Health Canada | Santé Canada

Environment Canada | Environnement Canada

NATIONAL AMBIENT AIR QUALITY OBJECTIVES FOR GROUND-LEVEL OZONE

SCIENCE ASSESSMENT DOCUMENT

A Report by
the Federal-Provincial Working Group
on Air Quality Objectives
and Guidelines

July, 1999

This document has been divided into a series of files for easier downloading from our web site.

Part 5 of 7

Canada

9 EFFECTS ON BIRDS AND MAMMALS

Since the NO_x/VOC Science Assessment Program did not address the issue of ozone effects on wildlife or domesticated birds and mammals, an independent review of the literature, up to the end of 1996, was undertaken. This chapter reviews the effects of ozone on birds and mammals and clearly reflects the paucity of literature available. The available information does indicate though that effects are likely not negligible under episodic (i.e. high concentrations) and, perhaps, lower ambient conditions.

Information contained in journal articles and other sources of primary literature that were considered in this report were identified through the following computerized databases:

Medline

Agricola

CAB Abstracts

Life Sciences

RTECS

HSDB

PolTox

In addition, toxicological abstracts from the past ten years and biological abstracts from the last three years were examined. Current Contents, which lists papers published, was also reviewed.

9.1 DOMESTIC BIRDS

For birds, the only significant exposure route to ozone is inhalation, and exposure can be represented by the concentration of ozone in inhaled air. Information on the effects associated with exposure via inhalation is summarized below. Indirect effects of ozone on birds, for example, due to adverse effects on plants upon which birds may depend, were not considered in this review.

9.1.1 Toxicological Assessment

Ozone's biological effect is attributed to its ability to cause oxidation or peroxidation of biomolecules directly and/or via free radical reactions. A sequence of events may include lipid peroxidation and loss of functional groups of enzymes, alteration of membrane permeability, and cell injury or death (Mustafa, 1990).

There are many distinct differences between the bird's lung-air sac respiratory system and the mammalian bronchoalveolar lung. The respiratory apparatus of birds has evolved from the primitive sac-like alveolar lungs of reptiles in response to the large metabolic demands of flight. This evolutionary process has produced a respiratory system with substantial physiological differences relative to other vertebrates. Among the most important of these differences are that birds have a higher mass-specific minute ventilation, higher mass specific effective ventilation of gas-exchange tissues, cross-current and counter-current gas-exchange mechanisms and a gas diffusion barrier half the thickness of that of mammals (Brown et al., 1997). While these differences may predispose birds to greater sensitivity to inhaled toxicants, there is insufficient information to make any predictions concerning relative sensitivities. There appears to be a complex relationship between a species' respiratory physiology, its pathophysiologic response to a toxic gas and other physiologic factors. For instance, Japanese quail appear to respond to ozone exposure in a different way than mammals (Rombout et al., 1991). No signs of repair in the air capillary epithelium of Japanese quail were observed after seven days of ozone exposure, indicating that the quail lacks the morphological and biochemical repair ability observed in mammals (Rombout et al., 1991). The development of tolerance to ozone has been reported in mammals. Pre-exposure to ozone did not confer tolerance in chicks (Quilligan et al., 1958).

Three studies which examined the effects of ozone exposure to birds were identified. These studies are described below, and are summarized in Table 9.1.

Rombout et al. (1991) exposed 8-week old male Japanese quail (*Coturnix coturnix japonica*) to 0, 0.3, 1.0 and 3.0 mg/m^3 (0, 0.15, 0.50 and 1.50 ppm respectively) of ozone in closed inhalation chambers continuously for seven days. Pulmonary effects were determined by light and electron microscopy, as well as by biochemistry. Focal areas of haemorrhage were observed in the birds exposed to 0.5 ppm ozone. Loss of cilia in the trachea and bronchi, an inflammatory response, and necrosis of air capillary epithelial cells were also seen after exposure to 0.5 ppm. Following exposure to 1.50 ppm ozone, many atria of tertiary bronchi were completely obstructed by extensive haemorrhage, and metaplasia of atrial wall cells and hypertrophy of smooth muscle cells were also observed. The cilia in the airways disappeared almost completely in the1.50 ppm group. No histological differences were seen in the heart, liver, kidneys, spleen, and bursa of Fabricius between the animals of the control group and the group exposed to 1.50 ppm ozone. Lung biochemistry data revealed that in the 1.50 ppm group, lactate dehydrogenase, glucose-6-phosphate dehydrogenase and glutathione reductase activities were significantly increased. No effects on lung antioxidant enzymes were observed in the 0.15 and 0.50 ppm exposure groups.

Table 9.1 Summary of Scientific Studies and Results for Ozone Effects on Domestic Birds

Study author(s), year, journal	Species, life stage, age	Ozone concentration and exposure time	Protocol	Endpoint measured	Significant ($p < 0.05$) effect? Y/N
Rombout et al., (1991)	Japanese Quail (*Coturnix coturnix japonica*) 8-week old males	0, 0.15, 0.50 and 1.50 ppm for 7 days	Continuous exposure in closed chambers	Morphology and biochemical analyses for protein content, G6PDH, GSH-Px, GSH-Red1	N at 0.15 ppm Y at 0.50 ppm Y at 1.50 ppm
Bartov et al., (1981)	Crossbred New Hampshire x White Leghorn male chicks, ages ranged from 1-11 days at start of experiment	0.3 or 0.7 ppm	Continuous exposure in a closed chamber 7-21 days; fed various diets	Growth, nutritional encephalopathy, fatty acid composition of the cerebellum and lungs	No consistent effect on severity of nutritional encephalopathy. N for liver α-tocopherol content and fatty acid composition of the cerebellum. Y for increased palmitic acid in total lung lipids at 0.7 ppm, and for lung congestion in all ozone treated chicks
Quilligan et al., (1958)	Young chicks (species not specified)	1-4 ppm for 8 days (mean = 1.96 $\pm$ 0.19 ppm) 12-13 ppm for 3.5 to 5.5 hour intervals	Continuous exposure Limited acute exposure	Death Death	Y: mean survival time = 1.2 days Y

[1]6PDH = glucose-6-phosphate dehydrogenase; GSH-Px = glutathione peroxidase; GSH-Red = glutathione reductase

The effect of ozone on the development of nutritional encephalopathy, one of the vitamin E-deficiency symptoms in chicks, was evaluated by Bartov et al., (1981). Young crossbred New Hampshire x White Leghorn male chicks, receiving diets low in vitamin E and containing a source of linoleic acid, were exposed at various ages to 0, 0.3 and 0.7 ppm ozone for various time periods. No consistent effect was established on the severity of the nutritional encephalopathy. Ozone had no clear effect on the liver α-tocopherol content of chicks fed vitamin E-supplemented diets, nor did it affect the fatty acid composition of the cerebellum. However, in all cases, ozone increased the proportion of palmitic acid in total lung lipids. On macroscopic examination, all ozone-treated chicks showed congested lungs of a darkened colour. Microscopic examination showed haemorrhage within the bronchi and alveoli. In addition, abundant leukocytes were seen. Ozone exposure to 0.3 ppm and 0.7 ppm improved the weight gain of young chicks, provided that exposure was started on the day of hatching. When exposure was begun one week or later after hatching, ozone either inhibited weight gain or had no effect. The authors speculate that the growth promotion of ozone-exposed chicks could be due either to a disinfecting effect of ozone or, since accelerated growth is associated with a higher rate of protein degradation and turnover, ozone-induced enhanced protein turnover and consequently increased growth rates (Bartov et al., 1981).

In an early study, (Quilligan et al., 1958), newly hatched chicks died after three days of continuous exposure to 1-4 ppm ozone (mean ozone concentration = 1.96 ± 0.19 ppm, mean survival time = 1.2 days). The pathological findings were principally those of edema and haemorrhage of the lungs. In an additional experiment in which ozone concentrations were kept constant and the exposure time varied, an LD50 of 53.5 ppm-hours was calculated, which corresponds to 8.9 ppm for a six-hour exposure. In a final series of experiments, several conditions of pre-exposure and challenge were studied. No evidence of tolerance on re-exposure of chicks to ozone could be demonstrated (Quilligan et al., 1958).

Table 9.2 outlines the effects reported and scores them according to the frequency and consistency of occurrence in the studies reviewed.

Table 9.2 Categories of Effects and Scores

Effect	Score[1]	Comment
Reduced growth and/or weight gain	-	One study found growth enhanced following exposure to ozone at a specific time of growth; exposure beyond that age had no effect or a negative effect.
Reduced reproductive vitality	-	Effect not measured in the studies reviewed.
Increased susceptibility to pathogens	+	Increased presence of leukocytes found in one study, significance not clear.
Increased susceptibility to physical stressors	+++	Hemorrhage found in the lungs of test animals in all studies.
Mortality	++	One study examined mortality as an endpoint. Effect found at various concentration/duration combinations.

[1]scores range from - (effect not found/not reported) to +++ (effect consistently found over various exposure scenarios)

Based on this analysis, increased susceptibility to physical stressors (in this case, lung hemorrhage) is the most appropriate endpoint for which adequate data is available to identify an effect level. Although lung hemorrhage is consistently found, little information on other potential relevant endpoints is available. This adds uncertainty to the selection of the endpoint. Other sources of uncertainty include the lack of information documenting ozone's effects on wild birds; the fact that all of the captive domestic birds in these studies had no flight exercise, therefore it is difficult to extrapolate these results to wild avifauna; and the lack of information on the effectiveness of the avian respiratory tract repair mechanism. Lung hemorrhage still appears to be the most appropriate dose-response metric, however, as it was consistently found and was the most sensitive endpoint from the reviewed studies.

9.1.2 Summary

Three studies were reviewed that studied the effects of ozone on birds. These included one which examined the acute lethal effects of ozone to chicks, a second which examined the effects on growth, nutritional encephalopathy and fatty acid composition of chicks following continuous exposure to 0.3 or 0.7 ppm ozone for 7-21 days, and another which examined the morphology and biochemical changes in Japanese quail exposed to 0.15, 0.50 and 1.50 ppm ozone for seven days.

Lung haemorrhage was noted in all of the studies. Lung haemorrhage was seen in chicks exposed to 0.3 ppm and in Japanese quail exposed to 0.50 ppm, but not when exposed to 0.15 ppm. There are insufficient data to identify the potential effects to birds due to chronic exposure to ozone. In addition, no data were identified which examined the effects of ozone on wild birds. The Quilligan et al. (1958) study examined the lethal effects of acute exposure to ozone, which is not an appropriate endpoint for ambient exposures, which are typically chronic and low level.

The major differences between avian and mammalian lung systems make it difficult to extrapolate mammalian findings to birds. Avian lungs may respond differently, both qualitatively and quantitatively, to oxidant gas exposure than lungs from mammalian species for several reasons. The gas exchange area per unit body weight is small in birds compared to mammals, since birds possess a higher surface area to body weight ratio. Furthermore, birds have a higher diffusing capacity than mammals that accommodates the high metabolic rate during flight, resulting in a higher demand for oxygen. The consequent high respiration rate leads to an increased ozone flux to the tissue when ozone polluted air is inhaled. Unlike some experimental mammals, birds do not appear to develop tolerance to ozone exposure and some birds, such as Japanese quail, lack the morphological and biochemical repair ability seen in mammals. The unknown effectiveness of avian repair mechanisms in the respiratory tract constitutes yet another highly uncertain element in the qualitative risk assessment of ozone exposure of birds.

In conclusion, to protect domestic birds from short-term adverse effects, ambient ozone concentrations should not exceed 0.15 ppm. The potential effects to birds due to chronic exposure to ozone are unknown. The damage induced by ozone in the gas exchange area of

avian lungs, together with the apparent lack of adequate morphological and biochemical repair capacity, gives rise to concern regarding the potential health effects on this order of animals.

9.2 DOMESTIC MAMMALS

As for birds, the only significant exposure route for mammals is inhalation, and exposure can therefore be defined as the concentration of ozone in inhaled air. Information on the direct effects associated with exposure via inhalation is summarized in the following sections. Indirect routes of exposure, for instance adverse effects on plants upon which mammals may depend, were not considered in this review.

9.2.1 Toxicological Assessment

Ozone's biological effect is attributed to its ability to cause oxidation or peroxidation of biomolecules directly and/or via free radical reactions. A sequence of events may include lipid peroxidation and loss of functional groups of enzymes, alteration of membrane permeability, and cell injury or death. Acute exposure to ozone causes lung injury involving the ciliated cells in the airways and the type 1 epithelial cells in the alveolar region. The effects are particularly localized at the junction of the terminal bronchioles and alveolar ducts (Mustafa, 1990). In a typical short-term exposure, the lung tissue response is biphasic: an initial injury-phase characterized by cell damage and loss of enzyme activities, followed by a repair-phase associated with increased metabolic activities, which coincide with a proliferation of metabolically active cells, for example the alveolar type 2 cells and the bronchiolar Clara cells (Mustafa, 1990).

Eleven studies which examined the effects of ozone on domestic mammals were identified (Table 9.3). These could be subdivided into three categories: one study examined the adverse developmental effects of exposure to ozone, three examined the effects on red blood cells (RBC) and seven examined inflammatory responses. No studies were found which examined the effects of ozone on wild mammals. Most of the literature on domestic mammals examined the effects of ozone on sheep. This is most likely due to the potential use of this animal model as a surrogate for human studies. For instance, the Dorset sheep has an erythrocyte G-6-PD activity that is very low, being comparable to human A-variants with G-6-PD deficiency (Moore et al., 1981) and sheep generally have airway responses to noxious stimuli which tend to mimic human responses arising from allergic challenge or irritative stimuli (Allgera et al., 1991). In addition, the adult body weight of the ewe is 40-60 kg, comparable to humans, thus making techniques for comparing results to humans more easily applied. One study was found regarding effects on horses, one regarding *in vitro* effects on the erythrocytes of cows and pigs and one study which examined the effects on ferret tracheal glands.

Developmental Effects:

Exposure to ozone (1 ppm for 4 hours/day for 5 days) during the first week after birth retarded the normal development of the tracheal epithelium in lambs. Mucus and ciliated cell

percentages remained at new-born levels in treated lambs, indicating that ozone exposure during the first week of life retards the normal development of the mucociliary system in sheep, which may lead to prolonged impairment of lung mucociliary clearance and thus increase the incidence of chronic obstructive airway disease in adulthood (Mariassy et al., 1990). Mucociliary clearance removes particles and cellular debris from the conducting airways. Impairment of this system could prolong the retention of unwanted materials (e.g., inhaled particles) in the lungs, allowing them to exert their toxicity or physical damage for longer periods of time.

Effects on Red Blood Cells

Although a statistically significant biochemical change (i.e., a decrease in glutathione levels) was noted only at the 0.5 ppm level, ozone was found capable of producing systemic oxidant stress on the red blood cells of female sheep at concentrations as low as 0.12 ppm (Moore et al., 1984).

Ozone exposure may also adversely effect red blood cell survival. Female Dorset sheep exposed to 0.25 ppm ozone showed a significant decrease in hematocrit levels (Moore et al., 1981). This decrease was not noted in the 0.50 or 0.70 ppm exposure level. The authors hypothesize that the production of excess mucus at higher ozone exposure levels may act to decrease the effective concentration of ozone reaching the lungs, passing through the air/blood barrier and consequently stressing the red cells. The 0.25 ppm ozone concentration may have minimally elicited this mucus response such that higher concentrations of ozone actually contacted the red cells (Moore et al., 1981).

An *in vitro* investigation of bovine and porcine erythrocytes exposed to ozone in the presence and absence of uric acid, a free radical scavenger, found a reduction in hemolysis and methemoglobin formation in the presence of uric acid (Meadows and Smith, 1987).

Inflammatory Response

Exposure of female sheep (aged 1-2 years) to 0.5 ppm ozone for two hours resulted in an increased number of mast cells and lymphocytes in lavage fluid when compared to controls (Sielczak et al., 1983). The increase in the number of luminal mast cells and lymphocytes following ozone exposure signals an enhanced inflammatory response in the airways. These changes could contribute to ozone-induced non-specific airway hyper-responsiveness and susceptibility to allergic IgE-mediated airway reactions (Sielczak et al., 1983).

Both short-term clearance (mucociliary) and long-term clearance (alveolar) were depressed by ozone in a dose-dependent fashion in female sheep following exposure to 1.0 ppm for 2 hours and for 5 hours per day for 4 days, respectively (Allgera et al., 1991). Ozone caused a dose-dependent decrease in tracheal mucus velocity (TMV), a marker of mucociliary function. Ciliary function, measured by Ciliary beat frequency *in vitro*, was not found to be defective, suggesting that ozone-induced mucociliary dysfunction resulted from alterations in mucus secretion (Allgera

et al., 1981). Pre-treatment with N-acetylcysteine, a mucolytic substance with antioxidant activity, significantly protected the sheep from TMV decreases.

Abraham et al., (1984) investigated the relationship between airway responsiveness and the permeability of histamine through the airways in female sheep after exposure to either 0.5 or 1.0 ppm ozone for two hours. Increased airway responsiveness and airway permeability were observed in the sheep exposed to 0.5 ppm. Approximately half of the sheep exposed to 1.0 ppm ozone showed increases in airway responsiveness and airway permeability; the other half showed decreases in these measures. The authors concluded that since ozone-induced directional changes in airway responsiveness paralleled the directional changes in airway permeability in both the positive and negative directions, that changes in airway responsiveness to inhaled histamine following exposure to ozone may be related to concomitant changes in airway permeability to this agent.

The acute exposure of female sheep to 3-4 ppm ozone for 3 hours was associated with dose-related increases in bronchial artery blood flow (Q_{br}), an acute inflammatory response (Schelegle, et al., 1990). Another study confirmed that exposure to 3.5 ppm ozone for 3 hours induced an increase in systemic blood flow to the lung and also found that exposure to 3.5 ppm ozone plus the corticosteroid methylprednisone for 3 hours completely blocked this vasodilatory response (Gunther et al., 1992).

Horses exposed to 0.5 ppm ozone for 12 hours showed a significant increase in reduced glutathione (GSH), glutathione disulfide (GSSG), the glutathione redox ratio (GRR = GSSG/[GSH + GSSG]) and total iron concentrations in bronchoalveolar fluid (Mills et al., 1996). Reduced GSH is the primary mechanism for the removal of hydrogen peroxide in the lung. GSSG is rapidly converted back to GSH. Oxidant injury or pulmonary disease will markedly increase GSSG concentration and is one of the most sensitive assays of oxidant injury. The authors conclude that oxidant injury in the lung will induce changes in the GSH status and iron homeostasis that could affect pathogenesis of respiratory tract diseases.

Ferrets exposed to 1 ppm ozone 24-hours per day for 3 days exhibited increased basal secretion of glycoconjugates. (McBride et al., 1991). The basal secretion by tracheal glands was not significantly different from controls following exposure to 1 ppm ozone for 7 days (McBride et al., 1991). To assess the oxidant stress on tracheal gland responsiveness to cholinergic stimulation, EC_{50} values, slopes and maximal responses were determined from individual dose-response curves to carbachol. The mean EC_{50} values were significantly lower in the ozone-exposed groups compared to controls, slopes of the dose-response curves did not change and maximal secretory responses were elevated, but not significantly different compared to controls (McBride et al., 1991).

