with default convergence criteria that have not been reanalyzed (with more than one non-parametric smoothing term) and focuses on the subset of studies listed on Table 12-6 for which risk estimates were available. Two parameters that can complicate the comparison of the risk estimates from different time-series studies include the time lag between exposure and death, and the O_3 exposure index used. An examination of the "most significant" lags extracted from the studies on Table 12-6 also suggests that the majority of the single-day associations were immediate (0-day lag: 21 studies; 1-day lag: 8 studies; 2-day lag: 3 studies; and, longer lag days: 3 studies). It should be noted that, when associations are found at multiple days (as in the case of O_3 risk estimates in the U.S. 90 cities analysis by Dominici et al. 2003), choosing only a single-day's lag results would also underestimate the multi-day effects. On the other hand, testing several lags and choosing the largest effect would tend to produce a positive bias. Thus, using a single lag day's risk estimate can result in bias in either direction.

With respect to exposure indices, the three used most often are the daily 1-hr maximum (1-hr max.), the daily maximum 8-hr running average (8-h max.), and the 24-hr average (24-hr avg.) concentrations. Of the 47 studies listed in Table 1, 29 studies used 1-hr max., 12 used 8-hr max., 17 used 24-hr avg. concentration, and 2 used alternative O_3 exposure indices in addition to

commonly used indices. Thus, the 1-hr max. O_3 concentration is the most frequently used index. Eight studies used two or more indices. An examination of these eight studies suggest that, in general, the significance of associations was similar for these indices, and for the same distributional increment (e.g., inter-quartile range), the excess mortality risks computed were also similar. This is expected because, for most urban locales, the daily fluctuations of these three indices are highly correlated ($r > 0.9$). However, in comparing relative risk estimates based on these alternative indices, it is important to be aware of the differences in relevant exposure increments. The exposure increment is the arbitrarily chosen exposure range for which the relative risk (i.e., percent change in mortality) is computed. Because day to day changes in concentrations of short term O_3 indices like the 1-hr max are much more variable than those of longer term indices like the 24-hr mean, it makes sense to scale the exposure increments to account for these differences. Using the nationwide data for O_3 monitors in MSA (Langstaff and Pinto, personal communication, 2003), the difference between the mean and the 95th percentile concentrations (that is, "average" to "high" O_3 increment) were approximately 40, 30, and 20 ppb, for 1-hr max, 8-hr max., and 24-hr avg., respectively. This, to facilitate comparisons across studies in the following sections, we express O_3 mortality relative risks using an increment of 40 ppb if the original risk estimate was computed using the 1-hr max.; we use an increment of 30 ppb for the 8-hr max, and we use an increment of 20 ppb for the 24-hr average in the tables and discussion for this section.

As listed in Table 12-6, there are over 40 studies that reported short-term mortality risk estimates for O_3. Table 12-6 does not include the additional 20+ studies that used GAM with default convergence criteria (with more than one non-parametric smoothing terms) that have not been reanalyzed. Among the reanalyzed GAM studies that presented O3 risk estimates, there was a tendency to find similar or slightly lower O_3 effect estimates using GAM than alternative analysis methods. For example, in the analysis of Santa Clara County, CA data (Fairley 1999; re-analysis Fairley D 2003), the O_3 non-accidental mortality risk estimate increased slightly from 2.7% (default GAM) to 2.8% (stringent GAM), and to 3.0% (GLM) per 30 ppb increase in 8-hr max. O_3. In Hoek et al.'s analysis of the Netherlands data (Hoek et al. 1997; re-analysis, Hoek G 2003), the O_3 non-accidental mortality risk estimates increased from 1.8% (default GAM) to 2.1% (stringent GAM), and to 2.2% (GLM) per 40 ppb increase in 1-hr max. O_3. In the analysis of the largest 90 U.S. cities (Samet, et al, 2000; re-analysis, Dominici et al. 2003), the year-round combined estimate of O_3 non-accidental mortality risk changed from a non-significant negative (~ -0.1% per 20 ppb change in 24-hr avg. O_3) to a significantly positive value (0.4%), while the estimates for winter (~ -1%) and summer (~ 1%) did not change appreciably. This limited evidence suggests that use of GAM tends to underestimate effects of O_3 on acute mortality.

Figure 12-3 shows O_3 non-accidental mortality risk estimates for all ages and for all season data in single pollutant models from 33 studies listed in Table 12-6. The majority of the estimates are positive with a few exceptions. Two multi-city

studies (the U.S. 90 cities study and the APHEA European 4 cities results) both showed positive and significant estimates, but the APHEA estimate was larger (4.5% per 40 ppb increase in 1-hr max O_3) than that for the 90 cities study (0.9%). Alternative analyses of data from the same city sometimes resulted in differing results. For example, Anderson et al.'s (1996) analysis of London, England data showed a larger O_3 mortality risk estimate (3.3% per 40 ppb increase in 1-hr max O_3) than Bremner et al. (1999) estimate (-0.8%). Another example is the Amsterdam data analyses for which Verhoeff et al.'s (1996) estimate (3.8%) was larger than that by Roemer and van Wijnen's (2001) estimate (-0.2%). Clearly, differences in analytical approaches can produce differing results. These results do suggest that more sensitivity analyses using alternative model specifications should be conducted. Despite these discrepancies, overall, the range of estimates were relatively narrow, with 27 out of the 33 estimates being between 0 and 6.6% per 40 ppb increase in 1-hr max O_3 (median = 2.9%) concentration. A new meta-analysis of time-series studies has been undertaken by WHO (Anderson et al. 2004). Using results from only European cities, this study reports an effect estimate of about 0.6% per 40 ppb change in 8 hr O_3 concentration.