Table 9.3 Summary of Scientific Studies and Results for Ozone Effects on Mammals

Study author(s), year, journal	Species, life stage, age	Ozone concentration, and exposure time	Protocol	Endpoint measured	Significant (p = 0.05) effect? Y/N
Mariassy et al. (1990)	Lambs	1.0 ppm 4 hours/day for 5 days during the first week of life	For TMV[1] study, measurements were obtained at birth and after exposure. TMV was re-measured at 2, 4, 8, 16 and 24 weeks in control and exposure groups; Secretory functions, bioelectric properties and morphological assessments were made at birth and at 2 weeks.	TMV (*in vivo*), tracheal secretory function (*in vitro*), morphology of tracheal mucosa	Y, TMV decreased, N for macromolecule secretion but a partial prevention of the age-dependent decrease normally present, normal development of tracheal epithelium was retarded.
Moore et al. (1984)	Female Dorset Sheep aged between 1.5 and 7 years	0.12, 0.25, 0.50, 0.70 ppm ozone for 2.75 hours	Blood was drawn before and after ozone exposure	RBC[2] counts, G-6-PD activity, Heinz body counts, GSH, ACHE and MetHb were assayed	Y for GSH decrease at 0.50 ppm. N but a trend found for GSH decrease at 0.70 ppm, ACHE at 0.70 ppm , dose-dependent increase in MetHb, reduced RBC although no dose trend evident. G-6-PD increased up through 0.50 ppm with a slight reduction at 0.70 ppm
Moore et al. (1981).	Female Dorset sheep	0.25, 0.50 and 0.70 ppm for 2.75 hours	Blood samples tagged with Cr-51 were collected before and after ozone exposure	Effect of ozone on erythrocyte survival	Y for 0.25 ppm, N for 0.50 and 0.70 ppm
Meadows and Smith (1987)	Bovine and swine erythrocytes	Ozone flow of 1.7 μmole/min with a gas flow rate of 70 mL/min.	Erythrocyte suspensions in phosphate buffer were bubbled with ozone *in vitro* with and without uric acid.	Effect of ozone on hemolysis and methemoglobin formation of erythrocytes in the presence and absence of uric acid (an antioxidant)	Y, increase in hemolysis and methemoglobin formation with ozone; decrease in both with the addition of uric acid.
Sielczak et al., (1983)	Adult female sheep, aged 1 to 2 years	0.5 ppm for 2 hours	Lavage of a defined segment of the trachea in conscious sheep	Impact on mast cells and lymphocytes	Y, Increased mast cells and lymphocytes

Table 9.3 Cont'd Summary of Scientific Studies and Results for Ozone Effects on Mammals

Study author(s), year, journal	Species, life stage, age	Ozone concentration, and exposure time	Protocol	Endpoint measured	Significant (p = 0.05) effect? Y/N
Allgera et al., (1991)	Adult female sheep	1.0 ppm for 2 hours	Roentgenographic method	Impact on TMV	Y, Decreased TVM at 40 minutes and at 2 hours after exposure
		1.0 ppm for 5 hours/day for 4 days		Impact on TMV	Y, decrease the first and second day TMV was still significantly reduced 5 days after the last exposure
		Pre-treatment with NAC[3], an antioxidant	300 mg NAC administered intravenously twice a day in the 4 days prior to exposure to ozone	Effectiveness in protecting the sheep from TMV deficiency	Y
Abraham et al. (1984)	Adult female sheep	0.5 or 1.0 ppm for 2 hours, followed by a controlled 2 minute inhalation challenge with 1% histamine	Endotracheal tube for delivery of ozone, chronic carotid loop preparation for sampling arterial blood	Changes in airway responsiveness and permeability to histamine after exposure	Y
Schelegle et al. (1990)	Adult female sheep	3-4 ppm for 3 hours	Endotracheal tube for delivery of ozone, a pulsed Doppler flow transducer, secured around the common bronchial artery, to measure blood flow	Bronchial artery blood flow (an acute inflammatory response)	Y, dose-related increase in resting, unanesthetized sheep.
Gunther et al. (1992)	Adult Columbia-Suffolk female sheep	3.5 ppm for 3 hours, with and without steroids	Endotracheal tube for delivery of ozone, a pulsed Doppler flow transducer, secured around the common bronchial artery, to measure blood flow. 3 intravenous infusions of 25 mg/kg methylprednisone at 24 hr, 12 hr, and 15 min before initiation of ozone exposure	Role of corticosteroids in modulating the increase in Q_{br}[4]	Y, exposure to ozone caused increased Q_{br}, exposure to ozone and steroids blocked the increase in Q_{br}.

Table 9.3 Cont'd Summary of Scientific Studies and Results for Ozone Effects on Mammals

Study author(s), year, journal	Species, life stage, age	Ozone concentration, and exposure time	Protocol	Endpoint measured	Significant (p = 0.05) effect? Y/N
Mills et al. (1996)	Adult horses, clinically normal (n=5) or referred for poor performance (n=25)	0.5 ppm for 12 hours for clinically normal horses	Blood, tracheal wash and bronchoalveolar lavage fluid samples were collected before and after ozone exposure or from clinical cases	Impact of ozone exposure on GSH, GSSG, GRR[5] and free Fe concentrations in the body fluids examined	Y, significant increase for all parameters in bronchoalveolar fluid. N for plasma.
McBride et al. (1991).	Adult castrated males	1.0 ppm for 3 or 7 days	Animals were exposed *in vivo*, sacrificed and glycoconjugates were radio labeled and measured via liquid scintillation spectrometry	Secretory function of airway submucosal glands	Y for basal secretion of glycoconjugates after 3 days and for tracheal gland responsiveness to carbachol after 3 or 7 days.

[1]RBC = Red blood cells; G-6-PD = glucose-6-phosphate dehydrogenase; GSH = glutathione; ACHE = acetylcholinesterase; MetHb = methemoglobin;

[2]TMV = tracheal mucous velocity;

[3]NAC = N-acetylcysteine;

[4]Q_{br} = bronchial blood flow;

[5]GSSG = glutathione disulfide; GRR = glutathione redox ratio

Table 9.4 outlines the effects reported in the studies reviewed above and in Table 9.3 and scores them according to frequency and consistency of occurrence in the studies reviewed.

Table 9.4 Categories of Effects and Scores

Effect	Score[1]	Comment
Reduced growth and/or weight gain	+	One study found lambs exposed to ozone had retarded development of the tracheal epithelium
Reduced reproductive vitality	-	Effect not measured in the studies reviewed
Increased susceptibility to pathogens	-	Effect not measured in the studies reviewed
Increased susceptibility to physical stressors	+++	Three studies found adverse effects on RBC counts and biochemical evidence of oxidant stress; seven studies reported effects consistent with an inflammatory response.
Mortality	-	Effect not measured in the studies reviewed.

[1]Scores range from - (effect not found/not reported) to +++ (effect consistently found over various exposure scenarios)

Based on this analysis, increased susceptibility to physical stressors (in this case adverse effects on RBC counts/biochemical effects and inflammatory response) is the most appropriate endpoint for which adequate data is available to identify an effect level. An examination of Table 9.3 shows that of the two endpoints, adverse effects on RBC counts/biochemical effects appears to be the more sensitive endpoint, with significant effects evident at 0.25 ppm. Although this effect is consistently found and is the most sensitive endpoint from the reviewed studies, little information on other potential relevant endpoints is available and no information exists for ozone effects on wild mammals. This introduces uncertainty into the selection of the most appropriate endpoint.

9.2.2 Summary

Short-term exposure to ozone has been reported to cause adverse developmental effects, adverse effects on red blood cell counts, and inflammatory responses in domestic mammals. Adverse effects on RBC cells appeared to be the most consistent and sensitive endpoint for which effects were noted, with significant effects noted at 0.25 ppm but not at 0.12 ppm (although some evidence of oxidative stress of RBCs was observed at 0.12 ppm). The Meadows and Smith (1987) study examined the adverse effects of *in vitro* exposure of erythrocyte suspensions of cows and pigs to ozone, but this is not an appropriate endpoint for *in vivo* ambient exposures. There are insufficient data to identify the potential effects to mammals due to chronic exposure to ozone. In addition, no data were identified that examined the effects of ozone on wild mammals.

9.3 RESEARCH NEEDS

There is a notable lack of information on the effects of both acute and chronic ozone exposures on mammals and birds, both domestic and wild. More information concerning the vulnerability of birds to ozone-induced effects, and the mechanism of damage and repair of their respiratory tract during chronic exposure is required, given that there is some indication that birds may be particularly at risk.

REFERENCES

Abraham, W.M., Delehunt, J.C., Yerger, L., Marchette, B., & Oliver, Jr., W. (1984). Changes in airway permeability and responsiveness after exposure to ozone. Environmental Research, 34, 110-119.

Allgera, L., Moavero, N.E., and Rampoldi, C. (1991). Ozone-induced impairment of mucociliary transport and its prevention with N-acetylcysteine. The American Journal of Medicine. 91(suppl 3C): 67S-71S.

Bartov, I., Budowski, P., Dror, Y., Sandbank, U., and Bubis, J.J. (1981). Effect of ozone exposure on growth, nutritional encephalopathy, and fatty acid composition of cerebellum and lungs in the young chick. Poultry Science, 60:532-540.

Brown, R. E., Brain, J.D., & Wang, N. (1997). The avian respiratory system: a unique model for studies of respiratory toxicosis and for monitoring air quality. Environmental Health Perspectives. 105:188-200.

Gunther, R.A., Yousef, M.A.A., Schelegle, S., and Cross, C.E. (1992). Corticosteroid administration modifies ozone-induced increases in sheep airway blood flow. Am Rev Respir Dis. 146:660-664.

Mariassy, A.T., Abraham, W.M., Phipps, R.J., Sielczak, M.W., and Wanner, A. (1990). Effect of ozone on the postnatal development of lamb mucociliary apparatus. Journal of Appl. Physiol. 68 (6): 2504-2510.

McBride, R.K., Oberdoerster, G., and Marin, M.G. (1991). Effects of ozone on the cholinergic secretory responsiveness of ferret tracheal glands. Environmental Research. 55:79-90.

Meadows, J., and Smith, R.C. (1987). Uric acid protects erythrocytes from ozone-induced changes. Environmental Research. 43:410-416.

Mills, P.C., Roberts, C.A., and Smith, N.C. (1996). Effects of ozone and airway inflammation on glutathione status and iron homeostasis in the lungs of horses. Am J Vet Res. 57:1359-1363.

Moore, G.S., Calabrese, E.J., and Labato, F.J. (1981). Erythrocyte survival in sheep exposed to ozone. Bull. Environ. Contam. Toxicol. 27:126-138.

Moore, G.S., Calabrese, E.J., and Schulz, E. (1984). The effect of in vivo ozone exposure to Dorset sheep, an animal model with low levels of erythrocyte glucose-6-phosphate dehydrogenase activity. Journal of Environ. Pathol. Toxicol. Oncol. 5:71-78.

Mustafa, M.G. (1990). Biochemical basis of ozone toxicity. Free Radical Biology and Medicine. 9 :45-265.

Quilligan, J.J., Boche, R.D., Falk, H.L., and Kotin, P. (1958). Toxicity of ozone for young chick. Amer. Med. Ass. Arch. Ind. Health. 18:16-22.

Rombout, P.J.A., Dormans, J.A.M.A., VanBree, L., and Marra, M. (1991). Structural and biochemical effects in lungs of Japanese quail following a 1-week exposure to ozone. Environmental Research. 54:39-51.

Schelegle, E.S., Gunther, R., Parsons, G.H., Colbert, S.R., Yousef, M.A.A., and Cross, C.E. (1990). Acute ozone exposure increases bronchial blood flow in conscious sheep. Respir Physiol. 82: 325-336.

Sielczak, M.W., Denas, S.M., and Abraham, W.M. (1983). Airway cell changes in tracheal lavage of sheep after ozone exposure. Journal of Toxicology and Environmental Health, 11:545-553.

10 HUMAN HEALTH EFFECTS:

ANIMAL TOXICOLOGY STUDIES

A summary of the salient points from animal toxicity studies based on Bates (1994; 1995a) is provided in this chapter with an addition of studies as of April 1997. For a more comprehensive review of the work on the toxicity of ozone in animal experiments, the U.S. EPA criteria document (1996) is recommended.

10.1 BIOCHEMICAL MECHANISMS OF TOXICITY

Ozone is a potent oxidizing agent. *In vitro* experiments have demonstrated that ozone interacts with a wide variety of cellular components, including polyunsaturated lipids, antioxidants, thiols, amine groups and proteins (U.S. EPA 1986). Since ozone is highly reactive, these reactions would be expected to occur at the area of initial contact (i.e., the respiratory tract). There are virtually no data available examining the degree to which ozone itself can penetrate into tissue. It has been calculated that due to this high reactivity, ozone would, most likely, not penetrate beyond the surface mucous and surfactant fluids of the lung, except in the terminal airway regions where the epithelial cells have only minimal protection (Pryor, 1992).

Thus it has been hypothesized that a “reactive cascade”, starting from interaction of ozone with the lining of the lung, forms reactive oxygen intermediates that penetrate into the cells and cause the biological effects observed. Free radicals generated from the cascade and oxygenated biomolecules that result from reaction with ozone may mediate the effects of ozone. Ozone-initiated free radical formation has been detected in rat lung tissues (Vincent *et al.,* 1996), and in rat and human blood (Liu et al., 1996, 1997), which support this “reactive cascade” notion.

With respect to target molecules, most of the attention has been centred on polyunsaturated fatty acids and carbon-carbon double bonds as the prime targets of ozone. Reactions with sulfhydryl, amino, and some electron-rich compounds may be equally important. Damages on these macromolecular structures ultimately lead to cell injury and cell death.

10.2 SUMMARY, ANIMAL AND CELLULAR STUDIES

10.2.1 Ozone Dosimetry

A considerable body of work has been published in relation to ozone dosimetry during the past few years; the major conclusions include:

Although somewhat different kinetic assumptions and geometric airway data have been used, all the models indicate that in all species, the tissue dose of inhaled ozone (expressed as micrograms/square centimetre/minute per microgram of inhaled ozone) reaches a maximum in the terminal bronchiolar region. The net dose is highest at the first point of impact, namely the trachea.

The maximally affected zone, in terms of tissue dose, in the human and in the rat is the 17th generation in the airway bronchiole branching. For the human data, this was based on a tidal volume of 800 ml, and a breathing frequency of 15 breaths/minute (Overton & Miller 1988).

The effect of exercise or increased ventilation is to increase the terminal bronchiolar regional dose slightly, and to significantly increase the pulmonary regional total dose and the dose in the proximal alveolar region.

Differences between animal species have been considered in detail (Overton & Miller, 1988); discussion of these is beyond the scope of this review, except to note that humans absorb significantly more of a given concentration of airborne ozone than rodents, and this appears to give rise to an increased response per unit of ambient ozone.

Theoretical dosimetric calculations have been compared with measurements of ozone in the respiratory tract under different conditions. Gerrity et al. (1988) measured ozone uptake in the extrathoracic airways of normal subjects, and found that this was as high as 40% on inspiration, and 92% on both inspiration and expiration. Both of these values fell as respiratory rate was increased. A surprising finding was that there was only a small difference between the uptake that occurred with nose breathing and with mouth breathing. Hynes et al (1988) found little difference between nose and mouth breathing in pulmonary function response to ozone.

These studies, collectively, indicate that deposition modelling of inhaled ozone emphasizes the terminal bronchiolar and centriacinar regions as sites of maximal tissue deposition of the gas. Exercise, by increasing both the tidal volume and breathing frequency, would be expected to increase the tissue dose significantly.

10.2.2 Acute Exposures

The principal effects observed after acute exposures of a variety of species to ozone concentrations less than 1.0 ppm, are lung inflammation and changes in lung permeability; increased airway responsiveness; and increased mortality if the animal is subsequently challenged with a bacterial aerosol, such as *streptococcus zooepidemicus*, commonly seen bacteria. The initial target of ozone appears to be lung fluid lining components and cell membranes (Pryor et al.1991). Increased permeability has been demonstrated after exposure of rats to 0.12 ppm or 0.4 ppm for two days (Guth et al. 1986) or in guinea pigs exposed to 1.0 ppm for one hour (Miller et al. 1986). The increased airway responsiveness is generally considered to be a consequence of the induced inflammation. However, a recent series of experiments on an isolated rat lung preparation (Joad et al. 1993) showed that ozone was capable of increasing airway responsiveness and damaging airway epithelium in the absence of neutrophils (polymorphonuclear leukocytes or PMN), and before a microvascular leak was caused. The presence of neutrophils was shown to increase the magnitude of these effects of ozone.

The increase of mortality in mice most likely occurs when ozone exposure precedes administration of a bacterial aerosol. This is primarily due to the adverse effect of ozone on macrophage function. Experiments using *Streptococcus zooepidemicus*- infected mice have shown that 3 hours of exposure to either 0.3 or 0.8 ppm ozone led to increased mortality, which was greater in one strain of mice than in another (Gilmour et al. 1993). The difference in sensitivity was shown to be due to a difference in the sensitivity of the alveolar macrophages in the two different strains of mice. It is the phagocytic efficiency of the macrophage that is impaired by the prior ozone exposure. Ozone exposure has also been shown to decrease the T-lymphocyte and antiviral response (Jakab & Hmieleski 1988).

Evidence that ozone impairs the clearance mechanism of the lung was provided by the observation in rats that ozone exposures to base levels of 0.06 ppm, with daily excursions to 0.25 ppm, increased the retention of asbestos fibres (Pinkerton et al. 1989). This was interpreted to be attributable to interference with macrophage function.

Plopper and colleagues (1990) have pointed out that non-human primates appear to be more responsive to ozone at concentrations less than 1.0 ppm than are rats. This may be due to differences in nasal structure (and hence in delivered dose) or to differences in lung structure, since rats do not possess the many generations of respiratory bronchioles that exist in human and primate lungs.

This brief summary of the effects of acute animal exposures shown in animal experiments indicate that aggravation of inflammatory lesions and worsening of respiratory infections are effects that should be evaluated in the human population.

Other phenomena described following acute ozone exposures to animals or to cells, usually to higher concentrations than those in ambient air, include:

Morphological changes in the nasal cavity and nasopharynx;

Greater sensitivity, in general, of younger rats versus older;

No differences in effects between normal rats and rats with elastase-induced emphysema (only one study reported);

A wide range of biochemical effects, including damage on polyunsaturated fatty acids, depletion of endogenous antioxidants, and damage on proteins; and effects on the production of arachidonate metabolites (inflammation mediators);

Cardiovascular effects including reduction of heart rate, arterial blood pressure and core temperature, and increase in frequency of arrhythmia in rats (Watkinson et al., 1993; Arito et al., 1990; Uchiyama & Yokoyama, 1989)

10.2.3 Long-term exposures

Human controlled exposures for longer than a few hours are not feasible, hence the results of longer term animal exposures are very important. Since the primate lung appears to be more sensitive to ozone than does the rat lung, the evidence from primate exposures (*Macaca fascicularis*) will be considered first. Almost all of this work has originated from the Primate Centre in Davis, California. It has been elegantly summarised and well illustrated by this research group (Tyler et al. 1992). The exposures were generally to 0.25 ppm ozone for 8 hours/day, 7 days/week for 18 months. A very brief summary of their findings and conclusions follows:

1. The initial inflammatory response is modified in subsequent exposures, but inflammatory cells in the peribronchiolar connective tissues persist during exposures that last up to one year;

2. Epithelial and interstitial changes in distal airways are the most striking morphologic changes in animals exposed for a year or more. There is a proliferation of non-ciliated bronchiolar and Type 2 alveolar epithelial cells. These changes, which impair the clearing function of the lining, start early, and are evident after 50 hours of exposure to 0.8 ppm of ozone.

3. Necrosis of ciliated and type 1 pneumocytes occurs, and these cells are replaced by the cells noted in 2) above. The long-term effect is therefore a "remodelling" of the centriacinar airways by extension of bronchiolar cell types in airways that were formerly alveolar ducts.

4. There is an increase of collagen localized in the peribronchiolar and centriacinar regions. This confirms earlier studies from the same laboratory in rats exposed to higher concentrations of ozone.

5. The morphological changes induced by the ozone exposure were still present after a 6 month post-exposure period of living in filtered air. Quantitative morphology studies indicated that the lungs were more abnormal after the post exposure period than immediately after the ozone exposure.

Rat exposures of longer than 2 weeks have been studied in a number of centres. Using a 78 week exposure protocol of a base exposure of 0.06 ppm with spikes to 0.25 ppm, Chang et al. (1992) showed that an acute response occurred in the centriacinar region; that these changes partly resolved that Type 2 cells increased; that the interstitium was increased; that the basement membrane was thickened; but that centriacinar remodelling did not occur. Earlier observations from the same laboratory using a 6 week exposure protocol of similar concentrations (Barry et al. 1988) had noted that there was an increase in interstitium thickness, and an increase in number of alveolar macrophages. Rat exposures to 0.95 ppm for 8 hours/day for 90 days were found by Barr et al. (1988) to have resulted in airway remodelling, and the lesions resulting from chronic versus daily episodic exposures were similar (Barr et al. 1990).

Costa et al. (1988) were able to demonstrate changes in lung function in rats after brief exposures, and in a more recent paper reported reductions in forced vital capacity (FVC) of rats after repetitive ozone exposures (Costa et al. 1994).

Saldiva et al (1992) in Sao Paolo exposed 60 rats for 6 months to the ambient air, and compared them to controls kept for the same length of time in a clean area. A monitoring station 200 metres from the exposure location showed that ozone went up to 0.4 ppm; particulates up to 90 $\mu g/m^3$; SO_2 up to 0.025 ppm; and CO up to 4 ppm. The exposed rats had secretory cell hyperplasia in the airways, ultrastructural cilliary alterations, and a more rigid mucus, changes that caused mucociliary clearance impairment. Nasal resistance and inflammatory cells in bronchoalveolar lavage (BAL) were also increased in the exposed group.

Genotoxicity, mutagenicity, and effects on carcinogenicity have also been observed. Although such effects can be shown, for instance on deoxyribonucleic acid (Hamelin 1985), there is no human correlate. Witschi (1988) reviewed these observations, pointing out that the radiomimetic activity of ozone did make it a potential contributor to human lung cancer.

10.2.4 Ozone in combination with other chemicals

Since human exposures are rarely to a single pollutant, the question of interaction with other chemicals assumes some importance. In very detailed experiments on rats, Last (1990) studied the effects of ozone and sulphuric acid aerosol alone and in combination. On all outcomes related to an inflammatory response, the two were additive in their effects, but were not clearly synergistic. Nishikawa et al. (1992) studied the effects of ozone and cigarette smoke in guinea pigs. They showed that airway responsiveness and permeability were affected when both irritants were inhaled, although single exposures to each separately at the same concentration produced no effect. This suggests that there may be a synergistic effect.

10.2.5 Applicability of animal toxicity studies to humans

Animal exposures are valuable for elucidating mechanisms of action; although recent human experimentation using bronchial lavage and even bronchial biopsies have added to this knowledge. Animal exposure data in relation to the effects of longer term exposures are of more contemporary importance, as human clinical studies of that phenomenon are impractical, and epidemiological studies of chronically exposed populations are few at present and usually involve co-exposure to other chemical substances.

The question of "biological plausibility" in reaching a judgement of causality, is complex. It requires consideration of dosimetry; of physiological and biochemical differences between species; and of the pathology and pathophysiology of common conditions such as asthma. A recent comparative dosimetric study has provided some very interesting and valuable data in this respect. Hatch et al. (1994) exposed both human subjects (with intermittent exercise) and resting rats to 400 ppb ^{18}O-labelled (a stable isotope of oxygen) ozone for 2 hours. Another group of rats was exposed to 2000 ppb ^{18}O-labelled ozone. The researchers analyzed the amount of recovered ^{18}O in bronchoalveolar lavagable cells from the human subjects and the rats. Data demonstrate that the exercising humans had four- to five-fold higher ^{18}O concentrations in all of their bronchioalveolar lavage constituents than did the resting rats. The humans also had significant increases in all of the airway inflammatory markers after 400 ppb ozone, whereas the rats did not. Rats that were exposed to 2000 ppb ^{18}O-labelled ozone had levels of ^{18}O in bronchoalveolar lavage that were more comparable to but still lower than those of humans. A detailed analysis of the distribution of ^{18}O-labelled ozone in rats shows that 49.6% was taken up by the head, 6.7% by the larynx/trachea, and 43.6% by the lungs (Hatch et al., 1989).

These studies provide a dose and effect linkage between rats and humans, which should assist in extrapolating animal toxicity data to humans. When taking into account the much lower sensitivity of rats to ozone in comparison with that of humans, it is conceivable that the doses used in experimental animals (as low as 100 ppb) that produced pathological changes are similar to the concentrations encountered by human population during ozone episodes. When ozone is delivered into deep airways, the relationship between the pulmonary tissue dose (normalized to body weight) and the pulmonary inflammation (measured as bronchoalveolar lavage protein) among rats, guinea pigs, rabbits and humans is approximately in the same pattern (US EPA, 1996).

With respect to regulatory issues such as establishment of objectives, exposure response data from animal studies on rodents are of value qualitatively, since all species appear to respond

similarly, but they are of limited use quantitatively due to differences in morphology of both the nose and the bronchial tree/lung area. These lead to a difference (reduction) in net uptake of inhaled ozone in rodents and a resulting reduction of response for a given concentration of ozone in rodents compared to primates, assuming the same time of exposure and ventilation rate.

10.3 RESEARCH NEEDS

- Few studies have demonstrated cardiorespiratory effects at ozone concentrations below 0.12 ppm, although epidemiological studies have shown increased mortality and morbidity below 0.12 ppm ozone. It is not clear whether failure to detect adverse effects in animals at ambient ozone concentration is because of species differences in responses to ozone, or due to the biological markers used that are not sensitive for minor injuries. It would be desirable to examine the effects of ambient levels of ozone on animals with compromised cardio-respiratory systems.

- Studies should be extended to include the effects and the mechanism of ozone on animals with asthma-like diseases or with chronic obstructive pulmonary diseases.

- More systemic effects, such as cardiovascular and developmental effects, should be investigated to elucidate the mechanism(s) of ozone-associated cardiovascular mortality observed in epidemiological studies.

REFERENCES

Arito, H., Uchiyama, I., Arakawa, H., Yokoyama, E. (1990). Ozone-induced bradycardia and arrhythmia and their relation to sleep-wakefulness in rats. Toxicol. Lett. 52:169-178.

Barr, B.C., Hyde, D.M., Plopper, C.G., & Dungworth, D.L. (1988). Distal airway remodelling in rats chronically exposed to ozone. Am Rev Respir Dis 137, 924-938.

Barr, B.C., Hyde, D.M., Plopper, C.G., & Dungworth, D.l. (1990). A comparison of terminal airway remodelling in chronic daily versus episodic ozone exposure. Toxicol Appl Pharmacol 106, 384-407.

Barry, B.E., Miller, F.J., & Crapo, J.D. (1988). Effects of inhalation of 0.12 and 0.25 parts per million ozone on the proximal alveolar region of juvenile and adult rats. Lab Invest 53, 692-704.

Bates, D.V. (1994). Ozone: a review of recent experimental, clinical and epidemiological evidence, with notes on causation. Contract prepared for Environmental Health Directorate, Health and Welfare Canada, contract No. 4346. March 1994.

Bates, D.V. (1995a). Ozone: a review of recent experimental, clinical and epidemiological evidence, with notes on causation, Part I. Can. Respir. J., 2:25-31.

Costa, D.L., Hatch, G.E., Highfill, J., Stevens, M.A., & Tepper, J.S. (1988). Pulmonary function studies in the rat addressing concentration versus time relationships in ozone, In: Atmospheric Ozone research and its policy implications: proceedings of the 3rd. US-Dutch international symposium; May 1988: Nijmegen, the Netherlands, Edited by Schneider, T., Lee, S.D., Woplters,

G.J.R., & Grant, L.D. Elsevier Science Publishers, Amsterdam, The Netherlands.

Costa, D.L., Tepper, J.S., Stevens, M.A., Fitzgerald, S., & Last, J.A. (1994). Functional evidence of a restrictive lung lesion in rats after chronic exposure to a simulated urban profile of ozone. In press 1994.

Chang, L-Y., Huang, Y., Stockstill, B.L., Graham, J.A., Grose, E.C., Menache, M.G., Miller, F.J., Costa, D.L., & Crapo, J.D. (1992). Epithelial injury and interstitial fibrosis in the proximal alveolar regions of rats chronically exposed to a simulated pattern of urban ambient ozone. Toxicol Appl Pharmacol 115, 241-252.

Gerrity, T.R., Weaver, R.A., Berntsen, J., House, D.E., & O'Neil, J.J. (1988). Extrathoracic and intrathoracic removal of O_3 in tidal-breathing humans. J Appl Physiol 65, 393-400.

Gilmour, M.I., Park, P., & Selgrade, M.K. (1993). Ozone-enhanced pulmonary infection with streptococcus zooepidemicus in mice. Am Rev Respir Dis 147, 753-760.

Guth, D.J., Warren, D.L., & Last, J.A. (1986). Comparative sensitivity of measurements of lung damage made by bronchoalveolar lavage after short-term exposure of rats to ozone. Toxicology 40, 131-144.

Hatch,G.E., Wiester, M.J., Overton, J.H., Jr., Aissa, M. (1989). Respiratory tract dosimetry of [18]O-labeled ozone in rats: implications for a rat-human extrapolation of ozone dose. In: Schneider, T., Lee, S.D., Wolters, G.J.R., Grant, L.D., eds. Atmospheric ozone research and its policy implications: proceedings of the 3rd US-Dutch internationals symposium. May 1988, Nijmegen, the Netherlands. Amsterdam, the Netherlands: Elsevier Science Publishers; pp. 553-560. (Studies in environmental science 35).

Hatch,G.E., Slade,R., Harris,L.P., McDonald,W.F., Devlin,R.B., Koren,H.S., Costa,D.L., McKee,J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labeling and bronchoalveolar lavage. Am.Rev.Respir.Cell Mol.Biol. 150. 676-683.

Hamelin, C. (1985). Production of single- and double-strand breaks in plasmid DNA by ozone. Int J Radiat Oncol Biol Phys 11:253-7

Hynes, B., Silverman, F., Cole, P., Corey, P. (1988). Effects of ozone exposure: a comparison between oral and nasal breathing. Arch. Environ. Health 43: 357-360.

Jakab, G.C., & Hmieleski, R.R. (1988) Reduction of influenza virus pathogenesis by exposure to 0.5 ppm ozone. Toxicol Environ Health. 23:455-72.

Joad, J.P., Bric, J.M., Pino, M.V., Hyde, D.M., & McDonald, R.J. (1993). Effects of ozone and neutrophils on function and morphology of the isolated rat lung. Am Rev Respir Dis 147, 1578-1584.

Last, J.A. (1990). Synergistic effects of air pollutants: ozone plus a respirable aerosol Health Effects Institute Research Report No. 38.

Leikauf, G.D., Zhao, Q., Zhou, S., Santrock, J. (1993). Ozonolysis products of membrane fatty acids activate eicosanoid metabolism in human airway epithelial cells. Am. J. Respir. Cell. Mol. Biol. 9(6):594-602

Liu, L., Kumarathasan, P., Guenette, J., Vincent, R. 1996. Hydroxylation of salicylate to 2,3-dihydroxybenzoic acid in the respiratory tract: Effects of aging and ozone exposure in Fischer 344 rats. *Am. J. Physiol.* 271:L995-L1003.

Liu, L., Leech, J. A., Urch, R. B., Silverman, F. S. 1997. *In vivo* salicylate hydroxylation: A potential biomarker for assessing acute ozone exposure and effects in humans. *Am. J. Respir. Crit. Care Med.* 156:1405-1412.

Miller, P.D., Gordon, T., Warnick, M., & Amdur, M.O. (1986). Effect of ozone and histamine on airway permeability to horseradish peroxidase in guinea pigs. J Toxicol Environ Health 18, 121-132.

Nishikawa, M., Ikeda, H., Nishiyama, H, Yamakawa, H., Suzuki, S., & Okubo, T. (1992). Combined effects of ozone and cigarette smoke on airway responsiveness and vascular permeability in guinea pigs. Lung 170:311-322.

Overton, J.H., & Miller, F.J. (1988). Dosimetry modelling of inhaled toxic reactive gases, In: Air Pollution, the automobile, and public health; Edited by Watson, A.Y., Bates, R.R., & Kennedy, D. National Academy Press, Washington, DC. 1988

Pinkerton, K.E., Brody, A.R., Miller, F.J., & Crapo, J.D. (1989). Exposure to low levels of ozone results in enhanced pulmonary retention of inhaled asbestos fibres. Am Rev Respir Dis 140, 1075-1081.

Plopper, C.G., Harkema, J.R., Last, J.A., Pinkerton, K.E., Tyler, W.S., St. George, J.A., Wong, V.J., Nishio, S.J., Weir, A.S., Dungworth, D.L., Barry, B.E., & Hyde, D.M. (1990). The respiratory system of nonhuman primates responds more to ambient concentrations of ozone than does that of rats. In: Tropospheric Ozone and the Environment: Papers from an International conference: March 1990, Los Angeles, CA. Eds: Berglund, R.L., Lawson, D.R., & McKee, D.J. Air & Waste Management Assoc, Pittsburgh, PA.

Pryor, W.A., Das, B., & Church, D.F. (1991). The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. Chem Res Toxicol 4, 341-348.

Pryor W.A. (1992). How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic. Biol. Med. 12(1):83-88.

Saldiva, P.H.N., King, M., Delmonte, V.L.C., Macchione, M., Parada, M.A.C., Daliberto, M.L., Sakae,

R.S., Criado, P.M.P., Silveira, P.L.P., Zin, W.A., & Bohm, G.M. (1992). Respiratory alterations due to urban air pollution: an experimental study in rats. Environ Research 57, 19-23.

Tyler, W.S., Julian, M.D., & Hyde, D.M. (1992). Respiratory Bronchiolitis following exposures to photochemical air pollutants. Seminars in Resp Med 13, 94-113.

Uchiyama, I., Yokoyama, E. (1989). Effects of short- and long-term exposure to ozone on heart rate and blood pressure of emphysematous rats. Environ. Res. 48:76-86.

U.S. Environmental Protection Agency. (1986). Air Quality Criteria for Ozone and Other Photochemical Oxidants. (EPA Report No. EPA/600/8-78/004). Research Triangle Park, NC: Environmental Criteria and Assessment Office. (NTIS No. PB80-124753).

U.S. Environmental Protection Agency. (1986). Air quality criteria for ozone and other photochemical oxidants. Research Triangle Park, NC: Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office; EPA report nos. EPA-600/8-84-020aFe-eF. 5v. Available from: NTIS, Springfield, VA; PB87-142949.

U.S. Environmental Protection Agency (EPA). (1996). Air Quality Criteria for Ozone and Related Photochemical Oxidants. (NTIS 600/p-93/004bF).

Vincent, R., Janzen, E. G., Chen, G., Kumarathasan, P., Haire, D. L., Guenette, J., Chen, J. Z., Bray, T. M. (1996). Spin trapping study in the lungs and liver of F344 rats after exposure to ozone. *Free Radical Res.* 25:475-488.

Watkinson, W.P., Aileru, A.A., Dowd, S.M., Doerfler, D.L., Tepper, J.S., Costa, D.L. (1993). Acute effects of ozone on heart rate and body temperature in the unanesthetized, unrestrained rat maintained at different ambient temperatures. Inhal. Toxicol. 5:129-147.

Witschi, H. (1988). Ozone, nitrogen dioxide, and lung cancer: a review of some recent issues and problems. Toxicology 48, 1-20.

11 HUMAN HEALTH EFFECTS: CONTROLLED HUMAN STUDIES

The previous chapter presented the data of ozone studies using experimental animals, in order to elucidate the underlying mechanisms of ozone toxicity and the basic exposure–response relationships for ozone. However, whenever possible, risk assessment of pollutants should be based upon direct evidence of their health effects in the human population. Controlled human exposure studies and epidemiological studies provide extensive information on human health responses to environmental pollutants.

Controlled human exposure studies document the health effects that result from breathing known concentrations of pollutants in controlled atmospheres under laboratory atmospheric conditions. There are some advantages in performing this type of study. Since the atmosphere is highly controlled for individual pollutants, experiments can be designed to explicitly characterize the dose-response relationship. Such a controlled environment also provides the opportunity to examine interactions among pollutants or with other environmental variables such as humidity, temperature or exercise. Subjects from special groups, such as young children or elderly people, or individuals with pre-existing cardio-respiratory diseases, may also be studied to identify potentially susceptible subpopulations.

The limitations of controlled human exposure studies lie in the practical and ethical aspects of such studies. This type of study has to be limited to short duration and only to concentrations of pollutants that are expected to produce mild and transient responses. An endpoint assessment has to be relatively non-invasive, and invariably includes pulmonary function, which sometimes may not be sensitive enough to reflect the adverse effects. Furthermore, transient responses in controlled human exposure studies have never been validated as predictors of more chronic and persistent effects. Nevertheless, controlled, quantitative human studies provide a complementary approach to examine the biological plausibility of the associations that have been observed in epidemiological investigations.

The information on controlled human exposure to ozone and human response has been extensively reviewed by the United States Environmental Protection Agency (U.S. EPA) in their criteria document (1996) based on studies up to 1996. The results of their investigations were used to determine if a No Observed Adverse Effects Level (NOAEL) or Lowest Observed Adverse Effects Level (LOAEL) could be set from these data. Recently, a large body of literature on controlled human exposure studies has come to light, providing further evidence on the impacts of ambient ozone on human health.