As discussed above, the O_3 risk estimates computed for year-round data may be misleading. A smaller number of studies conducted season specific analyses. Figure 12-4 shows the studies that reported O_3 risk estimates by season. For those studies that calculated O_3 risk estimates for each of the four seasons, only summer and winter results are shown. The estimates for year-round data analyses, when available, are also shown for comparisons. In all the studies, except the Brisbane data (for which the estimates are essentially the same for summer, winter, and year-round subsets), the O_3 risk estimates are larger for summer than for winter, with the year-around estimates in between the summer and winter estimates. It is not surprising that the summer and winter estimates were similar in Brisbane, as the O_3 levels do not differ greatly between summer and winter (22 ppb and 27 ppb, respectively, for 1-hr max O_3 concentration). These results, showing that the O_3 risk estimates are in fact larger in summer when O_3 is higher, are consistent with causal association, and also question the value of interpreting O_3 risk estimates obtained from year-round data.

Confounding between "winter type" pollution (i.e., CO, SO_2, and NO_2) and O_3 is not a great concern because the peaks of these pollutants do not strongly coincide. The main confounders of interest for O_3, especially for the northeast US, are "summer haze" type pollutants such as acid aerosols and sulfate. Since few studies had these chemical measurements, PM (especially PM2.5), may serve as a surrogate. However, due to the expected high correlation among the constituents of the "summer haze mix", multi-pollutant models including these pollutants may result in unstable coefficients, and therefore, interpretation of such results requires some caution. Only one study, which was conducted in Philadelphia, PA, (Lipfert et al. 2000) reported O_3 risk estimates with and without sulfate (as well as PM2.5). The O_3 risk estimate (2.8% per 45 ppb increase in avg. of lag 0 and 1 day 1-hr max O_3 concentration in the single pollutant model) in this study was not substantially affected by the addition of sulfate (3.4%, an

increase). Figure 12-5 shows the O_3 risk estimates with and without PM indices from 16 studies (note: these results are for year-round data). In general, the O_3 mortality risk estimates were not affected by addition of PM indices, with the exception of the Los Angeles data with PM10 and Mexico City data with TSP.

There are several limitations to this literature. As noted earlier, one limitation is that, in most cases the O_3-mortality associations were reported in studies whose main focus was PM. Consequently, the sensitivity of the O_3 mortality risk estimates to alternative model specifications has not been well explored. Therefore, there remains some uncertainty regarding the extent of confounding that could bias the current estimates of O_3 mortality risks. Also, the majority of the available O_3 mortality risk estimates have been computed using year-round data. The results from the studies that conducted analysis by season suggest that the O_3 risk estimates are larger in the warm season. Some of the risk estimates in cold season are negative, possibly reflecting the negative correlation between low-level O_3 and PM (and other primary pollutants) within the cold season observed in some U.S. cities. Thus, the O_3 risk estimates obtained for year-round data may be misleading and likely underestimate the effects during warm season.

With respect to confounding by co-pollutants, the results from two pollutant models published to-date generally suggest that co-pollutants do not substantially alter the O_3 risk estimates. Information on the possible confounding/synergism between summer haze PM (e.g., sulfate) and O_3 in summer remains limited and inconclusive.

Few studies have examined sub-categories of cause-specific mortality, and most of those that have had limited statistical power to detect associations in small daily counts databases. The results from a study of a relatively large data set from the Netherlands suggest that certain cardiovascular sub-categories had larger relative risk estimates than that for all cardiovascular categories combined, but the same pattern was also seen for other pollutants. This remains an issue requiring further study.

12.4.4 Conclusions

Though limited in some ways, a large and growing body of data now exists examining whether there is a relationship between daily mortality and O_3 concentrations. These data support a preliminary conclusion that warm season O_3 concentrations represent an independent risk factor for premature mortality, controlling for weather effects and other air pollutants. Current data do not enable distinguishing among alternative O_3 exposure metrics (1-hr, 8-hr, 24-hr) with respect to risk. Given the high correlations among the three, mortality risk can be addressed using a standard based on any of these indices. The median risk from current studies appears to be about 3% pr 40 ppb change in 1 hr O_3 concentration.

Figure 12-3 Ozone excess non-accidental mortality risk estimates <u>for all season data</u>. All age, unless otherwise noted.