This chapter is built upon the information from the U.S. EPA criteria document (U.S. EPA, 1996), with additional publications as of April 1997, in an attempt to assess ozone concentrations and exposure durations which impose adverse health effects on humans. Ambient air pollution is composed of a complex mixture of particulate matter and gases, therefore, potential synergistic interactions between ozone and various other pollutants are considered in this document. A key issue in the evaluation of the health effects of air pollution is to identify the individuals who are at

greatest risk from exposure to air pollutants. We have, therefore, placed considerable emphasis on characterizing susceptible subpopulation(s).

11.1 PULMONARY FUNCTION EFFECTS AND SYMPTOMS OF ACUTE OZONE EXPOSURE

11.1.1 Healthy Subjects

As summarized in the U.S. EPA document (1996), the major human pulmonary physiological responses to acute ozone exposure include a decreased inspiratory capacity, a mild bronchoconstriction, and a rapid shallow breathing pattern during exercise. The pulmonary function changes observed appear to act in sequence and are inter-related: the decreased inspiratory capacity results in a decrease in forced vital capacity (FVC) and total lung capacity (TLC). These effects combined with bronchoconstriction lead to a decrease in the forced expiratory volume in one second (FEV_1). Decreased FEV_1 values are closely related to decreased FVC and also to increases in airway resistance (R_{aw}). Ozone-induced pulmonary function changes demonstrate a concentration-dependent pattern.

Subjective symptoms following ozone exposure include cough, substernal soreness, and pain on deep inspiration. Other observations include airway hyperresponsiveness as demonstrated by increased physiological response to a nonspecific stimulus. The relationship between spirometry, resistance measurements, symptoms and nonspecific bronchial responsiveness is not fully determined. The weak associations suggest that several response mechanisms may be involved.

The Ozone Concentration-Response Relationship

At Rest Exposures

Since the publication of the 1996 U.S. EPA criteria document, no new studies have been reported on ozone-induced pulmonary function changes and symptoms in resting healthy individuals. The observed lowest concentration at which significant reductions in FVC and FEV_1 were reported was 0.50 ppm for a 2-hour exposure (Folinsbee et al.,1978; Horvath et al., 1979). Results on ozone-induced increase in airway resistance are inconsistent in resting human subjects at doses <1.00 ppm.

A recent study utilizing hydrogen peroxide concentration in breath as a biomarker for ozone exposure (Madden et al., 1997) did not show any significant change of hydrogen peroxide concentration following exposure of 11 resting healthy subjects to 0.4 ppm ozone for 2-hours, although the same dose of ozone caused a significant increase in breath hydrogen peroxide during intermittent exercise.

In 15 subjects with mild asthma, an 1-hour resting exposure to 0.12 ppm ozone did not initiate significant changes in pulmonary function or symptoms reported, nor did it potentiate an immediate bronchoconstrictive response to grass allergen (Ball et al., 1996).

Exposure with Exercise

Early studies on the effects of increased ventilation through exercise during ozone exposure were carried out in 1972-73 (Hazucha et al., 1973). Most of the exercise achieved a doubling of the ventilation rate. The concentration-related effects noted were decreased forced expiratory endpoints at 0.37 and 0.75 ppm. Subjective complaints included substernal soreness, chest tightness and cough. During the following years, Silverman et al. (1976) conducted further examination concerning the effects of exercise on ozone-induced pulmonary function changes. The workloads on the subjects were expressed as fraction of maximum oxygen uptake ($\underline{V}O_{2max}$), and studies were conducted at 45, 60 and 75% of $\underline{V}O_{2max}$. They found that pulmonary function responses were related to the total inhaled dose of ozone (0.37, 0.5 or 0.75 ppm, 2-hours) calculated as the product of concentration x time x $\underline{V}_E$. Moreover, exposure to increasing doses of ozone did not result in any effects on $\underline{V}O_{2max}$ or minute ventilation ($\underline{V}_E$), while the breathing frequency (f) was increased and tidal volume (V_T) was decreased at the 75% $\underline{V}O_{2max}$.

The important findings of these early studies were that at a given ozone concentration, the increase in ventilation results in increased pulmonary function responses, and that increased ventilation lowers the concentration of ozone required for a given pulmonary response. This introduces the concept of 'effective dose' which is a result of the product of the ventilation rate, the concentration of ozone and the exposure time.

McKittrick and Adams (1995) examined the effects of exercise pattern on ozone-induced changes of pulmonary function and symptoms. Twelve healthy young subjects were exposed to air or 0.3 ppm ozone for 1-hour with continuous exercise (mean $\underline{V}_E$ 60L/min), or 2-hours with intermittent exercise (mean $\underline{V}_E$ 45 to 47 L/min; protocol one with alternating 15 minutes of rest/exercise; protocol two with alternating 30 minutes of rest/exercise). The data show that FVC, FEV_1 and $FEF_{25-75\%}$ (forced expiratory flow between 25 and 75% of vital capacity) were significantly decreased after all three ozone exposure protocols. There were no significant differences in ozone-induced lung function changes among three ozone exposures, although symptom severity in the continuous exercise group was higher than in the two intermittent exercise groups. The total effective dose of ozone for the 3 protocols was not significantly different.

Subsequent studies demonstrated that an ozone concentration of 0.12 to 0.18 ppm is required to elicit significant pulmonary function decrements and increases of symptoms in healthy young adults performing moderate to severe intermittent or continuous exercises for 1 to 4-hours at $\underline{V}_E$ 30 to 90 L/min (Linn et al., 1986; Kulle et al., 1985; Schelegle and Adams, 1986; McDonnell et al., 1983; McDonnell, III et al., 1985b; Gong et al., 1986; Avol et al., 1984; McDonnell et al., 1993; Seal et al., 1993b; Balmes et al., 1996; Hazbun et al., 1993; Fox et al., 1993; Jorres et al., 1996; Aris et al., 1993; Basha et al., 1994; Foster and Stetkiewicz, 1996; Seal, et al., 1996; Aris et al., 1995; McDonnell et al., 1995; McBride et al., 1994; Liu et al., 1997b).

However, a retrospective analysis by Hazucha (1987) demonstrated that data from 2-hour ozone exposure with intermittent exercise best fit a model that was a quadratic function of the

concentration of ozone. A further conclusion was that he could not define a concentration of ozone below which no pulmonary function response would be elicited (i.e., no threshold).

In order to identify personal characteristics that predict individual differences in acute pulmonary function response to ozone exposure, McDonnell et al. (1993) examined FEV_1 decrements among 290 young white male subjects (18 to 32 years old) after exposure to 0, 0.12, 0.18, 0.24, 0.3 or 0.4 ppm ozone for 2-hours with intermittent exercise ($\underline{V}_E$ - 60 to 70 L/min). Linear, quadratic, cubic and two sigmoid-shape models (the logistic and Gompertz functions) were used to describe decrements in FEV_1 as a function of ozone. The researchers found that a sigmoid-shaped concentration-response function is plausible for their data, however a stimulus 'threshold' could not be defined. Their model also predicts a decreasing response with increasing age for all nonzero ozone concentrations. For exposure to 0.4 ppm, the model predicts decrements in FEV_1 of 1.07 and 0.47 L for 18 and 30-year old subjects, respectively. They concluded that for white males, age was a significant predictor of response, with older subjects being less responsive to ozone.

In a subsequent study by the same research group (Seal et al., 1996), a less homogeneous sample population containing 371 young (18 to 35 years old) male and female, African American and white subjects was utilized. Subjects were exposed to 0, 0.12, 0.18, 0.24, 0.3, or 0.4 ppm ozone for 2.3-hours while doing intermittent exercise ($\underline{V}_E$ - 40 to 50 L/min). Personal and family histories were recorded before exposure. Lung function was recorded before, during and after exposure. Logistic function was used to model exposure-response relationship, and to test the statistical significance of age, socio-economic status, and menstrual cycle phase to the pulmonary response to ozone exposure. The overall fit of the data to the logistic model in this study was comparable to that observed by McDonnell et al. (1993). FEV_1 decrements revealed a ozone dose-dependent relationship, with a concave upward pattern over a range of low concentrations of ozone.

Individual Sensitivity

There is a large intersubject variability in response to ozone exposure. Data provided by Kulle et al. (1985) shows that in 5 subjects, exposure to ozone at doses ranging from 0.10 to 0.25 ppm for 2-hours (exercise $\underline{V}_E$ 70 L/min) resulted in no changes in FEV_1 to changes ranging from a 4% reduction to a 22% reduction, each individual having a different dose-response curve.

The factors that may contribute to this variability have not yet been defined. McDonnell et al. (1985a) studied the reproducibility of individual responses to ozone exposure (0.12 to 0.4 ppm) in healthy human subjects (18 to 30 years of age) exposed twice with intermittent exercise ($\underline{V}_E$ -70 L/min), with from 21 to 385 days separating exposures. FVC, FEV_1, $FEF_{25-75\%}$, airway resistance, tidal volume, breathing frequency, and symptoms were monitored. The authors found a significant reproducibility in FVC, FEV_1, $FEF_{25-75\%}$, cough, airway resistance and shortness of breath at ozone concentrations $\geq$0.18 ppm. They concluded that the reported large intersubject variability in magnitude of response was due to large differences in the intrinsic responsiveness of individual subjects to ozone exposure.

In a recent study, Liu et al. (1997b) studied changes of plasma salicylate hydroxylation (an indicator for the production of hydroxyl radicals), pulmonary function and symptoms in young, healthy subjects following exposure to 0, 0.12, or 0.4 ppm ozone for 2-hours with intermittent exercise ($\underline{V}_E$ 45 L/min). They found that 2 out of 16 subjects had extraordinarily larger decrements of FEV_1, FEF_{50} and FEF_{75} than other subjects (>mean + 2SD), and higher plasma levels of salicylate hydroxylation (>mean + 3SD) than did other subjects. This implies that the high sensitivity of some subjects to ozone might be due to higher levels of ozone uptake or lower endogenous antioxidant defence systems in their airways.

Torres et al. (1996) studied inflammatory responses in bronchoalveolar lavage (BAL) fluid of healthy, nonsmoking responders (>15% fall in FEV_1 after ozone exposure) and non responders (<5% fall in FEV_1). Bronchoalveolar lavage fluid was obtained immediately and 18 hours after a 4-hour exposure to filtered air or 0.22 ppm ozone with exercise. Production of superoxide anion by the total lavage cell population was determined with and without phorbol myristate acetate stimulation. The concentration of polymorphonuclear leukocytes (PMN) in BAL increased nearly five fold 18 hours after ozone exposure in both responders and non-responders. In responders, stimulated superoxide anion in total cells increased progressively. Unstimulated hydrogen peroxide production in alveolar macrophages from responders decreased progressively. There were no significant changes in reactive oxygen intermediate production by cells from non-responders. The data support the observation by McDonnell et al. (1985b) who suggest that the individual sensitivity to ozone may be due largely to differences in the intrinsic responsiveness, such as macrophage function, of individual subjects to ozone.

However, a study conducted by Balmes et al. (1996) shows that ozone-induced pulmonary function changes were not correlated with airway inflammatory responses. In this study, healthy adult subjects (22 to 38 years old) were divided into least sensitive (mean ozone-induced change in FEV_1 = -7%, n = 12) and most sensitive (mean ozone-induced change in FEV_1 = -36%, n = 8) groups, and were exposed to 0.2 ppm ozone or filtered air for 2-hours during intermittent exercise ($\underline{V}_E$ -50 L/min). Ozone exposure significantly increased airway resistance, small airway respiratory symptoms, percent neutrophils, total protein, and interleukin-8 in airway lavage fluids for all subjects combined. However, there were no significant differences between least and most sensitive groups. The data indicate that spirometric responses (such as FEV_1 and FVC) do not predict ozone-induced inflammatory changes, and may be due to separate mechanisms.

Mechanism of Acute Pulmonary Responses

It has been hypothesized that there may be an overall sequence of events influencing the outcome of ozone exposure on lung toxicity. First, ozone is delivered to the lung tissue. Second, ozone reacts with components in airway surface liquid and/or epithelial cell membranes. Third, local tissue responses including injury and inflammation are triggered. The secretion of prostaglandins may result in an inhibition of the α-adrenergic receptors which are responsible for the stimulation of the sympathetic system (relaxation of lung fibres). Finally, there is stimulation of the neural afferents (bronchial C fibres) which results in pulmonary function responses. The stimulation of C fibres, possibly through an increased sensitization of the cholinergic receptors or prostaglandins, results

in the contraction of the lung smooth muscle which causes increased airway resistance and rapid shallow breathing.

An experimental animal study using isolated guinea pig lung (Joad et al., 1996) has demonstrated that acute ozone exposure increased responsiveness to methacholine, an effect which was reduced by depletion of C-fibers. The authors concluded that ozone, in the absence of systemic effects and the blood components of inflammation, increased muscarinic reactivity in part via the local effects of C-fibers.

A study conducted by Hazbun et al. (1993) has provided evidence of ozone-induced stimulation of bronchial C fibers in human subjects. In this study, healthy, nonsmoking subjects (22 to 30 years, n = 7) were exposed to filtered air and 0.25 ppm ozone for 1-hour with heavy exercise ($\underline{V}_E$ ~60 L/min). Their data demonstrate a significant increase in substance P, the neurotransmitter released from the afferent endings of bronchial C fiber by stimulus, in airway lavage fluids after ozone exposure in comparison with air control. This study also demonstrated that airway substance P levels were significantly correlated with an elevated airway concentration of 8-epi-prostaglandin $F_{2\alpha}$ ($r^2 = 0.89$). Lung C fibers have been shown to be stimulated by prostaglandin E_2 and other lung autacoids (Coleridge et al., 1976; Coleridge et al., 1978). Recent studies by Liu et al. (1997b) and Hazucha et al. (1996) have shown that pre-treatment with the cyclooxygenase inhibitors which inhibit the synthesis of prostaglandins, can reduce ozone-induced pulmonary function decrements in human subjects. In the study by Liu et al. (1997b) pre-treatment of acetylsalicylic acid (ASA, 0.975 g) significantly reduced 0.12 and 0.4 ppm ozone-induced increments in airway resistance, and 0.4 ppm ozone-induced decrements of peak expiratory flow rate. In the study by Hazucha et al. (1996), pre-treatment of ibuprofen (800 mg) significantly reduced 0.4 ppm ozone-induced decrements of FEV_1 and FVC, but did not alter ozone-induced increase in airway resistance. Ibuprofen pre-treatment significantly decreased post-ozone BAL levels (obtained 18 hours post exposure) of prostaglandin E_2 by 60.4%, and thromboxin B_2 by 25.5%. However, there was no correlation between post exposure prostaglandin concentrations in BAL and FEV_1 or FVC, presumably due to differences in the time courses of the development of functional and biochemical responses.

Some authors (Gerrity et al., 1994; Liu et al., 1997b) have postulated that the rapid shallow breathing may in fact be a defence mechanism that prevents ozone from reaching deep airways. Data from Gerrity et al. (1994) show that ozone-induced decrease in tidal volume (25%) is significantly correlated with a decreased ozone uptake efficiency of the lower respiratory tract. Data from Liu et al. (1997b) also demonstrate a more rapid and shallow breathing following exposure to 0.4 ppm ozone compared with exposure to air control and 0.12 ppm ozone. Concomitantly, the increase of *in vivo* hydroxyl radical production was not proportional to exposure concentrations of ozone; a threefold increase in ozone concentration did not cause an increase of hydroxyl radical production to the same extent, probably due to the modification of breathing pattern at high ozone concentration (Liu et al., 1997b). More studies are required to examine the effects of ozone responsiveness, the distribution in the airways and the resulting airway inflammation.

11.1.2 Subjects with Pre-existing Disease

This section includes subjects with (1) chronic obstructive pulmonary disease (COPD), (2) asthma, (3) allergic rhinitis, and (4) ischemic heart disease.

Subjects with Chronic Obstructive Pulmonary Disease

To summarize the U.S. EPA criteria document (1996), in previously reported studies (Linn et al., 1982a; Linn et al., 1983; Solic et al., 1982; Kehrl et al., 1985), no significant changes in pulmonary function or symptoms were detected in COPD patients in response to ozone exposure. In four of these five studies (Linn et al., 1982a; Linn et al., 1983; Solic et al., 1982; Kehrl et al., 1985), researchers studied patients with mild to moderate COPD who were exposed to 0.1 to 0.3 ppm ozone for 1 to 2-hours with intermittent light exercise with a $\underline{V}_E$ of 14 to 28 L/min. No consistent changes of arterial oxygen saturation were observed in these studies. However, based on the assumption that the response of pulmonary function to ozone may be a protective mechanism for the lungs from receiving more ozone in deep airways, failure of COPD patients to have pulmonary function responses to ozone might render these patients more susceptible to the pulmonary injury.

Very recently, Gong et al. (1997b) compared the responses to ozone exposure between COPD patients and the same age healthy subjects. Healthy subjects (60 to 69 years, n = 10) and subjects with severe COPD (59 to 71 years, n = 9) were exposed to air or 0.24 ppm ozone for 4-hours during intermittent light exercise ($\underline{V}_E$ 20 L/min). Symptoms, lung function, and arterial oxygen saturation were monitored. Overall COPD subjects had a 19% loss of FEV_1, while healthy subjects had a 2% loss ($p<0.001$). There were no significant changes in airway resistance in both groups following ozone exposure. Ozone exposure caused moderate changes in symptoms, and blood oxygenation in COPD patients, but not in healthy subjects. COPD patients appeared to be more sensitive to 0.24 ppm ozone-induced pulmonary function decrements and symptoms than healthy subjects.

Overall, more studies are required before a conclusion can be drawn regarding the susceptibility of COPD patients to ozone exposure.

Subjects with Asthma

In summary of the U.S. EPA criteria document (1996), studies by Linn et al. (1978), Silverman (1979), Koenig et al. (1985, 1987, 1988) have shown that no significant decrements in group pulmonary function could be elicited with ozone exposures ranging from 0.12 to 0.25 ppm for 40 minutes to 2-hours at rest or using light to moderate intermittent exercise ($\underline{V}_E$ 30 to 40 L/min) protocols in both adults and adolescents. Kreit et al. (1989) and Eschenbacher et al. (1989) used a heavy exercise protocol ($\underline{V}_E$ -60 L/min) and ozone exposure at 0.40 ppm for 2-hours. Their data demonstrate that significant decreases in FVC, FEV_1, ratio of FEV_1 to FVC, and $FEF_{25-75\%}$ can be elicited in both normal and asthmatic subjects. Asthmatic patients were found to have greater decrements in all parameters except FVC and subjective symptoms when compared with normal subjects. Bronchial responsiveness as measured by the concentration of methacholine bronchoconstrictor required to increase 100% of airway resistance ($PC_{100SRaw}$) was also evaluated in these studies. While the asthmatic group had a lower baseline than the normal subjects, the percentage increase of bronchial responsiveness after ozone exposure was similar in normal and asthmatic subjects. These findings indicate that if the total inhaled dose (or effective dose) is

increased significantly by either increasing $\underline{V}_E$ during exposure or ozone concentration, mild to moderate asthmatics will respond with a greater obstructive response than will normal subjects.

Using a prolonged exposure protocol, Linn et al. (1994) showed that exposure of asthmatic patients (18 to 50 years old, n = 30) to 0.12 ppm ozone for 6.5-hours with exercise ($\underline{V}_E$ 29 L/min), resulted in a statistically significant decrease in FEV_1. Exposure of healthy subjects (22 to 41 years old, n = 15) to the same protocol did not cause any spirometric changes. There was a greater but not statistically significant reduction in FEV_1 following ozone in asthmatics than in healthy subjects (-8.6% versus -1.7%).

Horstman et al. (1995) compared pulmonary function responses and symptoms of asthmatic (19 to 32 years old, n = 17) and non asthmatic (18 to 35 years old, n = 13) subjects to ozone. Subjects were exposed to 0.16 ppm ozone or air for 7.6-hours with intermittent light exercise ($\underline{V}_E$ 30 L/min). In this prolonged exposure study using low ozone concentration, asthmatic patients revealed significantly more decrements in FEV_1 and FEV_1/FVC than did healthy subjects. Nine of 17 asthmatics experienced wheezing with ozone, while no healthy subjects experienced wheezing. Six of 17 asthmatics requested inhaled β-agonist bronchodilator prior to and/or during ozone exposure and experienced some temporary alleviation of decrements. At the end of exposure, asthmatics who were medicated had greater ozone-induced decrements than those who were not medicated. Asthmatic subjects who had the larger ozone-induced FEV_1 decrements had lower baseline FEV_1/FVC and lower baseline % predicted FEV_1. It appears that in asthmatics, unlike non asthmatics, some portion of ozone-induced pulmonary function decrements experienced is related to bronchoconstriction, and that ozone-responsiveness for asthmatics depends upon baseline airway status.

Jorres et al. (1996) compared ozone-induced pulmonary function changes, symptoms and responsiveness to allergens among patients with mild stable allergic asthma (21 to 31 years old, n = 24), patients with allergic rhinitis without asthma (25 ± 3 years old, n = 12), and healthy subjects (23 ± 2 years old, n = 10). Subjects were exposed to filtered air or 0.25 ppm ozone for 3-hours during intermittent exercise ($\underline{V}_E$ 30 L/min). The concentration of methacholine ($PC_{20}FEV_1$) and the dose of allergen ($PD_{20}FEV_1$) producing a 20% fall in FEV_1 were determined. In asthmatic patients, FEV_1 decreased by 12.5 ± 2.2%, $PC_{20}FEV_1$ of methacholine by 0.9 ± 0.19 doubling concentrations, and $PD_{20}FEV_1$ of allergen by 1.74 ± 0.25 doubling doses, after ozone exposure. In the subjects with rhinitis, mean FEV_1 decrease was 14.1 ± 3.0% following ozone exposure. While allergen treatment worsened ozone-induced FEV_1 decrement, the responsiveness to methacholine was not altered by ozone exposure. In healthy subjects, mean FEV_1 decreased by 10.2 ± 3.5% following ozone exposure. Allergen did not substantiate ozone-induced FEV_1 decrement in healthy subjects, and the responsiveness to methacholine was not altered by ozone exposure. There were no differences in ozone-induced symptoms among three groups. The data indicate that short term exposure to ozone can enhance bronchial responsiveness to inhaled allergens in asthmatic subjects, and likely in rhinitis subjects without asthma, but not in healthy subjects.

In the study conducted by McBride et al. (1994), researchers exposed 10 asthmatic subjects with histories of allergic rhinitis and 8 healthy subjects to 0, 0.12 and 0.24 ppm ozone for 90 minutes

with light intermittent exercise ($\underline{V}_E$ 23 L/min). The authors did not find significant changes in pulmonary and nasal function in two groups of subjects. However, in asthmatic patients, a significant increase in the number of white blood cells in nasal lavage fluid was detected both immediately and 24 hours after exposure to 0.24 ppm ozone, as was a significant increase in epithelial cells immediately after exposure. A significant correlation was observed between interleukin-8 (IL-8) and white blood cell counts in the nasal lavage fluids after exposure to 0.24 ppm ozone (r = 0.76), although the levels of IL-8 were not significantly elevated after exposure to ozone. In healthy subjects, no significant changes in pulmonary or nasal function, or biochemical and cellular components were found in non asthmatics. The above studies suggest that asthmatic subjects have an exaggerated airway inflammatory response to acute ozone exposure, even when lung function is not affected.

However, in a study conducted by Hiltermann et al. (1995), data regarding ozone-induced hyperresponsiveness in asthmatic subjects are not quite consistent with reports by other groups. In this study, six asthmatic patients (22 to 27 years old) and 6 healthy subjects (20 to 40 years old) were exposed to air or 0.4 ppm ozone during 2-hours of exercise ($\underline{V}_E$ ~40 L/min). The authors found that FEV_1 dropped following ozone exposure to a similar extent in both asthmatic (15.3 ± 3.7%) and non-asthmatic (15.2 ± 4.0%) subjects. The percent changes of responsiveness to methacholine were significantly smaller in asthmatic subjects than in healthy subjects. However, asthmatic patients had higher baseline values for FEV_1 and responsiveness to methacholine challenge, indicating a pre-compromised physiological status. There was a significant rise in percent PMN in sputum in all subjects; however no correlation could be found between the changes in pulmonary function tests and those in cellular parameters. Since asthmatic patients have been 'pre-disposed' to a higher baseline value of responses, ozone exposure might facilitate the induction of an exacerbation of asthma, while the maximal plateau of responses induced by ozone may be independent of an underlying respiratory disease state.

Fernandes et al. (1994) investigated the effect of pre-exposure to 0.12 ppm ozone on exercise-induced asthma. Non smoking (26 ± 7 years old, n = 15) subjects with mild stable asthma who had exhibited a fall in FEV_1 >15% after a standard 6 minute treadmill exercise challenge test were exposed to ozone or air for 1-hour at rest. The exposure was followed by a 6 minute exercise challenge; the treadmill speed and inclination were such that patients achieved their maximal exercise. The percent fall in FEV_1 and partial expiratory flow at 40% of vital capacity (V_{40p}) were not significantly different following exposure to ozone or air. The effects of exposure on the time course of spirometric responses after exercise showed no significant difference between air and ozone exposure. A study conducted by Weymer et al. (1994) comparing the responses of exercise-induced asthmatic versus non exercise-induced asthmatic patients to ozone reached a similar conclusion. In this study, of 21 asthmatic subjects, nine showed exercise-induced asthma positive, and 12 showed exercise-induced asthma negative. Subjects were exposed to 0, 0.1 or 0.25 ppm ozone for 1-hour with intermittent light exercise ($\underline{V}_E$ 27 L/min). Subjects then rested for 1-hour followed by a post exposure standardized exercise challenge in air. The researchers did not find significant changes in FEV_1 or FVC following 1-hour exposure to 0, 0.1 and 0.25 ppm ozone (regardless of exercise-induced asthma status). Increasing ozone concentration did not produce significant differences in FEV_1 response to post exposure exercise challenges for either the complete group or the subgroups with and without exercise-induced asthma. Thus, the researchers

concluded that pre exposure to ozone does not sensitize the airways to subsequently produce greater exercise-induced airflow obstruction. Ozone-induced effects on airway mechanics are not additive or synergistic with exercise-related responses in asthmatics.

In summary, recent studies show that asthmatic subjects do not reveal substantially higher response in pulmonary function compared with healthy subjects when exposed to 0.12 to 0.4 ppm ozone for less than 3-hours. Pre-exposure to ozone (0.1 to 0.25 ppm for 1-hour at rest or with light exercise) does not aggravate exercise-induced asthma. However asthmatic subjects appear to have higher airway cellular responses (McBride et al., 1994) and higher responsiveness to allergens (Jorres et al., 1996; Hiltermann et al., 1995) than healthy subjects under these exposure conditions. With prolonged exposure (6.5 to 7.6-hours) to low concentrations of ozone (0.12 to 0.16 ppm) (Linn et al., 1994; Horstman et al., 1995), asthmatic subjects reveal higher pulmonary function response and symptoms than healthy subjects. These suggest that FEV_1 may not be a sensitive indicator for individual susceptibility to ozone insult. Because of the pre-existing compromised pulmonary function and an elevated value for bronchial responsiveness to methacholine in asthmatic patients compared with healthy people, ozone exposure might facilitate the induction of an exacerbation of asthma. The underlying mechanism that would explain a possible increased responsiveness of asthmatic subjects to ozone requires additional study.

Subjects with Allergic Rhinitis

Data on subjects with allergic rhinitis (inflammation of the nose or mucous membrane) and asthmatic subjects suggest that both of these groups have a greater rise in airway resistance to ozone (0.18 ppm for 2-hours with intermittent exercise) with a relative order of airway responsiveness to ozone being normal < allergic < asthmatic (McDonnell et al.,1987). Other early results have shown that in subjects with allergic rhinitis, acute exposure to ozone does not alter acute response to nasal challenge with antigen.

Jorres et al. (1996) investigated ozone-induced pulmonary function changes, symptoms and responsiveness to allergens in patients with mild stable allergic asthma (21 to 31 years old, n = 24), patients with allergic rhinitis without asthma (25 $\pm$ 3 years old, n = 12), and healthy subjects (23 $\pm$ 2 years old, n = 10). Subjects were exposed to filtered air or 0.25 ppm ozone for 3-hours during intermittent exercise ($\underline{V}_E$ = 30 L/min). The concentration of methacholine ($PC_{20}FEV_1$) and the dose of allergen ($PD_{20}FEV_1$) producing a 20% fall in FEV_1 were determined. In the subjects with allergic rhinitis, mean FEV_1 decrease following ozone exposure was equivalent to the FEV_1 decrements found in asthmatic patients. While allergen treatment worsened ozone-induced FEV_1 decrement, the responsiveness to methacholine was not altered by ozone exposure in rhinitis patients. In healthy subjects, mean FEV_1 decreased by 10.2 $\pm$ 3.5% following ozone exposure. Allergen did not substantiate ozone-induced FEV_1 decrement in healthy subjects, and the responsiveness to methacholine was not altered by ozone exposure. There were no differences in ozone-induced symptoms among the three groups. The data indicate that short term exposure to 0.25 ppm ozone can enhance bronchial responsiveness to inhaled allergens in allergic rhinitis subjects as well as in asthmatic subjects, but not in healthy subjects.

Subjects with Ischemic Heart Disease

Only one study has been reported in the U.S. EPA criteria document (1996) concerning the cardiopulmonary effects of acute ozone inhalation in patients with ischemic heart disease. Ischemic heart disease is caused by a deficiency of blood supply to the heart muscle due to obstruction or constriction of the coronary arteries. Six middle-aged male patients were exposed to air and 0.2 to 0.3 ppm ozone for 40 minutes with angina-symptom-limited exercise (mean $\underline{V}_E$ = 35 L/min). No significant pulmonary function impairment or evidence of cardiovascular strain induced by ozone inhalation was detected, but limitations in the study preclude any final conclusion.

In a study conducted by Drechsler-Parks (1995a), eight elderly healthy subjects (56 to 85 years of age) were exposed to either air or 0.45 ppm ozone, or 0.6 ppm nitrogen dioxide, or two pollutants in combination, for 90 minutes with intermittent exercise. Cardiac output was monitored by non-invasive impedance cardiography method, before and during the last 5 minutes of each exercise period. Exposure to ozone or nitrogen dioxide alone did not significantly modify cardiac output in comparison with air control. The exercise-induced increase in cardiac output was significantly smaller when subjects were exposed with nitrogen dioxide/ozone exposure than with air or ozone alone. The author speculates that the observed effects of nitrogen dioxide/ozone might be because nitrogen dioxide reacted with ozone and formed nitrate and nitrite, which might function as a vasodilator and subsequently caused a reduction of cardiac output.

Thus, due to the limited data reported, the responsiveness of subjects with ischemic heart disease to ozone exposure is inconclusive.

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

Gender Differences.

Based on the U.S. EPA criteria document (1996), the question as to whether there is a difference in responsiveness to ozone between men and women remains unresolved. Different conclusions can be drawn depending on whether $\underline{V}_E$ is normalized to body weight or lung size when the inhaled doses of ozone are calculated.

Frampton et al. (1997) exposed 56 never-smoking (age 25 $\pm$ 4 years, including 20 females) and 34 smoking (age 28 $\pm$ 1 years including 6 females) healthy subjects to 0.22 ppm ozone and air for 4-hours with exercise. By using multiple logistic regression analysis the authors found that gender, age and methacholine responsiveness were not predictive of responder status, although pack-year (annual consumption) of smoking was associated with decreased ozone responsiveness (OR = 0.87, p = 0.017).

Seal et al. (1996) investigated the effects of gender, age, socio-economic status and menstrual cycle phase on the pulmonary response to ozone exposure. Healthy African American and Caucasian subjects (18 to 35 years old, n = 372) were exposed once to air or 0.12, 0.18, 0.24, 0.3, or 0.4 ppm ozone for 2.3-hours with intermittent exercise ($\underline{V}_E$ -45 - 55 L/min). Logistic function was used to model the exposure-response relationship, and the significant impact of gender, age, social economic status and menstrual cycle phase. While the researchers found that there were

age and socio-economic effects on ozone-induced pulmonary function alterations, they did not find a significant gender effect, nor menstrual cycle effect, on individual sensitivity to ozone.

Data from a study conducted by Weinmann et al. (1995b) also show that there were no gender differences in pulmonary function responses to ozone. The authors exposed 24 healthy nonsmokers (12 men, age 27 ± 1 years; 12 women, age 25 ± 1 years) to 0.35 ppm ozone and air for 130 minutes with intermittent exercise ($\underline{V}_E$ 38 L/min). In order to detect the effects of ozone on small airway function, the researchers measured isovolumetric FEFs (forced expiratory flows), helium-oxygen ($He\text{-}O_2$), volume of isoflow (V_{isoV}), the multiple breath nitrogen washout (MBNW) curve, functional residual volume (FRC), and residual volume (RV) 24 hours after exposure. FEFs, $He\text{-}O_2$ and V_{isoV} were adjusted for any changes in FVC that occurred as a consequence of exposure, so that the pre and post exposure comparisons are isovolumetric and are designated as isoV. No gender differences in ozone responsiveness in small airway function were found, although isovolume (isoV) $FEF_{25\text{-}75}$, $Vmax_{50}$, $Vmax_{75}$ were reduced in all subjects combined at 25 minutes after ozone exposure, and showed no recovery at 24 hours.

Bush et al. (1996) studied the longitudinal distribution of ozone absorption in airways, and found that the absorption distribution of ozone in resting men and women was indistinguishable, although women absorbed ozone at smaller penetration volume than the men, and women had smaller dead space volume than the men. They concluded that previously reported gender differences may be due to a failure in properly accounting for tissue surface within the conducting airways.

Overall, the current literature does not suggest that there is a substantial gender difference in the responsiveness to ozone.

Hormonal Influences

Data reviewed in the U.S. EPA document (1996) did not permit any final conclusion on the influence of the menstrual cycle. The group sizes were again small for 2 (Fox et al., 1993; Gerbase et al., 1993) of the 3 studies and resulted in opposite conclusions. The third study (Seal et al.,1993a) compared race (African-American and Caucasian) and menstrual phase, obtaining a significant interaction between race and menstrual phase, but post hoc analysis failed to establish a basis for the interaction.

In a recent study by Seal et al. (1996), healthy women (18 to 35 years old, n = 150) were studied for the menstrual cycle effects on ozone-induced responses. The women were grouped into the appropriate menstrual cycle phase according to a menstrual history obtained immediately prior to ozone exposure. Subjects were exposed once to air or 0.12, 0.18, 0.24, 0.3, or 0.4 ppm ozone for 2.3-hours with intermittent exercise ($\underline{V}_E$ -45 - 55 L/min). Logistic function analysis did not show any effects of menstrual cycle phase on ozone-induced pulmonary function responses.

Weinmann et al. (1995b) examined the effects of menstrual cycle phase on the responsiveness of small airway function parameters to ozone. Nine women were exposed to 0.35 ppm ozone for 130 minutes with intermittent exercise ($\underline{V}_E$ = 38 L/min), during the follicular and luteal phases. Ozone induced significant small airway dysfunction in all the subjects indicated by the reduction of

isovolume FEF_{25-75}, $Vmax_{50}$, $Vmax_{75}$, as well as FVC and FEV_1. Neither the spirometric nor symptomatic responses differed significantly as a function of the menstrual cycle phase.

Age Differences

It has been hypothesized that age may be a factor in responsiveness to ozone. Few chamber studies exist on subjects under 18 (U.S. EPA, 1996), but field and epidemiology studies attempting to relate ambient air pollutant exposure to pulmonary function in children have suggested that children may be more responsive to ambient air pollution than young adults.

The response of adolescents and adults are not substantially different based on studies reported in the U.S. EPA document (1996). One of the problems that the authors noted seems to be the difficulty for children to perform consistent, reproducible pulmonary function tests.

Frampton et al. (1997) recently conduced exposure tests of healthy subjects (25 to 28 years of age, n = 90) to 0.22 ppm ozone or air for 4-hours with exercise ($\underline{V}_E$ for smokers and nonsmokers was 39 to 45 L/min and 43 to 46 L/min, respectively). Multiple logistic regression analysis did not show that age had any impact on individual pulmonary function responses to ozone exposure, although the amount of cigarette smoking was significantly associated with decreased ozone responsiveness (OR = 0.87). It should be noted that the narrow range of age in this study precludes any final conclusion on age effects.

Seal et al. (1996) exposed healthy African American and Caucasian subjects (18 to 35 years old, n = 372) once to air or 0.12, 0.18, 0.24, 0.3, or 0.4 ppm ozone for 2.3-hours with intermittent exercise ($\underline{V}_E$ -45 - 55 L/min). Logistic function was used to model the exposure-response relationship, and the significant impact of age, social economic status and menstrual cycle phase. The ages of subjects within the studied range had an effect on sensitivity to ozone; younger subjects appear to be more responsive to ozone-induced decrements in FEV_1.

McDonnell et al. (1993) examined factors affecting ozone-induced FEV_1 decrements using a more homogeneous population, and found similar results. The authors exposed 290 white male subjects(18 to 32 years of age) to 0, 0.12, 0.18, 0.24, 0.3 or 0.4 ppm ozone for 2-hours with intermittent exercise ($\underline{V}_E$ -70 L/min). Linear, quadratic, cubic and two sigmoid-shape models (the logistic and Gompertz functions) were used to describe decrements in FEV_1 as a function of ozone. Their model predicted a decreasing response with increasing age for all nonzero ozone concentrations. For exposure to 0.4 ppm, the model predicts decrements in FEV_1 of 1.07 and 0.47 L for 18 and 30 year old subjects, respectively. They concluded that for white males, age within the study range was significant predictor of response, with older subjects being less responsive to ozone.