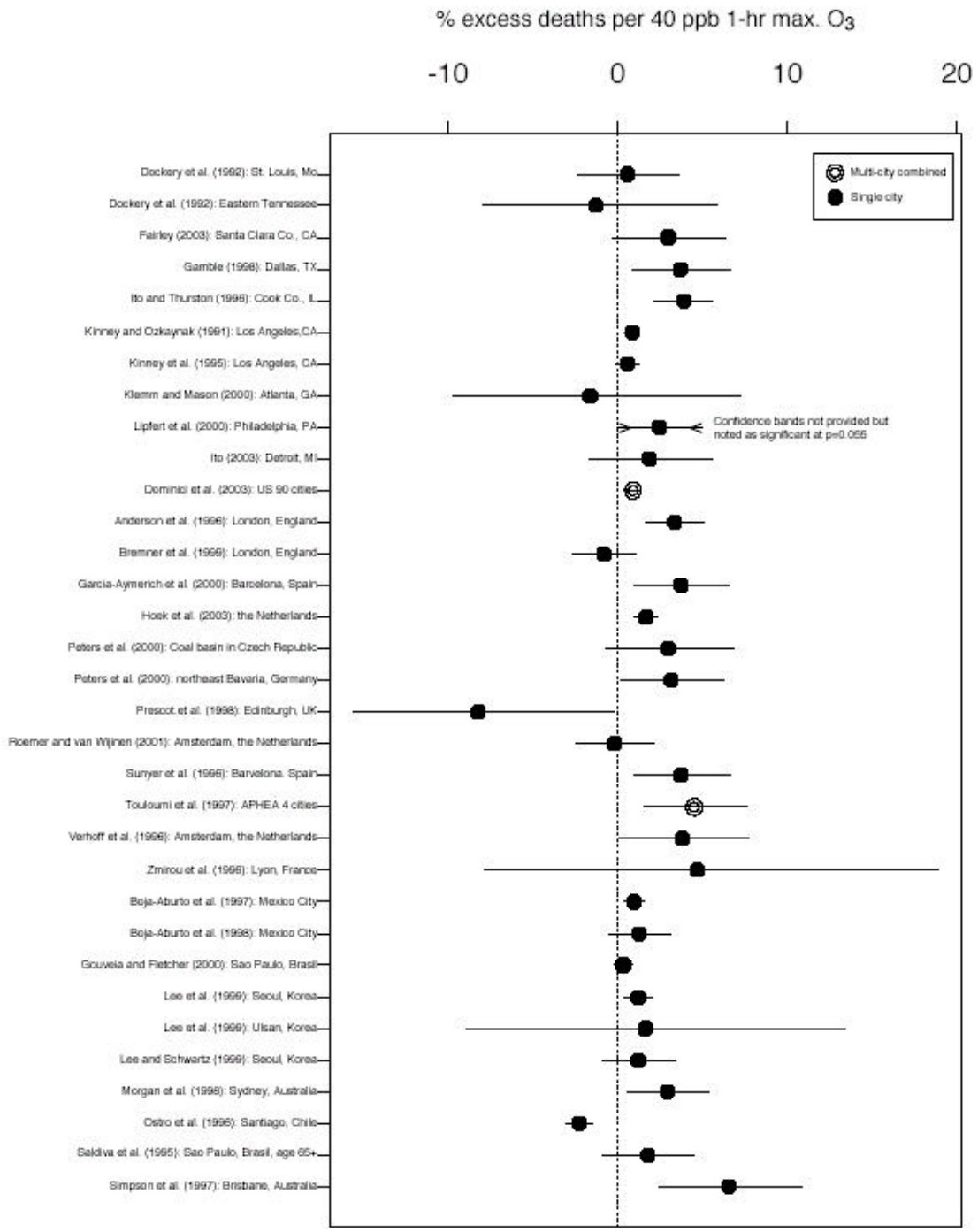

Figure 12-4 Ozone mortality risk estimates <u>by season</u>. All age non-accidental mortality, unless otherwise noted.

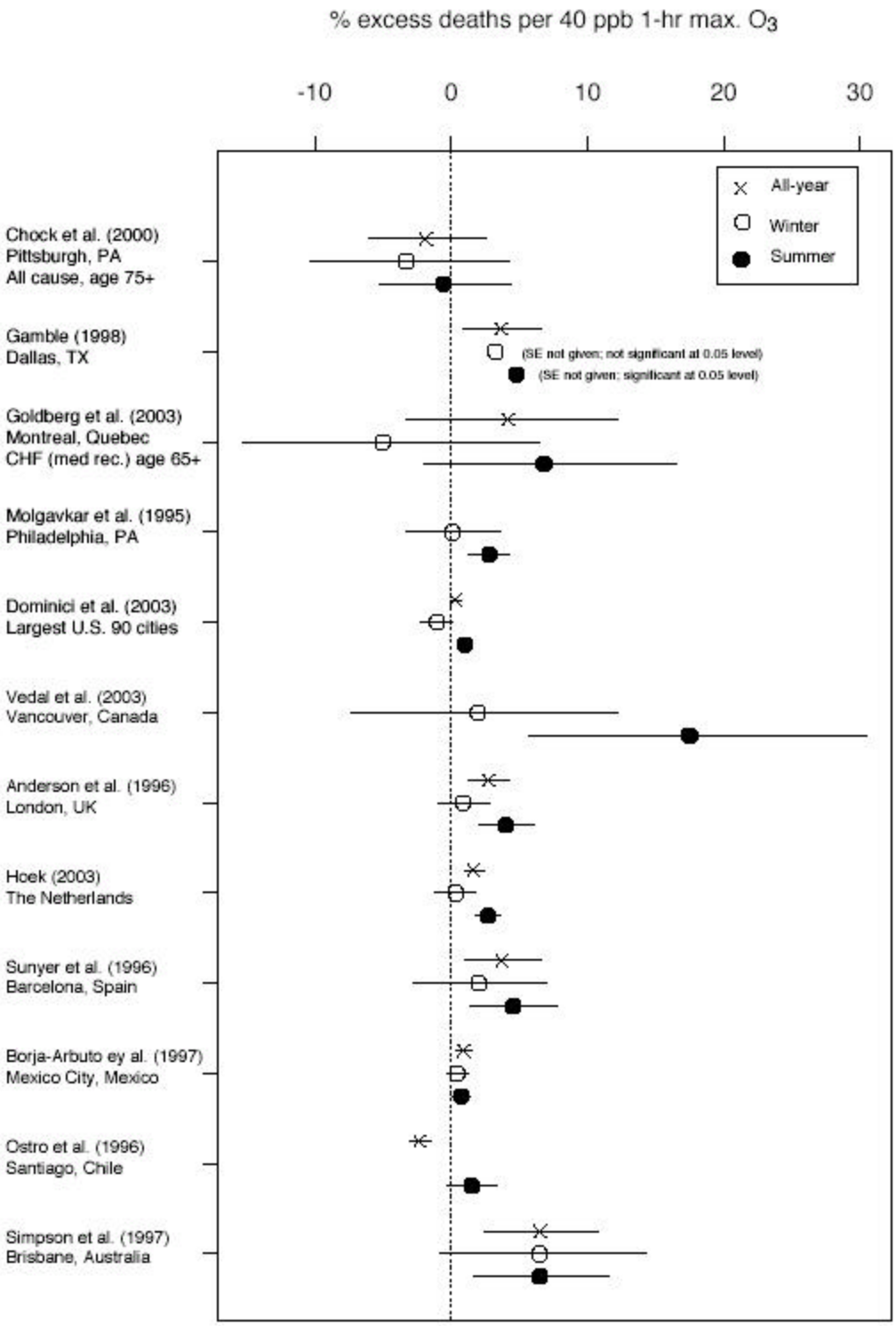

Figure 12-5 Ozone mortality risk estimates <u>with and without PM index</u>. All age nonaccidental mortality, unless otherwise noted.