The available data in the U.S. EPA document (1996) indicate that responsiveness to ozone is decreased in persons over 50 years of age compared to young adults. Recently Drechsler-Parks (1995b) investigated pulmonary function responses in 9 male healthy elderly subjects (56 to 71 years) following exposure to air and 0.45 ppm ozone for 2-hours. Exposure concentrations of ozone were altered by varying $\underline{V}_E$ through exercise. At $\underline{V}_E$ of 25, 40 and 55 L/min, ozone exposure

induced small, significant decrements in FVC (-5.31%, -6.47% and -5.29%, respectively) and FEV_1 (-7.54%, -7.59% and -10.43%, respectively), but no significant changes in $FEF_{25-75\%}$. There were no significant differences among responses to the three ozone exposures. When taking into account the effective dose of ozone (concentration x $\underline{V}_E$ x duration), ozone-induced decrements of pulmonary function in older subjects were much smaller than those typically observed in young males exposed to similar inhaled doses of ozone.

More recently, Gong et al. (1997b) studied the effects of ozone on elderly healthy subjects (60 to 69 years, n = 10) and subjects with severe COPD (59 to 71 years, n = 9). Subjects were exposed to air or 0.24 ppm ozone for 4-hours during intermittent exercise ($\underline{V}_E$ = 20 - 30 L/min). Symptoms, lung function, and arterial oxygen saturation were monitored. While COPD subjects had 19% loss of FEV_1, healthy subjects had only 2% loss of FEV_1. There were no significant changes in airway resistance in both groups following ozone exposure. Ozone exposure did not cause changes in symptoms, and blood oxygenation in healthy subjects. Four-hour exposure to 0.22 ppm ozone has been shown to initiate >15% of decrements in FEV_1 in young healthy subjects with moderate exercise ($\underline{V}_E$ -40 - 46 L/min) (Torres et al., 1996; Frampton et al., 1997). Thus, overall elderly subjects are revealed to be less sensitive to ozone-induced pulmonary function decrements and symptoms than young healthy subjects.

Ethnic and Racial Factors

Only one study (Seal et al., 1993b) has been conducted to investigate these potential factors in the U.S. EPA document (1996). Although 372 subjects (approximately 90/sex/race—African American & Caucasian), each subject only participated in one experimental session (6 dose groups ranging from 0.00 to 0.40 ppm ozone). A multiple comparison analysis was used to identify specific differences among the gender-race groups when an overall group effect was detected. African American males experienced significant decrements in FEV_1 following exposure to 0.120 ppm of ozone, whereas black women and white men and women did not have significant decrements in FEV_1 at ozone concentrations below 0.180 ppm. The results are suggestive of an ethnic difference, but more subjects must be studied before a final conclusion can be reached.

In recent years, no new studies have been reported regarding the race effects on ozone responsiveness.

Environmental Factors

A number of environmental factors, such as ambient temperature and humidity, season of the year, route of inhalation, and smoking history have been hypothesized to potentially impact on responses to ozone exposure in additive or synergistic ways. To date, none of these factors have been studied adequately to reach final conclusions. The few studies performed to examine the effects of temperature and humidity produced, at best, equivocal results.

The issue of nasal versus oral inhalation of ozone has been studied to some extent. This issue is important since chamber studies have been performed with subjects breathing freely while others used mouthpiece exposure systems. The results from the studies by Hynes et al. (1988) and

Adams et al. (1989) would tend to demonstrate that there is no difference in pulmonary function regardless of the route of inhalation exposure.

Two studies reported in the U.S. EPA document (1996) demonstrated that current smoking may blunt responsiveness to ozone exposure (Emmons & Foster, 1991) and that smoking cessation for 6 months leads to improved baseline pulmonary function and possibly the re-emergence of ozone responsiveness (Frampton et al., 1993).

Data published recently by Frampton et al. (1997) support these observations. In this study, 56 never-smoking (age 25 $\pm$ 4 years) and 34 smoking (age 28 $\pm$ 1 years) healthy subjects were exposed to 0.22 ppm ozone and air for 4-hours with exercise ($\underline{V}_E$ for smokers and nonsmokers was 43 to 46 L/min and 39 to 45 L/min, respectively). Multiple logistic regression analysis reveals pack-year of smoking to be associated with decreased ozone responsiveness (OR = 0.87, p = 0.017); healthy smokers had smaller decrements of FEV_1 in response to ozone exposure than did nonsmokers. Smokers reported fewer respiratory symptoms than did nonsmokers following ozone exposure, but the difference was significant only for cough. The researchers speculate that the decreased responsiveness of smokers to ozone might be because the oxidative chemicals in cigarette smoke attenuated irritant-receptor responsiveness and increased the mucous layer lining the airways in smokers.

11.1.4 Co-pollutants

Ambient air pollution is composed of a complex mixture of gases and particles, and therefore potential interactions among various pollutants should be taken into consideration.

The U.S. EPA criteria document (1996) reviewed the evidence for the interactions between ozone and co-pollutants, and concluded that no significant enhancement of respiratory effects (more than additive) in healthy subjects has been consistently demonstrated for mixtures of ozone (0.12 to 0.16 ppm, 1 to 2-hours) with ambient concentrations of SO_2, NO_2, H_2SO_4, HNO_3, and peroxyacetyl nitrate (PAN). At higher concentrations, PAN (0.30 ppm) combined with ozone exposure (2-hours) has resulted in greater pulmonary function responses than ozone (0.485 ppm) alone in healthy subjects (Horvath et al., 1986).

In asthmatic adolescents, pre exposure to ozone (0.12 ppm, 45 minutes, $\underline{V}_E$ = 30 L/min) followed by low level of SO_2 (0.1 ppm, 15 minutes) elicited a greater degree of bronchial responsiveness than did pure air followed by SO_2 or by two exposures to ozone alone, indicating some synergism between the effects of SO_2 and ozone (Koenig et al.1990). Both ozone and SO_2 concentrations were at subthreshold levels for the experimental design used.

Recent studies support the previous observations. Frampton et al. (1995) evaluated symptoms and pulmonary function responses of 30 healthy (28 $\pm$ 5 years old) and 30 allergic asthmatic (28 $\pm$ 7 years old) subjects to sulphuric acid aerosol and ozone. Each subject was studied 4 times, receiving 100 $\mu g/m^3$ sulphuric acid (MMAD [mass median average diameter] 0.66 μm) or NaCl aerosols (MMAD 0.45 μm), for 3-hours as pre-exposure. One day later (24 hours), the subject was exposed to 0.08, 0.12, or 0.18 ppm ozone for 3-hours. During each exposure, subjects performed

intermittent exercise ($\underline{V}_E$ 33 to 40 L/min). In healthy subjects, there was no convincing evidence for a symptomatic or physiologic effect of exposure to either NaCl or sulphuric acid aerosols or ozone alone, and there was no clear evidence for an effect of aerosol pre-exposure on the ozone response. In asthmatic patients, ozone (starting from 0.08 ppm), NaCl and sulphuric acid aerosols independently caused decrements of FVC and FEV_1. Sulphuric acid aerosol altered the pattern of spirometric response to ozone in comparison with NaCl pre-exposure and appeared to enhance the small mean decrements in FVC and FEV_1 that occurred in response to 0.18 ppm ozone. Individual responses among asthmatics varied markedly, some demonstrating reductions in FEV_1 of more than 35% following ozone exposure.

Pre exposure of healthy subjects (18 to 35 years old, n = 21) to NO_2 followed by ozone has shown to enhance ozone-induced spirometric changes (Hazucha et al., 1994). Subjects were exposed to 0.6 ppm NO_2 or air for 2-hours, and 3-hours later exposed to 0.3 ppm ozone for 2-hours. Subjects performed intermittent exercise ($\underline{V}_E$ - 40 L/min). NO_2 alone did not reduce FEV_1, but did significantly enhance ozone-induced spirometric changes. No significant effects were observed in airway resistance. Following the NO_2/ozone exposure, the median bronchial responsiveness to methacholine was significantly reduced compared with the air/ozone exposure.

Utell et al. (1994) studied the effect of pre exposure to acid aerosols on ozone-induced pulmonary function and symptoms. Healthy (18 to 45 years, n = 30) and asthmatic (18 to 45 years, n = 30) nonsmoking subjects were exposed to particulate aerosols (sulphuric acid 100 $\mu g/m^3$ or NaCl 100 $\mu g/m^3$) for 3-hours. Twenty-four hours later, subjects were exposed to ozone (0.08, 0.12 and 0.18 ppm) for 3-hours. Intermittent exercise was performed during both exposures ($\underline{V}_E$ for healthy subjects was 40 L/min, for asthmatics was 30 to 36 L/min). In healthy subjects, no lung function changes or symptoms were seen in either aerosol or ozone exposures. No interaction of either particle with ozone was found in healthy subjects. In asthmatic subjects, pre exposure to sulphuric acid had no direct effect on lung function but appeared to enhance the small mean decrements in FVC that occurred in response to 0.18 ppm ozone. Therefore, exposure to ozone and other pollutants in a sequential fashion seems to reveal a synergistic effect on ozone-induced spirometric responses.

Linn et al. (1995) exposed asthmatic children (11 to 18 years, n = 24) to clean air, or 0.2 ppm ozone + 0.3 ppm NO_2, or ozone + NO_2 + sulphuric acid aerosol (MMAD 0.66 μm, concentration equivalent to 127 $\mu g/m^3$ sulphuric acid) with intermittent exercise ($\underline{V}_E$ = 32 L/min) for 90 minutes. Subjects gargled lemonade to minimize acid neutralization by oral ammonia. Differences in group mean FEV_1 among ozone/NO_2/acid aerosol, ozone/NO_2 and air exposures were not significant. An earlier study conducted by the same research group (Linn et al., 1994) has shown that repeated, multi-hour exposure of healthy and asthmatic adults to a low level of ozone (0.12 ppm with exercise $\underline{V}_E$ at 29 L/min) plus sulphuric acid aerosol (100 $\mu g/m^3$ respirable acid aerosol, MMAD 0.5 μm) did not cause substantially higher spirometric and symptomatic changes than exposure to ozone alone. However both asthmatic and healthy subjects showed substantial diversity with respect to the individual responsiveness to the combined exposure.

In addition to pulmonary function, Aris et al. (1993) also examined airway inflammatory responses in 10 healthy subjects following exposure to 500 $\mu g/m^3$ of HNO_3 alone, or HNO_3 + 0.2 ppm ozone, or ozone alone, for 4-hours. During exposure, subjects performed intermittent exercise (40 L/min). BAL, PAL (proximal airway lavage) and bronchial biopsy (to see proximal airway injury) were performed 18 hours after exposure. While exposures to HNO_3/ozone and ozone alone were associated with significant decrements in FEV_1 and FVC and increases in airway resistance, and increases of neutrophils, proteins, albumin, fibronectin, and α1-antitrypsin in BAL; there were no significant differences in these endpoints between the HNO_3/ozone and ozone exposure. There were no differences in pulmonary function parameters or in the cellular or biochemical constituents in either BAL or the PAL between HNO_3 and air exposure. There were no significant differences in the bronchial biopsy specimens between the HNO_3 and air exposures, or between the HNO_3/ozone and ozone exposures. Thus, the data do not suggest that HNO_3 gas causes either proximal airway or distal lung injury or that HNO_3 potentiates the inflammatory response produced by ozone in healthy individuals.

The assessment of such mixtures is, therefore, far from complete and much work needs to be done. Few studies have included more than two chemicals, and the endpoints measured are often limited to pulmonary function variables.

11.1.5 Repeated Exposures to Ozone

A repeated exposure study is designed to determine health effects after several consecutive days of exposure to ozone. As summarized in the U.S. EPA document (1996), the spirometric responses to repeated ozone exposure typically show that the response is increased on the second exposure day to concentrations in the range of 0.40 to 0.50 ppm ozone in exposures accompanied by moderate exercise. This phenomenon was not observed at lower concentrations or in groups with relatively small ozone-induced decrements in spirometry.

Two studies (Bedi et al., 1985; Folinsbee et al., 1986) indicated that enhanced spirometric responsiveness was present within 12 hours after exposure to ozone (0.25 to 0.45 ppm for 1 to 2-hours), and lasted for at least 24, and possibly 48, hours, but was clearly absent after 72 hours. After 3 to 5 days of consecutive daily exposure to ozone, responses were markedly diminished or absent. Horvath et al. (1981) had previously observed similar attenuation of lung function decrements following repeated exposure to 0.42 ppm ozone for 5 consecutive days. The authors suggested that the rapidity of this decline in response was related to the magnitude of the subjects' initial responses to ozone or their sensitivity.

Other observations by Gliner et al. (1983) and Brookes et al. (1989) suggest that although pre exposure to low concentrations of ozone (0.2 ppm) may not influence response to higher concentrations, pre exposure to a high concentration of ozone (0.35 ppm) may significantly increase response to a lower concentration (0.2 ppm) the following day.

Schonfeld et al. (1989) also showed that the responses to a second exposure (0.35 ppm ozone) were clearly increased at 24 hours and possibly at 48 hours, but with a 3 or 4 day interval, the responses were similar. The persistence of the attenuation of spirometric and symptom responses

is relatively short-lived, being partially reversed within 3 to 7 days and typically abolished within 1 to 2 weeks. Linn et al. (1982b) have shown that repeated exposure (0.47 ppm ozone) separated by a week for up to 6 weeks did not cause any lessening of the spirometric response.

Repeated prolonged (6 to 7-hours) exposure to low concentrations of ozone has also been examined (Horvath et al., 1991; Linn et al., 1991, 1992). The results appear to show an attenuation of symptoms and pulmonary function changes with repeated exposure to low levels of ozone. Folinsbee et al. (1994) recently exposed 17 healthy adults (25 $\pm$ 4 years of age) to 0.12 ppm ozone for 6.6-hours/day for 5 consecutive days, using an intermittent exercise protocol ($\underline{V}_E$ = 39 L/min). FEV_1 changes averaged -12.79, -8.73, -2.54, -0.6, and +0.18% on the 5 exposure days. Airway responsiveness to methacholine challenge was significantly increased after each ozone exposure. The mean ratios were 2.00, 3.67, 4.55, 3.99, 3.24, and 3.74 for air and ozone days 1 to 5, respectively. Respiratory symptoms increased significantly on ozone day 1 only. Thus, repeated prolonged exposure to low levels of ozone results in progressive but transient attenuation of pulmonary function responses.

In a very recent study conducted by Gong et al. (1997a), mild asthmatic subjects (20 to 48 years old, n = 10) were exposed to 0.4 ppm ozone, 3-hours/day for 5 days, with intermittent exercise ($\underline{V}$= 32 L/min). Follow-up exposures to 0.4 ppm ozone were performed 4 and 7 days after the most recent consecutive exposure to study the tolerance to ozone. Symptoms and decrements of FEV_1 (-35% on day 1, and -34% on day 2) progressively diminished after the third day exposure, with only 6% decrement of FEV_1 on day 5. The tolerance was partially lost 4 and 7 days later, with mean FEV_1 losses of 15% and 17% respectively. Bronchial reactivity to methacholine peaked after the first ozone exposure, and remained elevated after all subsequent ozone exposures relative to the levels in air control. The data indicate that asthmatics can develop tolerance to high level ozone in a similar pattern as normal subjects.

Linn et al. compared healthy subjects versus asthmatics in their adaptation to repeated ozone exposure (Linn et al., 1994). Healthy (22 to 41 years, n = 15) and asthmatic (18 to 50 years, n = 30) subjects were exposed to filtered air, 0.12 ppm ozone, ozone + 100 $\mu g/m^3$ respirable sulphuric acid aerosol (MMAD 0.5 μm), or acid aerosol alone, with six 50-minute exercise periods (29 L/min), total 6.5-hours/day for 2 days. Exposure to ozone alone or ozone plus acid aerosol caused a progressive, significant decline in FEV_1 and a progressive increase in airway resistance, smaller on the second day than the first day in both asthmatic and healthy subjects. Bronchial reactivity to methacholine challenge increased significantly after exposure to ozone with or without acid aerosol on the first day, and declined after the second day exposure. Changes in mean lung function and bronchial reactivity with ozone plus acid exposure were modestly but not significantly larger than changes with ozone alone. A minority of asthmatics and non asthmatics showed substantially greater declines in function with exposure to ozone plus acid relative to ozone alone. Overall ozone-induced changes of FEV_1 and bronchial responsiveness in healthy and asthmatic subjects were attenuated to a similar extent; however asthmatics were more sensitive to ozone or ozone plus acid than healthy subjects.

The U.S. EPA criteria document (1996) summarized a series of studies from the Rancho Los Amigos group. The studies were designed to examine changes in the spirometric and symptomatic responses to ozone in a year including the 'smog season' in the Los Angeles/South Coast Air Basin. The results suggest a seasonal variation in physiological responses that may be attributed to increased ambient ozone exposure during the summer months. In agreement with the findings from repeated ozone exposure studies, 'responders' became less responsive to 0.18 ppm ozone after 'smog season'. However, 'responders' included subjects who had a history of complaints from ambient air pollution. The group also included a significant proportion of allergic individuals, which may have contributed to their varying responses. Historically, studies with subjects from L.A. have reported reduced responses to ozone in the laboratory compared to non-residents (Hackney et al., 1976, 1977).

Besides the absence of pulmonary function responses after repeated ozone exposure, a study by Devlin et al. (1997) has demonstrated that airway inflammatory responses are also attenuated. In this study, healthy young nonsmoking male subjects (20 to 48 years old, n = 16) were exposure to 0.4 ppm ozone, 2-hours/day for 5 days, with intermittent exercise ($\underline{V}$= 60 L/min). Follow-up exposures to 0.4 ppm ozone were performed 10 (9 subjects) or 20 (7 subjects) days after the last exposure day. Bronchoalveolar lavage was performed after 5 days of exposure to ozone and air, and a third time after the 10 or 20 day follow-up ozone exposure. Cell differential, interleukin-6, (IL-6) IL-8, PGE_2 , elastase, fibronectin, protein, α1-antitrypsin, lactate dehydrogenase (LDH), were determined in BAL. Alveolar macrophage phagocytosis of yeast was also measured. Their data demonstrate that after 5 days of exposure, ozone-induced increases in percent PMN, IL-6, prostaglandin E_2, elastase and fibronectin were diminished. Ozone-induced decreases in recovery of viable BAL cells and alveolar macrophage phagocytosis of yeast were blunted. Within 10 to 20 days, percent PMN, IL-6, PGE_2, fibronectin, and recovery of viable cells showed at least partial reversal, while other mediators did not return to the normal response to ozone. It is concluded that biochemical mediators in response to ozone were attenuated after 5 consecutive days of ozone exposure. However, markers of cell injury were not attenuated and may indicate a persistent but not perceived effect of ozone. Inflammatory responses could be invoked to explain at least a portion of the pulmonary function responses.

Airway responsiveness to methacholine is increased with an initial ozone exposure (Holtzman et al., 1979; Folinsbee et al., 1988; Folinsbee et al., 1994; Gong et al., 1997a). One interesting finding is that at low (0.12 ppm) or high (0.4 ppm) concentrations of ozone, responsiveness to methacholine was increased over the 5 days of multi-hour exposure period when compared to air control (Folinsbee et al., 1994; Gong et al., 1997a). However, Kulle et al. (1982) and Dimeo et al. (1981) reported a tendency for the increased responsiveness to diminish with repeated exposure to ozone.

Overall, several conclusions can be drawn about repeated exposure to ozone:

1. Repeated exposure to high concentrations of ozone (0.35 to 0.45 ppm) for 1 to 2-hours can cause an enhanced spirometric response on the second day of exposure. This enhancement appears to be dependent on the interval between the exposures, with 24

hours causing the greatest increase, and absent with intervals ≥ 3 days. An enhanced response also appears to depend to some extent upon the magnitude of the initial response. Small responses to the first ozone exposure are less likely to result in an enhanced response on the second day of ozone exposure.

2. Repeated daily exposure also results in attenuation of spirometric responses, typically after 3 to 5 days of exposure. This attenuation may persist for less than 1 or 2 weeks. In temporal conjunction with the spirometry changes, symptoms induced by ozone, such as cough and chest discomfort are also attenuated with repeated exposure.

3. Ozone-induced changes in airway responsiveness to methacholine attenuate somewhat more slowly than do spirometric and symptom responses. Attenuation of the changes in airway responsiveness may also persist longer than changes in spirometry, although this has only been studied on a limited basis.

4. Exposures at lower concentration for longer duration do not cause an enhanced second-day response. The attenuation of response to ozone appears to proceed more rapidly.

5. Asthmatics can develop tolerance to high level ozone in a similar fashion as normal subjects. When compared with healthy subjects, asthmatics appear to be more sensitive to repeated exposure to ozone than healthy subjects.

6. Limited studies on BAL have also shown that several inflammatory markers may be attenuated following repeated exposure to ozone. This may impair the host defense system, and render subjects susceptible to infectious diseases.

11.1.6 Effects on Exercise Performance

An early epidemiological study examining race performances in high school cross-country runners (Wayne et al., 1967) suggested that exercise performance is depressed by inhalation of ambient oxidant air pollutants. Controlled human studies regarding the effects of ozone inhalation on exercise performance can be divided into two categories, those that examine the effects of acute ozone inhalation on maximal oxygen uptake ($\underline{V}O_{2max}$), and those that examine the effects of acute ozone inhalation on subjects' ability to complete strenuous continuous exercise protocols up to 1-hour in duration.

Effects on Maximal Oxygen Uptake ($\underline{V}O_{2max}$)

To summarize studies reviewed by the U.S. EPA document (1996), when the exercise is preceded by an ozone exposure entailing a sufficient total inhaled dose of ozone to result in significant pulmonary function decrements and/or subjective symptoms of respiratory discomfort, maximal oxygen uptake appears to be reduced. Simply stated, the effective dose is what counts. Exposure to ozone at a concentration of 0.75 ppm for 2-hours with intermittent exercise can result in reductions of $\underline{V}O_{2max}$, maximum ventilation, maximum attained workload, and maximum heart rate

(Folinsbee et al. 1977), whereas the same concentration at rest did not induce any effects except a significantly decreased FVC (Horvath et al., 1979). Other studies have confirmed these findings.

Effects on Endurance Exercise Performance

This effect is more subjectively assessed. Many investigators cited in the U.S. EPA document (1996) have concluded that the observed reductions in exercise performance appear to be due to symptoms limiting the ability of the subjects to perform. However, in every case, this conclusion is reached by exclusion and not by a demonstration of causal relationship.

Ozone concentrations as low as 0.06 ppm have been reported to cause small decrements in performance time during a progressive maximal exercise test (at $\underline{V}_E$ of 30 to 120 L/min for 16 to 28 min) (Linder et al., 1988). Avol et al. (1984a) reported that 3 out of 5 well-conditioned cyclists could not complete the 1-hour exposure to 0.16 ppm ozone, without a reduction in workload.

One hypothesis proposed for ozone-induced reduction of exercise performance is that the stimulation of neural receptors in the airways by ozone may result in an inhibition of α-motor nerve activity to respiratory muscles during inspiration (Koepchen et al., 1977; Schmidt and Wellhoner, 1970). This may result in the observed decrease in V_T (U.S. EPA, 1996), while at the same time increasing the subjects' sensation of respiratory effort. A reflex inhibition of the ability to inspire would be consistent with the reduced V_T following ozone exposure in subjects performing maximal exercise, and would be consistent with the development of a physiologically induced ventilatory limitation to maximal oxygen uptake.

11.2 PULMONARY FUNCTION EFFECTS OF PROLONGED (MULTI-HOUR) OZONE EXPOSURES

A series of studies has described the responses of healthy and asthmatic subjects exposed to relatively low ozone concentrations for durations of 4 to 8 hours. These studies have demonstrated statistically significant changes in lung function, symptoms and airway responsiveness during and after exposures. Unlike repeated-exposure studies, prolonged exposure studies demonstrated lung function decrements in a concentration-dependent fashion, and did not show an attenuation at the end of the exposure.

Two primary studies are described in the U.S. EPA document. The first one, conducted by Folinsbee et al. (1988), utilized an 'EPA prolonged-exposure protocol'. In order to simulate heavy outdoor work or play, healthy nonsmoking male subjects (18 to 33 years, n = 10) exercised at a moderate level ($\underline{V}_E$ at 40 L/min for 50 minutes and 10 minutes rest) for 3-hours, then had a 35 minute (0.6-hour) lunch break (with continuous exposure to ozone at rest) followed by another 3-hours of intermittent exercise. Total exposure time was 6.6-hours. During the intermittent exercise, subjects were exposed to 0.12 ppm ozone. FVC and FEV_1 decreased in a time-dependent pattern during the exposure. Increased symptoms were observed and airway responsiveness to methacholine was approximately doubled after ozone exposure. Likewise, Horstman et al. (1990) adapted the same exercise protocol to expose 22 healthy nonsmoking male subjects (18 to 33 years of age, n = 22) to clean air and 0.08, 0.10, and 0.12 ppm of ozone, and found a similar

response pattern. The decrements of FEV_1 were ozone concentration-dependent, with a concentration as low as 0.08 ppm causing a 7% reduction of FEV_1.

Larsen et al. (1991) applied that information (Horstman et al., 1990) to a lognormal multiple linear regression model to develop a dose—response relationship for percent changes in FEV_1 as a function of ozone concentration and exposure duration. The results suggest that FEV_1 responses were approximately linear with duration of exposure but that ozone concentration plays a more important role. The exponent of approximately 4/3 suggests that doubling ozone concentration would be similar to increasing exposure duration by about 2.5 times (see Figures 11.1 and 11.2).

Other studies such as that conducted by McDonnell et al. (1991a) have assisted in confirming the above findings. The duration-FEV_1 response data were fit to a three parameter logistic model, which significantly improved the amount of variance explained by the model compared to the linear model. The reasonably good fit to the logistic model suggests that the ozone-pulmonary function response relationship may have a sigmoid shape. The main importance of this observation is that it suggests a response plateau. That is, there is a level of response (i.e., plateau) to a given ozone concentration and exercise ventilation level (i.e., dose rate) beyond which decrements in FEV_1 response tend not to increase with increasing duration of exposure.

Folinsbee et al. (1991) utilized the data from 4 different studies conducted at the U .S. EPA Health Effects Research Laboratory using the U.S. EPA prolonged-exposure protocol, and examined the distribution of responses among subjects at 3 different concentrations. Decrements in FEV_1 as large as 30 to 50% were observed with prolonged exposure to 0.12 ppm of ozone or less. In fact, with respect to the distribution, it is noted that none of the subjects showed a drop of >10% in the clean air group, whereas in the 0.08, 0.10, and 0.12 ppm groups, 26, 31, and 46% of the subjects had >10% decrements in FEV_1, respectively. This serves to illustrate the variability of responses under similar exposure conditions.

Figure 11.1 Ozone dose-response curves of FEV1 lung function decrease—fitted using the lognormal distribution (from Larsen et al., 1991)

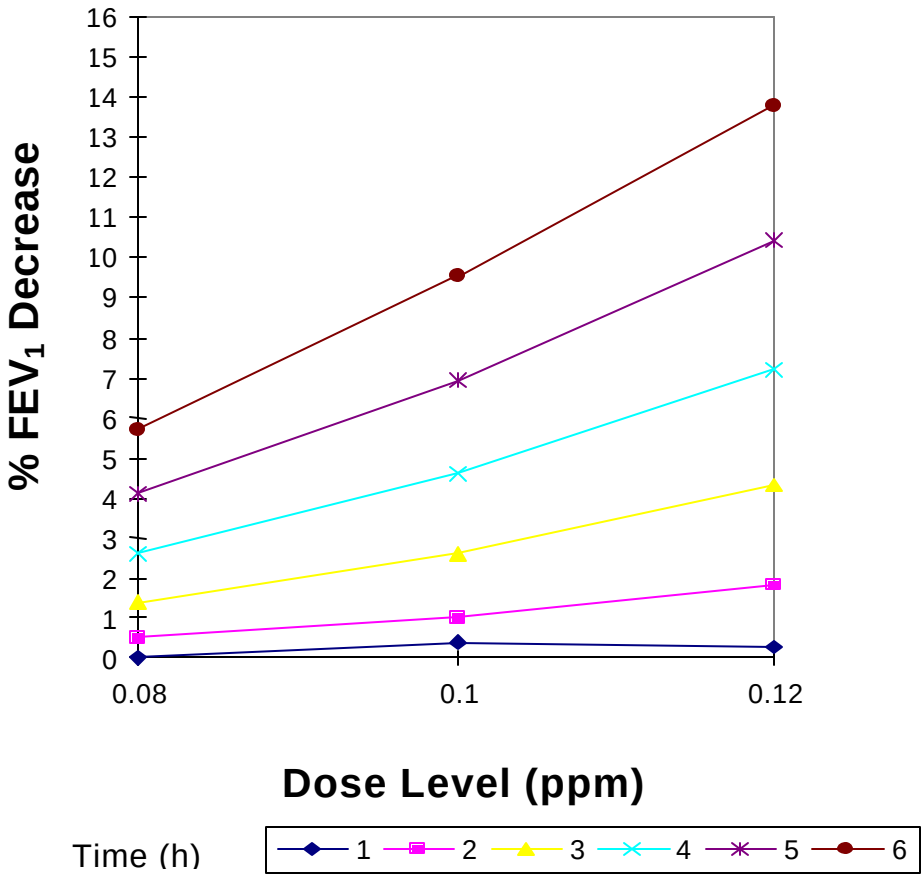
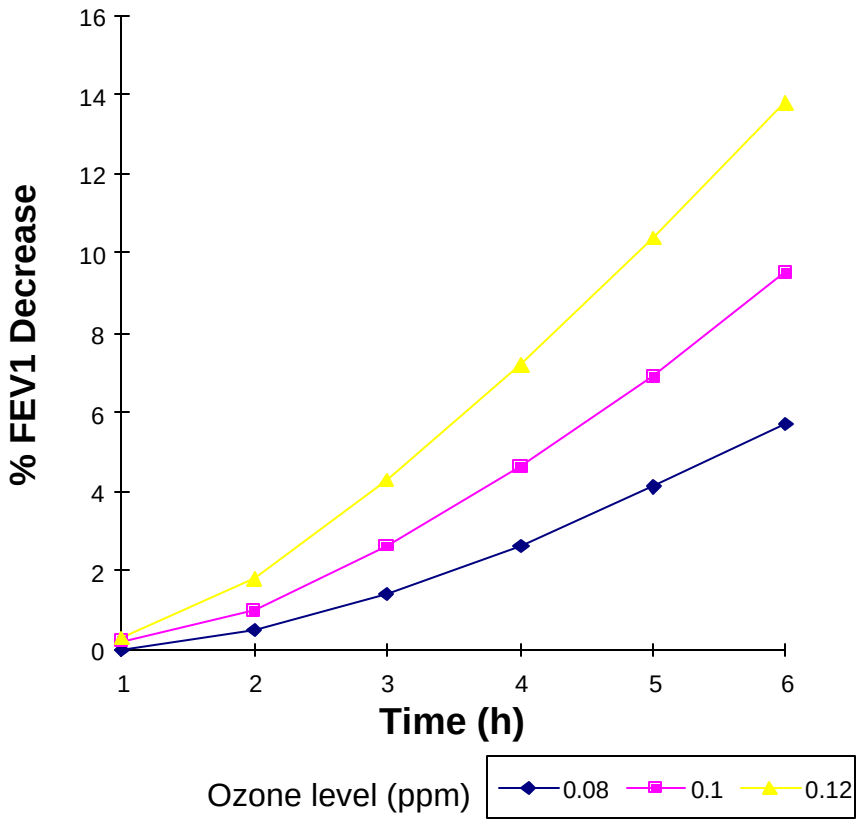


Figure 11.2 Decrease of FEV1 over time. (Lognormal values, from Larsen et al., 1991)



Hazucha et al. (1992) designed a protocol to examine the effect of varying, rather than constant, ozone concentration. In the study, male subjects (20 to 35 years old, n = 23) were exposed to filtered air, a constant concentration of 0.12 ppm ozone, or a linearly variable concentration of ozone ranging from 0 at the start to 0.24 ppm at 4-hours and back down to 0 ppm at 8-hours (end of experiment). Subjects performed intermittent exercise ($\underline{V}_E$ at 40 L/min). No effects were noted in the filtered air group. At a constant 0.12 ppm of ozone, FEV_1 was decreased by 5% at the fifth hour. In the variable concentration group, the FEV_1 decreased almost twice of that in the constant concentration group after 6 hours of exposure. FEV_1 began to improve and was reduced by 5.9% at the end of the 8-hour exposure. This study illustrates two important points for those modelling spirometric responses to ozone exposure. First, the model should include a response plateau and secondly, the response to ozone exposure is dependent on the dose rate as well as the cumulative dose, at least when the ozone concentration is varied.

More recently, several studies have been carried out using an exercise protocol similar to the 'EPA prolonged exposure protocol' (Basha et al., 1994; Folinsbee et al., 1994; McDonnell et al., 1995; Horstman et al., 1995), in order to investigate the effects of 6- to 8-hour exposure to ozone (0.08 to 0.2 ppm, with exercise at $\underline{V}_E$ of 30 - 40 L/min). The findings of spirometric and symptomatic responses were consistent with the previous reports (Folinsbee et al., 1988, Horstman et al., 1990). Healthy subjects (18 to 45 years old) experienced significant time-dependent and ozone concentration-dependent loss of FEV_1, at a concentration as low as 0.08 ppm (McDonnell et al., 1995). In the case of asthmatic patients (18 to 50 years old), most studies show that decrements in FEV_1 for asthmatics were significantly greater than for non asthmatics (Linn et al., 1994; Horstman et al., 1995). However one study conducted by Basha et al. (1994) demonstrates that asthmatic subjects were no more responsive to a 6-hour exposure of 0.2 ppm ozone than their healthy counterparts in pulmonary function decrements, although cellular and biochemical inflammatory parameters in BAL were more augmented in asthmatic subjects than in healthy ones.

In general, data from prolonged ozone exposure studies further confirm the essential concept of the 'effective dose', namely, ozone concentration x ventilation rate x exposure duration, although ozone concentration apparently contributes more to pulmonary responses than does ventilation or exposure duration. More importantly, on the grounds of the 'effective dose', there appears not to exist a lower threshold for ozone-induced spirometric responses; a prolonged ozone exposure at a concentration of $\leq$0.08 ppm (the current National Ambient Air Quality Objective for ozone) may still exhibit an adverse health effect.

11.3 INCREASED AIRWAY RESPONSIVENESS

Increased airway responsiveness is an important consequence of exposure to ozone. An elevated airway responsiveness to methacholine challenge indicates that the airways are predisposed to bronchoconstriction induced by a variety of stimuli (e.g., specific allergens, sulphur dioxide, cold air, etc.). The dose of a bronchoconstrictor (such as methacholine and histamine) that causes a 20% drop in FEV_1 or a 100% increase in airway resistance is referred to as the PD_{20} or the PD_{100} respectively.

A high level of bronchial responsiveness is a hallmark of asthma. Historically, the range of nonspecific responsiveness (expressed as PD_{20}) between the least sensitive healthy subject and the most sensitive asthmatic is about 1000 fold. All other types of 'responders' (e.g., atopic or allergic individuals) would fall between these values. The average changes in airway responsiveness induced by ozone range from 150 to 500% (U.S. EPA criteria document, 1996). This suggests that a healthy subject could move from the normal range to the mild asthmatic range of airway responsiveness when exposed to ozone.

Data summarized in the U.S. EPA document (1996) suggest that ozone-induced increases in airway responsiveness usually resolve 18 to 24 hours after exposure (Holtzman et al., 1979; Folinsbee & Hazucha, 1989; Konig et al., 1980), while the hyperresponsiveness may persist in some individuals for longer periods (Golden et al., 1978). Increased airway responsiveness to histamine and methacholine has been observed following exposure to 0.20 ppm ozone for 1-hour (vigorous exercise) (Gong et al., 1986), and 0.08 to 0.12 ppm ozone for 6.6-hours with intermittent exercise ($\underline{V}_E$ at 39 - 50 L/min) (Folinsbee et al., 1988; Horstman et al., 1990).

Data from the latest studies further demonstrate that 0.24 ppm ozone (4-hours with exercise at $\underline{V}_E$ of 20 L/min) did not cause a significant increase in bronchial reactivity in response to methacholine challenge in elderly healthy subjects (60 to 69 years) (Gong et al., 1997b). Based on the effective dose, elderly subjects appear to be less sensitive to ozone-induced airway hyperresponsiveness in comparison with young people. In allergic asthmatic subjects (21 to 31 years old), a 3-hour exposure to 0.25 ppm ozone ($\underline{V}_E$ at 30 L/min) initiated significant increases in bronchial responsiveness to methacholine and allergen (Jorres et al., 1996). No alteration in bronchial responsiveness to methacholine was observed in healthy subjects and subjects with allergic rhinitis without asthma, although subjects with allergic rhinitis did show a bronchial allergen response after ozone exposure (Jorres et al., 1996). While an 1-hour exposure to 0.12 ppm ozone at rest did not cause an increased responsiveness to methacholine challenge in asthmatic patients (Ball et al., 1996), prolonged exposure (6.6-hours) to this concentration with exercise ($\underline{V}_E$ at 29 L/min) did induce an enhanced bronchial reactivity in asthmatics (Linn et al., 1994).