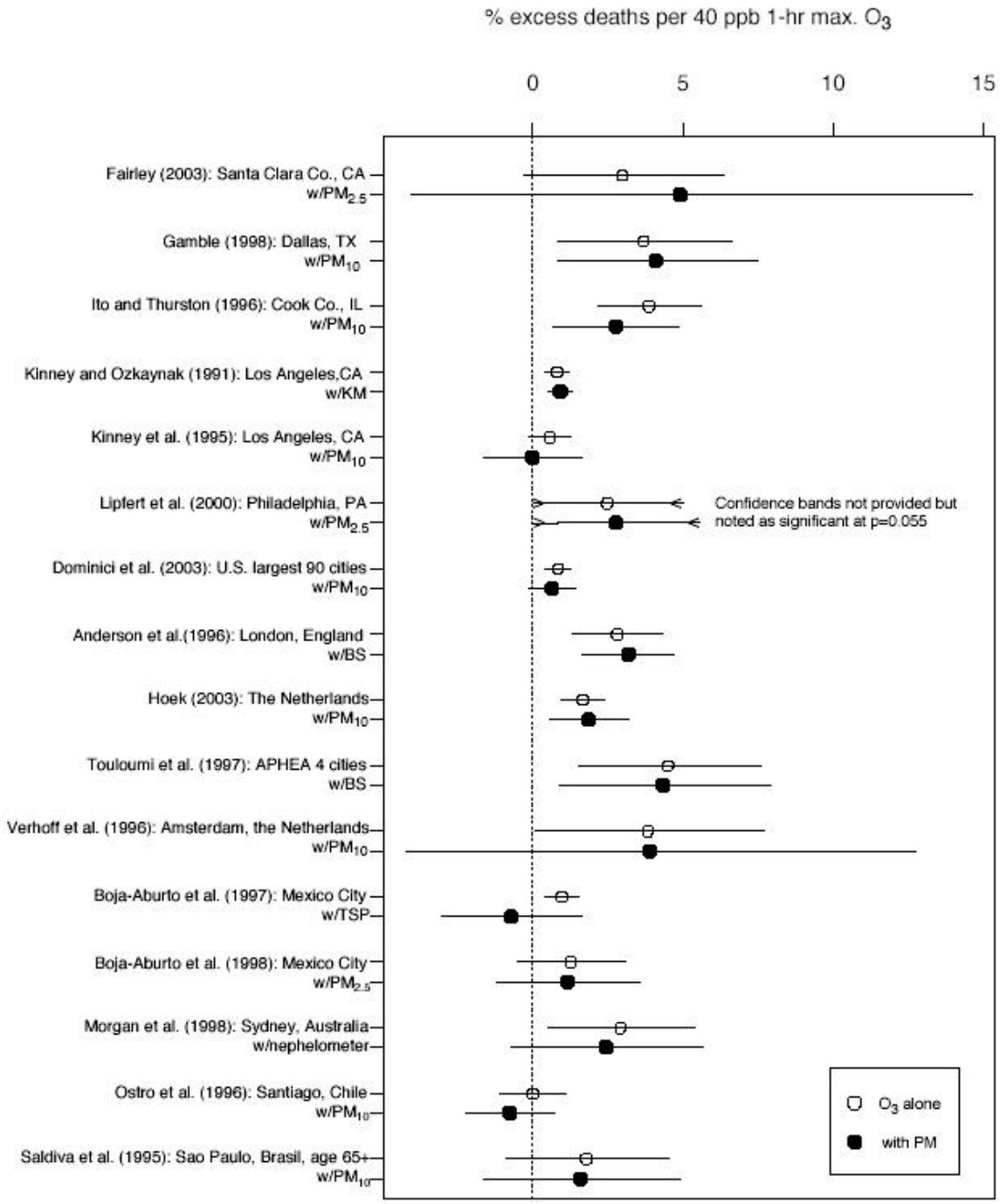

Table 12-6: Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Anderson et al. 1996 Greater London, UK, 1987-1992	All; respiratory; cardiovascular	1-hr max = 20.6; 8-hr avg. =15.5 (mean)	BS, NO_2, SO_2; also 2-poll model	Lag 0	Poisson GLM	8-hr avg.; 2.43% (1.11-3.76) per 26 ppb, all cause, all year; 1-hr max: 2.59% (1.30-3.89) per 31 ppb, all cause, all year
Borja-Aburto et al. 1997b Mexico City, 1990-1992	All (all age); ages < 5yr; ages > 65yr	1-hr max = 155; 24-hr avg. = 54; 8am-6pm avg. = 87; 8-hr moving avg. = 94 (median)	TSP, SO_2, CO; also 2-poll model	Lag 0	Poisson IWFLS	2.4% (1.1-3.9) per 100ppb daily 1-hr max.
Borja-Aburto et al. 1998 Mexico City, 1993-1995	All; age > 65yr; Cardiovascular; respiratory; other.	1-hr max = 163; 24-hr avg. = 44 (median)	PM2.5, NO_2, SO_2; also 2-poll model	Avg. of lag 1 and 2	Poisson GAM but with only smoother	0.63% (-0.27, 1.53) per 10 ppb increase for total; 1.76% (0.07,3.46) for cardiovascular; 0.82%(-0.39, 2.03) for age > 65yr; -0.74% (-3.58, 2.10) for respiratory; 0.30 (-0.85, 1.44) for others
Bremner et al. 1999 London, UK	All cause; respiratory; cardiovascular; and age specific (0-64; 65+; 65-74; 75+) of these causes; all cancer; all others.	1-hr max = 22.6; 8-hr avg. = 17.5 (median)	BS, PM10, NO_2, SO_2, CO; also 2-poll model	Lag 2 for all age group all cause, respiratory, and cardiovascular; lag varied for age-specific and cause	Poisson GLM	8-hr avg.; -0.7% (-2.3-0.9) per 26 ppb, all cause, all year; 3.5% (0.5-6.7) per 26 ppb, CV, all year;
Chock et al. 2000 Pittsburgh, PA	All cause by two age groups (0-74 and 75+ yr)	1-hr max (distribution statistics not reported)	PM10, NO_2, SO_2, CO; also 2-, 5-, and 6-poll model	Lag 0	Poisson GLM	For older age (> 75): beta = -0.459 (t=-0.82) per PPM in 1-pollutant model; even more negative with 2- and 5-pollutant models.

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Dab et al. 1996 Paris, 1987-1992	Respiratory deaths	1-hr max = 23.2; 8-hr avg. = 11.5 µg/m^3 (median)	BS, PM_{13}, NO_2, SO_2, CO	Lag 0	Poisson autoregressive	Beta = 0.000715 (se = 0.000713) for 8-hr avg.; Beta = 0.000390 (se = 0.000545) for 8-hr avg. (NOTE: µg/m^3)
De Leon et al. 2003 New York City, 1985-1994	Circulatory and cancer with and without contributing respiratory	24-hr avg. = 22	PM10, NO_2, SO_2, CO; 2-pollutant models also	Lag 0 or 1	Poisson GAM/GLM	For underlying circulatory, those with contributing respiratory had larger estimates than those without. For underlying cancer, opposite
Diaz et al. 1999 Madrid, Spain, 1990-1992	All cause; respiratory; cardiovascular	24-hr avg. (distribution statistics not reported)	TSP, NO_2, SO_2, CO	Lag 1, 4, 10	autoregressive linear	U-shaped O_3-mortality relationship
Dockery et al. 1992 St. Louis, MO; Eastern Tennessee, 1985-1986	All cause	24-hr avg. = 22.5 in St. Louis, 23.0 in Eastern Tennessee (mean)	PM10, PM2.5, $SO_4^{=}$, H^+, NO_2, SO_2	Lag 1	Poisson, GEE	Beta= 0.00029 (se = 0.00076) in St. Louis; beta = -0.00065 (se = 0.00178) in Eastern Tennessee
Fairley 1999; Fairley D 2003 Santa Clara County, CA	All cause; respiratory; cardiovascular	8-hr avg. = 29; 24-hr avg. = 16; O_3 ppb-hrs greater than 60 ppb (mean)	PM10, PM2.5, PM10-2.5, COH, $SO_4^{=}$, NO_3^-, NO_2, SO_2; 2-pollutant models	Lag 0	GAM (re-analyzed with stringent convergence criteria), GLM	3.3% (-0.3-7.0) per 33 ppb 8-hr max
Gamble 1998 Dallas, TX, 1990-1994	All cause; respiratory; cardiovascular; cancer; other	24-hr avg. = 22 (mean)	PM10, NO_2, SO_2, CO; 2-pollutant models also	Avg. of lag 1 and 2	Poisson GLM	2.7% (0.6-4.8) per 14.7ppb for total mortality. (larger for summer)

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Garcia-Aymerich et al. 2000 Barcelona, Spain	All cause; respiratory; CVD; general population and patients with COPD	1-hr max (distribution not reported)	BS, NO_2, SO_2,	Lag 5 for general population; lag 3 for COPD cohort	Poisson GLM	2.4% (0.6, 4.2) per 50µg/m^3 4.0% (-4.7, 13.4)
Goldberg et al. 2003 Montreal, Quebec, 1984-1993	Underlying congestive heart failure (CHF) vs. those classified as having CHF from medical records	24-hr avg. = 15 (mean)	TSP, COH, PM10, sulfate, SO_2, NO_2, CO	Avg. of lag 0, 1, and 2.	Poisson GLM	Underlying CHF: 4.5% (-5.6, 15.8) per 11 ppb avg. of lag 0-2; those classified as having CHF from medical records: 2.3% (-1.8, 6.6)
Gouveia and Fletcher 2000b Sao Paulo, Brazil	All cause; age under 5 (all cause, respiratory, pneumonia); age 65+ (all cause, respiratory, CVD)	1-hr max = 67.9 (mean)	PM10, NO_2, SO_2, CO	Lag 0 for	Poisson	0.8% (-0.11-2.7) per 106ppb for all cause and ages; 2.3% (0-4.6) per 106ppb for all cause, age 65+.
Hoek et al., 2000; Hoek G 2003 the Netherlands (entire country, 4 urban areas), 1986-1994	All cause; CVD, COPD, pneumonia	8-hr avg. = 24 (47 µg/m^3) (median)	PM10, BS, $SO_4^=$, NO_3^-, NO_2, SO_2, CO; also 2-poll models	Lag 1 or avg, of lag 0 through 6	Poisson GAM re-analyzed; GLM	4.0% (2.5-5.4) per 77 ppb at lag 1 day; 4.2% (2.6-5.7) per 61 ppb avg. of 0-6 days for all cause.

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Hoek et al. 2001; Hoek G 2003 the Netherlands, 1986-1994	Total CVD, MI, arrhythmia (ARH), heart failure (HF), cerebrovascular disease (CRB), thrombosis (TRM).	Same as above	PM10, NO_2, SO_2, CO	Lag 1	Poisson GAM re-analyzed; GLM	CVD: 6.2% (3.3, 9.2); MI: 4.3%(0.1, 8.6); ARH: 11.4% (-1.2, 25.5); HF: 10.2% (1.2, 19.9); CRB: 9.1% (2.9, 15.7); TRM: 16.6% (2.8, 32.2)
Ito and Thurston 1996 Cook County, IL	All cause, circulatory, respiratory, cancer; race/gender sub-categories	1-hr max. = 38 (mean)	PM10, NO_2, SO_2, CO	Avg, of 0 and 1 lag days	Poisson GLM	10% (6.0, 15.0) per 100ppb (avg. of 0 and 1 days).
Kinney and Ozkaynak 1991 Los Angeles, 1970-1979	All cause, circulatory, respiratory.	Total oxidants (O_x) 1-hr max. = 75 ppb (mean)	KM, B_{ext}, NO_2, SO_2, CO; multiple poll. model	Lag 1	OLS on high-pass filtered variables.	beta=0.03 (se=0.009)
Kinney et al. 1995 Los Angeles	All cause	1-hr max. = 70 (mean)	PM10, NO_2, SO_2, CO; also 2-poll, models	Lag 1	linear, log-linear, and Poisson	2.0% (0.0, 5.0) per 143ppb.
Klemm and Mason 2000 Atlanta, GA	All cause; age 65+; separate counties.	8-hr avg. = 46 (mean)	PM10, PM2.5, PM10-2.5, EC, OC, etc.; NO_2, SO_2, CO	Lag 0	Poisson GLM	beta=-0.00041 (se=0.00110)
Lee et al. 1999 Seoul and Ulsan, Korea, 1991-1995	All cause	1-hr max. = 32 in Seoul; 26 in Ulsan (mean)	TSP, SO_2	Lag 0	Poisson with GEE	Seoul: 1.5% (0.5, 2.5) per 50 ppb; Ulsan: 2.0% (-11.1, 17.0) per 50 ppb.
Lee and Schwartz 1999 Seoul, Korea, 1991-1995	All cause	1-hr max. = 32 in Seoul; 26 in Ulsan (mean)	TSP, SO_2	Lag 0	conditional logistic (case crossover)	1.5% (-1.2, 4.2) per 50ppbusing plus/minus 1 wk controls; 2.3% (-0.1, 4.8) per 50ppb using plus/minus 2 wks controls

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Lipfert et al. 2000 Philadelphia, PA, 1991-1995	All cause; CVD, respiratory, age 65+, age <65; various sub-regional boundaries	1-hr max. = 45; 24-hr avg. = 23 (mean)	PM10, PM2.5, PM10-2.5, $SO_4^{=}$, NO_3^{-}, and other PM indices; NO_2, SO_2, CO;	Avg. of lag 0 and 1	linear, with Shumway filters, SPSS	2.8% per 45 ppb 1-hr max. (avg. of 0 and 1 day), significant at $p < 0.055$, but no SE or confidence bands provided.
Lippmann et al, 2000; Ito K 2003 Detroit, MI, 1992-1994	All cause; CVD, respiratory	24-hr avg. = 25 (mean)	PM10, PM2.5, PM10-2.5, $SO_4^{=}$, H^{+}; NO_2, SO_2, CO; also 2-poll. model	Lag 0	Poisson, GAM, GLM	2.6% (-2.4, 7.8) per 28 ppb for GLM
Moolgavkar et al. 1995 Philadelphia, 1973-1988	All causes	1-hr max. = 26 in spring; 36 in summer; 16 in fall; 12 in winter (mean)	TSP, SO_2		Poisson, also GEE and nonparametric bootstrap methods	Spring: 2.0% (-6.7-11.5); Summer: 14.9% (6.8-23.6); Fall: -4.5% (-13.9-5.9); Winter: 0.4% (-15.6%-19.4) per 100ppb daily 24-hr avg.
Morgan et al. 1998 Sydney, Australia, 1989-1993	All cause, cardiovascular, respiratory.	1-hr max. = 24 (mean)	B_{scat}, NO_2	Lag 0	Poisson with GEE in SAS	All cause: 2.04% (0.37, 3.73) per 28ppb
Ostro 1995 2 Southern California counties	All cause, cardiovascular, respiratory, but O_3 results were reported only for all cause	1-hr max. = 14 (mean)	PM2.5	Lag 0	Autoregressive linear; Poisson, other in sensitivity	2.0% (0.0, 5.0) per 100ppb. Summer quarters only.

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Ostro et al. 1996 Santiago, Chile, 1989-1991	All cause, cardiovascular, respiratory	1-hr max. = 53 (mean)	PM10, NO_2, SO_2	Lag 1	OLS, several other methods	All season: -3.0% (-4.0, -2.0) per 53ppb; summer: 4.0% (0, 9.0) per 100ppb
Pereira et al. 1998 Sao Paulo, Brazil, 1991-1992	Intrauterine mortality	"major-hour mean" = 68 (mean)	PM10, NO_2, SO_2, CO	Lag 0	Poisson, linear with M-estimation	Beta = 0.0000 (se=0.0004)
Peters et al. 2000 northeast Bavaria, Germany and the coal basin in the Czech Republic, 1982-1994	All cause for the Czech Republic; all cause and cardiovascular for Bavaria	24-hr avg. = 40.3 in the Czech Republic; 38 in Bavaria (mean)	TSP, PM10, NO_2, SO_2, CO	Lag 2 in the Czech Republic; lag 0 in Bavaria	Poisson GLM	7.8% (-1.8, 18.4) per 51 ppb for all cause in the Czech Republic; 8.2% (0.4, 16.7) for all cause in Bavaria.
Ponka et al. 1998 Helsinki, Finland	All cause and CVD; age < 65 yr, age 65+	24-hr avg. = 18 (median)	TSP, PM10, NO_2, SO_2	Lag highly variable	Poisson, GLM	Estimate for all cause not reported.
Prescott et al., 1998 Edinburgh, 1992-1995	All cause, CVD, and respiratory by age (< 65 and 65 +)	24-hr avg. = 15 (mean)	BS, PM10, NO_2, SO_2, CO; also 2-poll. model	Lag 0	Poisson GLM	All cause all age: -4.2% (-8.1, -0.1) per 10 ppb.
Roemer and van Wijnen 2001 Amsterdam, 1987-1998	All cause	8-hr avg. = 41 in "background area"; 32 in "traffic area" (median)	BS, PM10, NO_2, SO_2, CO; also 2-poll. model	Lag 1, 2, and avg. of lag 0 through 6	Poisson GAM but only one smoother in the model	Total population using background sites: -0.3% (-4.1, 3.7) per 51 ppb; total population using traffic sites: 0.2% (-3.6, 4.2).

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Saez et al. 1999 Barcelona, Spain, 1986-1989	Asthma mortality	1-hr max., distribution not reported.	BS, NO_2, SO_2,	Avg. of lags 0 through 2	Poisson with GEE	beta = 0.021 (se=0.011) for 0-2 day average, for asthma mortality.
Saldiva et al. 1994 Sao Paulo, Brazil, 1990-1991	Respiratory mortality in children age under 5	24-hr avg. = 12	PM10, NO_2, SO_2, CO; all in the model	Avg. of lag 0, 1, and 2	OLS of transformed data	Beta = 0.01048 (se=0.02481)
Saldiva et al. 1995 Sao Paulo, Brazil, 1990-1991	All cause for age 65+	1-hr max. = 38; 24-hr avg. = 13 (mean)	PM10, NO_2, SO_2, CO; also 2-poll. model	Avg. of lag 0 and 1	OLS/autoregressive, Poisson/GEE	Beta = 0.0093 (se=0.0813) for 24-hr O_3; beta = 0.0280 (se=0.0213) for 1-hr max. O_3
Samet et al., 2000; Dominici et al. 2003 90 U.S. cities, 1987-1994	All cause, cardiorespiratory	24-hr avg. = ranged from 12 in Des Moines to 36 in San Bernardino (mean)	PM10, NO_2, SO_2, CO; also 2-poll. model	Lag 0	Poisson GAM (re-analyzed with stringent convergence criteria); GLM	All seasons: ~ 0.2%; Summer: ~ 0.5%; Winter: ~ -0.5% per 10ppb.
Sartor et al. 1995 Heat wave in Belgium, 1994	All cause in two age groups: 0-64 and 65+	24-hr avg. = 37 during heat wave; 27 and 20 before and after heat wave	TSP, NO, NO_2, SO_2	Lag 0 and 1	Data stratification, log-linear regression; correlation	No individual coefficient for O_3 alone; interaction with temperature suggested.
Simpson et al. 1997 Brisbane, Australia, 1987-1993	All cause, CVD, and respiratory in all ages and 65+	1-hr max. = 24; 8-hr avg. = 18 (mean)	PM10, nephelometry, NO_2, SO_2, CO	Lags 0	(method follows APHEA approach) Poisson with GEE	Daily 1-hr max: 1.6% (0.6, 2.6) per 10ppb; 8-hr average: 2.4% (0.8, 4.0) per 10ppb.

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Sunyer et al. 1996 Barcelona, 1985-1991	All cause (all age and 70+), CVD, respiratory	1-hr max. = 28 in winter 44 in summer	BS, SO_2, NO_2	Lag 0	(APHEA) autoregressive Poisson	4.8% (1.2, 8.6) per 51 ppb for all year; 2.6% (-3.5, 9.1) in winter; 5.8% (1.7, 10.1) in summer
Sunyer and Basagana 2001 Barcelona, 1990-1995	Mortality in a cohort of patients with COPD	1-hr max. mean not reported; IQR = 11	PM10, NO_2, CO	Lag 0	Conditional logistic (case-crossover)	-2.1% (-8.1-6.5) per 11 ppb
Sunyer et al. 1996 Barcelona, 1985-1995	Mortality in a cohort of patients with severe asthma	1-hr max. = 35; 8-hr avg. = 28 (median)	PM10, BS, SO_2, NO_2, CO, pollen	Avg. of lag 0, 1, and 2	Conditional logistic (case-crossover)	Patients with only 1 admission with asthma: 9.6% (-18.0, 46.6); patients with more than 1 admissions with asthma: 68.8% (-2.2, 264.3) per 24 ppb 1-hr max. O_3
Tellez-Rojo et al. 2000 Mexico City,1994	Respiratory mortality, COPD mortality; within medical unit and out of medical unit	1-hr max. = 24	PM10, NO_2, SO_2	Avg. of lag 1 through 5 days	Poisson, IWFLS	Respiratory mortality (outside medical unit): 14.0% (4.1-24.9) per 40ppb 5-day average; COPD mortality (outside medical unit): 15.6% (4.0-28.4) per 40 ppb 5-day average.
Touloumi et al. 1997 APHEA1 (subset: four cities: London, Athens, Barcelona, and Paris)	All cause	1-hr max. = 48 in Athens; 37 in Barcelona; 21 in London; 24 in Paris (mean)	BS, NO_2; 2-poll. model also considered.	Single-day best lag chosen in each city; average of lag 2 through 5	Poisson autoregressive	Combined across 4 cities: 2.9% (1.0, 4.9) per 25.5 ppb in random effects; 2.3% (1.4, 3.3) in fixed effects.

Table 12-6 (cont.): Ozone Effects on Acute Mortality

Reference, location, years.	Outcome measure	Mean O_3 levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Vedal et al. 2003 Vancouver, BC, 1994-1996	All cause, CVD, respiratory	1-hr max. = 27	PM10, NO_2, SO_2, CO	Lag 0	GAM, stringent with new SE methods	Summer: 4.2% (1.4, 7.0) per 10.2 ppb; Winter: 0.5% (-1.9, 3.0) per 10.2 ppb.
Verhoeff et al., 1996 Amsterdam	All cause, age 65+	1-hr max. = 43	PM10, NO_2, SO_2, CO	Lag 2	Poisson	4.9% (0.1-10.0) per 51 ppb
Zmirou et al. 1996 Lyon, France, 1985-199	All cause, CVD, respiratory, digestive	1-hr max. = 15; 24-hr avg. = 10 (mean)	PM_{13}, SO_2, NO_2	Lag 0 for all cause; lag 1 for CVD; lag 2 for respiratory	Poisson GLM	All cause: 3.0% (-5.0, 12.0) per 26 ppb.
Zmirou et al. 1998 APHEA (four cities: London, Lyon, Barcelona, and Paris)	CVD, respiratory	8-hr avg. (9am-5pm), for cold/warm seasons= 11/21 in London; 6/22 in Paris; 5/6 in Lyon; 27/46 in Barcelona (mean)	BS, SO_2, NO_2	Each city chose best 1-day lag fits	Poisson GLM	Combined CVD estimate: 2% (1, 3) per 25.5 ppb 1-hr max.; 2% (0, 3)

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