Publications on 'adaptation' or tolerance, of increased bronchial responsiveness to methacholine challenge following repeated exposure to ozone are not consistent. Data from two early studies (Kulle et al., 1982, Dimeo et al., 1981) exhibit a trend to the increased responsiveness to diminish with repeated exposure to ozone, starting on or after the third day of consecutive exposure. However, two later studies demonstrate that at low (0.12 ppm, 6.6-hours/day, $\underline{V}_E$ at 39 L/min) (Folinsbee et al., 1994) and high (0.4 ppm, 3-hours/day, $\underline{V}_E$ at 32 L/min) (Gong et al., 1997a) concentrations of ozone, bronchial responsiveness to methacholine remained elevated over the 5 days of multi-hour exposure period in both healthy (n = 17) (Folinsbee et al., 1994) and asthmatic (n = 10) (Gong et al., 1997a) subjects.

There is no doubt that ozone produces acute increases in airway responsiveness. However, it is not known if ozone exposure causes protracted increases in airway responsiveness in healthy individuals, or even induces or predisposes subjects to asthma. Observations of Koenig et al. (1990) and Molfino et al. (1991) suggest the possibility that acute exposure to ozone at a dose

(0.12 ppm with or without exercise) that does not produce measurable pulmonary function decrements may increase the responsiveness of asthmatics to subsequently inhaled SO_2 or allergens. Contrary to these findings, Bascom et al. (1990) found no increase in responses to antigen nasal challenge in a group of allergic subjects pre-exposed to 0.5 ppm ozone for 4 h when compared to air-antigen challenge.

The overall animal data provides strong evidence that the ozone-induced enhancement in responsiveness is mediated, at least in part, by cholinergic receptors on airway smooth muscle. Osebold et al. (1980) hypothesized that the increased epithelial permeability caused by ozone may allow greater penetration of bronchoconstriction substances, resulting in increased airway responsiveness.

It has also been postulated that ozone-induced acute inflammation may be important in the induction of the increased airway responsiveness (U.S. EPA, 1996). Ozone (0.4 ppm)-induced rapid increase in prostaglandin (PGE_2), a mediator involved in bronchoconstriction, in BAL has been reported in a number of studies (McDonnell et al., 1991a,b; Hazucha et al., 1996; Devlin et al., 1996; Devlin et al., 1997), except for one study by Coffey et al. (1996) who did not observe any alteration in PGE_2. It also appears that increased bronchial reactivity is a consequence of cellular or biochemical changes in the airway, and does not correlated with spirometric responses (Aris et al., 1995; Jorres et al., 1996). Because these alterations are part of a complex process, it comes as no surprise that the mechanistic studies on ozone-induced increases in airway responsiveness have not pinpointed an isolated derangement.

11.4 INFLAMMATION AND HOST DEFENCE

Inflammation can be considered as the host response to injury, and the induction of inflammation can be accepted as evidence that injury has occurred. Inflammation induced by exposure of humans to ozone can have several outcomes:

- inflammation induced by a single exposure (or several exposures over the course of a summer) resolves entirely;
- continued acute inflammation evolves into a chronic inflammatory state;
- continued inflammation alters the structure or function of other pulmonary tissue, leading to diseases such as fibrosis or emphysema;
- inflammation alters the body's host defence response to inhaled microorganisms, particularly in potentially vulnerable populations such as the very young and old;
- inflammation alters the lung's response to other agents such as allergens or toxicants.

It is also postulated (U.S. EPA, 1996) that the profile of response can be altered in persons with pre existing pulmonary disease (e.g., asthma or COPD) or smokers.

The presence of polymorphonuclear leukocytes (PMN) in the lung has long been accepted as a hallmark of inflammation and has been taken as the major indicator that ozone causes inflammation in the lungs of humans. Soluble mediators of inflammation such as cytokines (Interleukin-6, -8, -1,

and tumour necrosis factor) and arachidonic acid metabolites (e.g., PGE_2, $PGF_{2"}$, throboxanes, leukotrienes) have also been measured in the BAL fluid of humans exposed to ozone. In addition to their role in inflammation, many of these compounds have bronchoconstrictive properties, and may be involved in increased bronchial hyperreactivity observed following ozone exposure.

Under normal circumstances, the epithelia lining the large and small airways develop tight junctions and restrict the penetration of exogenous particles and macromolecules from the airway lumen into the interstitium and blood, as well as restricting the flow of plasma components into the airway lumen. Several studies report that ozone disrupts the integrity of the epithelial cell barrier in human airways, as measured by increased passage of radio labelled compounds out of the airways, as well as passage of markers of plasma influx such as albumin, immunoglobulin, and total BAL fluid protein into the airways. Markers of epithelial cell damage such as LDH have also been measured in the BAL fluid of subjects exposed to ozone.

Inflammatory cells of the lung such as macrophages, monocytes, and PMN also constitute an important component of the pulmonary host defence system. Upon activation, these cells not only are capable of generating free radicals and enzymes with microbicidal activities, but also have the potential of damaging nearby cells. On the other hand, damage of these cells may inhibit microbicidal function, and enhance the susceptibility to bacterial infection.

Other soluble factors that have been studied include those involved with fibrin deposition and degradation (tissue factor, factor VII, and plasminogen activator), potential markers of fibrogenesis (fibronectin, platelet derived growth factor), and components of the complement cascade (C3a).

It should be noted that existing evidence has shown that airway inflammatory responses are not correlated with decrements in FEV_1 (Aris et al., 1993; Balmes et al., 1996; Hazucha et al., 1996). 'Responders' (substantial decrement in FEV_1 in response to ozone) may not necessarily have higher inflammatory responses than 'non responders' (little decrement in FEV_1 after ozone exposure). The mechanism of FEV_1 and FVC decrements are thought to involve neurally mediated involuntary inhibition of inspiration and reduction of vital capacity (Hazucha et al., 1989). Therefore, assessment of ozone-induced health effects using spirometric parameters may have measured a reflexive rather than adverse physical response. Since the US National Ambient Air Quality Standard (NAAQS) for ozone was established largely based on spirometric responses to ozone inhalation, some researchers have suggested rethinking the NAAQS setting process for ozone.

11.4.1 Inflammation Assessed by Bronchoalveolar Lavage

Two early studies (Seltzer et al., 1986; Koren et al., 1989a,b) demonstrate that exposure of humans to high levels of ozone (0.4 to 0.6 ppm, with exercise for 2 h at $\underline{V}_E$ 70 L/min; BAL were performed 3 and 18 hours post exposure respectively for each study) results in an inflammatory reaction in the lung, as evidenced by substantial increases in neutrophils and proinflammatory compounds.

Numerous studies published during the following years have provided further evidence to support these observations (Hatch et al., 1994; Fahy et al., 1995; Weinmann et al., 1995a; Hazucha et al., 1996; Devlin et al., 1996; Coffey et al., 1996; Devlin et al., 1997). Moreover, new studies also demonstrate that at moderate (0.2 - 0.25 ppm for 1 - 4-hours, at $\underline{V}_E$ 40 - 60 L/min) concentrations of ozone, both inflammatory cells and mediators capable of damaging pulmonary tissue integrity increased significantly in healthy young subjects after ozone exposure, as did compounds that play a role in fibrotic and fibrinolytic processes (Hazbun et al., 1993; Aris et al., 1993; Scannell et al., 1996; Balmes et al., 1996; Torres et al., 1996).

Asthmatic patients are revealed more sensitive toward ozone-induced airway inflammation than healthy subjects (Basha et al., 1994; Scannell et al., 1996). At concentrations of 0.12 to 0.24 ppm (90 minutes at $\underline{V}_E$ ~50 L/min, and 6-hours at $\underline{V}_E$ ~25 L/min) ozone induced higher inflammatory responses in asthmatics than in healthy subjects (Basha et al., 1994; Scannell et al., 1996).

Kehrl et al. (1987) demonstrate that human pulmonary epithelial cell permeability was increased as measured by increased clearance of ^{99m}Tc-DTPA (diethylene triamine pentaacetic acid), following the exposure to ozone (0.4 ppm for 2-hours with exercise $\underline{V}_E$ 70 L/min). Kehrl et al. (1989) reported similar observation on an additional 16 subjects. For the combined group of 24 subjects, exposed to 0.4 ppm for 2-hours (with heavy exercise, $\underline{V}_E$ 70 L/min), the average clearance rate was 60% faster than that observed after air exposure, strongly suggesting increased permeability from the airway lumen and alveolar space to the blood and interstitial spaces. The average ozone-induced decrement in FVC in these subjects was 10%.

Recently published data by Foster and Stetkiewicz (1996), despite using a different dose regimen, observed similar results. Foster and Stetkiewicz (1996) exposed healthy subjects (mean age 26 years, n = 9) to filtered air or ozone (initiated at 0.15 ppm, ramped to 0.35 ppm, then returned to 0.15 ppm) during a 130 minute intermittent exercise ($\underline{V}_E$ at 40 L/min). ^{99m}Tc-labelled DTPA solute was deposited by aerosol on the epithelial surfaces 18 to 20 hours later. The lung clearance of ^{99m}Tc-DTPA over a 120 minute period was significantly faster compared with air control. Regional analysis demonstrated that ^{99m}Tc-DTPA clearance from the periphery (excluding the lung hilum) and lung apexes were significantly increased by ozone but changes in clearance for the base of the lung were not significant. Their data support the observations by Kehrl et al. (1987, 1989) indicating that epithelial permeability of the lung is altered by ozone exposure. Moreover, this injury happens at relatively low ozone concentrations, and is regional in lungs. These changes in permeability are likely associated with acute inflammation and may potentially allow better access of inhaled antigens and other substances to the mucosa.

Macrophages exposed *in vivo* showed changes in 123 proteins (Koren et al., 1989a). Exposure to 0.22 ppm ozone for 4-hours has been reported to initiate the generation of superoxide anion by the total BAL cells (Torres et al., 1996). Exposure to 0.4 ppm ozone for 2-hours ($\underline{V}_E$ at ~60 L/min) increased production of lipoxygenase metabolites by alveolar macrophages (Coffey et al., 1996). These data are suggestive of mediator activation of the macrophage *in vivo*. However, in terms of alveolar macrophage function, other researchers have observed an ozone-induced reduction of macrophage function, indicated by a decrease in the ability of alveolar macrophages to phagocytes

Candida albicans after exposure to 0.4 ppm ozone for 2-hours ($\underline{V}_E$ at 66 L/min) (Devlin et al., 1996), and a reduction in production of hydrogen peroxide in alveolar macrophages after exposure to 0.22 ppm ozone for 4-hours (Torres et al., 1996). Therefore, exposure to ozone may impair the endogenous microcidal function.

Based on data obtained from experimental animal studies, airways have been shown to be a major site of ozone-induced inflammation. However, few human studies have confirmed this finding because BAL primarily samples cells and fluid in the terminal alveoli and bronchioles (U.S. EPA, 1996). Recently, studies investigating inflammatory responses in bronchus proximal airway lavage (PAL), bronchial fraction and BAL (Aris et al., 1993; Scannell et al., 1996; Balmes et al., 1996) demonstrate that there is a regional difference in inflammatory effects in response to ozone insult. While exposure of healthy and asthmatic subjects to ozone (0.2 to 0.25 ppm ozone for 1 to 4-hours) initiated the influx of PMN occurs in all the airway regions, the increased release of small inflammatory mediators and molecules such as interleukins, total proteins, LDH (a cell death indicator), and fibronectin was more likely to take place in BAL (Balmes et al., 1996; Scannell et al., 1996). Additionally, Aris et al. (1993) found that more total cells, neutrophils and epithelial cells appeared in PAL fluids than in BAL, and a significant increase of LDH in PAL after exposure to 0.2 ppm ozone (4-hours), suggesting an ozone-induced neutrophil influx coupled with ozone-induced epithelial cell shedding. Ozone injures proximal airways as well as the distal lung.

Numerous studies have shown the 'adaptation' or 'tolerance' phenomenon in pulmonary function decrements following daily consecutive exposure to ozone (see 11.1.5). Experimental animal studies (Tepper et al., 1989; VanBree et al., 1994) have suggested that although some markers of inflammation may be diminished after repeated ozone exposure, underlying damage to lung epithelial cells may continue. There are very limited data on human 'adaptation' to ozone-induced inflammation. In a recently published report (Devlin et al., 1997), healthy human subjects (n = 16) were exposed to 0.4 ppm ozone for 5 consecutive days (2-hours/day) while undergoing intermittent exercise ($\underline{V}_E$ at 60 L/min). Ten or 20 days later, subjects were exposed to ozone a single time. The results show that numerous indicators of inflammation were attenuated. After 5 days of exposure, ozone-induced increases in %PMN, IL-6, PGE_2, elastase, fibronectin were diminished, when compared with a single exposure to the same dosage of ozone (Devlin et al., 1996). Ozone-induced decreases in recovery of viable BAL cells and alveolar macrophage phagocytosis of yeast were also blunted by the repeated exposure. Within 10 to 20 days, %PMN, IL-6, PGE_2, fibronectin, and recovery of viable cells showed at least partial reversal, while other mediators did not return to the normal response to ozone. Thus, biochemical mediators in response to ozone were attenuated after 5 consecutive days of ozone exposure. The attenuation of inflammation is similar to attenuation of symptoms and lung function responses. In agreement with animal studies, some cell injury markers (LDH, elastase) did not show adaptation, indicating that tissue damage may continue to occur during repeated exposure.

11.4.2 Inflammation Induced by Ambient Levels of Ozone

Devlin et al. (1991) reported an inflammatory response in humans exposed to levels of ozone at 0.12 ppm or below. In this study, healthy subjects were exposed to 0.08 and 0.1 ppm ozone for 6.6-hours with moderate exercise ($\underline{V}_E$ 40 L/min), and underwent BAL 18 hours later. Increased numbers

of PMN and levels of IL-6, fibronectin and PGE_2, and decreased alveolar macrophage phagocytic capability were found at both ozone concentrations. When data are normalized for concentration, exposure duration and $\underline{V}_E$, and compared with the data reported by Koren et al. (1989a,b) (0.4 ppm ozone exposure for 2-hours with intermittent exercise at $\underline{V}_E$ 70 L/min), the inflammatory changes observed in these studies suggest that lung inflammation from ozone may occur as a consequence of exposure to ambient levels while exercising.

There is a considerable variation of response among the individuals in the Devlin study, some showing little or no response to ozone and others having increases in IL-6 or PMN that were as large or larger than those reported by Koren et al. (1989a,b). Individuals with the largest increases in inflammatory mediators in this study did not necessarily have the largest decrements in pulmonary function, suggesting separate mechanisms underlying physiological and inflammatory responses to ozone.

These data further suggest that, although the population as a whole may have a small inflammatory response to low levels of ozone, there may be a significant subpopulation that is very sensitive to these low levels of ozone. Furthermore, even a small inflammatory response (if it recurs) in the population as a whole should not be discounted. Such a response in people with severely compromised respiratory systems may result in sustained adverse health effects. Although this has not yet been documented in chamber studies (only mild asthma cases have been used for the chamber studies), we cannot dismiss the potential of ozone to affect moderate and severe asthma through this process.

11.4.3 Time Course of Inflammatory Response

As summarized by the U.S. EPA (1996), some data show that the inflammatory response is quickly initiated 1 hour (Devlin et al., 1990; Koren et al., 1991) to 3 hours (Seltzer et al., 1986), after a 2-hour exposure to 0.4 ppm of ozone. Inflammatory mediators (IL-6) and cells (PMN) remain elevated 18 hours after exposure (Koren et al., 1989a,b). These studies also show that there is a difference in the magnitude of response of some indicators depending on when the BAL is performed after exposure to ozone. Increases of PMN, IL-6, and PGE_2 are greater after 1 hour, fibronectin and plasminogen activator are greater after 18 hours, and protein and Tissue Factor are equally elevated at both times. A study by Schelegle et al. (1991) suggests that the percent of PMN may be elevated early (1 to 6 hours post exposure to 0.3 ppm ozone for 1-hour with mouth piece) in proximal airways and later (6-24 hours post exposure) in distal airways and alveolar surfaces. These results are preliminary and include data from only 5 subjects.

In a recent study conducted by Coffey et al. (1996), the researchers investigated inflammatory changes in BAL at 0, 2 and 4 hours after a 2-hour exposure to 0.4 ppm ozone with intermittent exercise ($\underline{V}_E$ -60 L/min). The volunteers were 11 healthy nonsmokers, age 18 - 35 years. There was a time-dependent increase in the number of PMN, and a significant reduction in the number of alveolar macrophages following ozone exposure, with the highest changes occurring at 4 hours after exposure. The levels of leukotrienes [LTC_4 (a substance of anaphylaxis) and LTB_4 (a potent chemotactic agent inflammatory cells)] were significantly higher at 0 hour following ozone exposure

compared to air, with no change in prostaglandins. At 4 hours after the end of ozone exposure, LTB4 level returned to normal level.

Devlin et al. (1996) compared changes of inflammatory mediators in BAL 1 and 18 hours after a 2-hour exposure to 0.4 ppm ozone. Eight healthy subjects, at the age of 18 to 35 years, were studied during intermittent exercise ($\underline{V}_E$ 66 L/min). The data reveal that ozone induced rapid increase in PMN, protein, LDH, α-1 antitrypsin, fibronectin, PGE_2, thromboxane B_2, C3a, tissue factor and clotting factor VII one hour after exposure. There was a decrease in recovery of total cells and alveolar macrophages, and decreased ability of alveolar macrophages to phagocytes *Candida albicans*. Compared with data collected 18 hours after exposure, IL-6 and PGE_2 levels were higher 1 hour after exposure than 18 hours after exposure, while fibronectin and tissue-plasminogen activator levels were higher 18 hours after exposure, and the PMN, proteins, and C3a were present at essentially the same levels at both times. The researchers concluded that several inflammatory mediators are already elevated 1 hour after exposure, and some mediators achieve their maximal levels in BAL fluid at different times following exposure.

Torres et al. (1996) examined the time pattern of airway inflammation following exposure to a moderate level of ozone (0.22 ppm, 4-hours, with exercise). Healthy nonsmokers were classified as responders (>15% fall in FEV_1 following ozone exposure, n = 12) and non-responders (<5% fall in FEV_1 after ozone exposure, n = 12). Subjects were exposed to air and ozone in separate days. BAL was performed immediately and 18-hour after ozone exposure, and either early or late after air. The concentration of PMN in BAL increased nearly five fold 18 hours after ozone exposure in both responders and non-responders. In responders, stimulated superoxide anion production in lavage cells increased progressively, with the highest level at 18 hours after ozone exposure. Unstimulated hydrogen peroxide production in alveolar macrophage from responders decreased progressively, and in a time-dependent fashion. There were no significant changes in reactive oxygen intermediate production by cells from non-responders. Therefore, influx of PMN may increase the burden of reactive oxygen intermediate to the airway epithelium. The authors speculate that both the number of alveolar macrophages and the production of reactive oxygen intermediates in alveolar macrophages were down-regulated following exposure in ozone responders, thus limiting the potential for injury to the alveolar epithelium.

11.4.4 Effects of Anti-Inflammatory Agents on Ozone-Induced Inflammation

Hazucha et al. (1996) studied the effect of ibuprofen, a non-steroid anti-inflammatory agent, on ozone-induced inflammatory responses. Ten healthy subjects (20 to 32 years of age) were given 800 mg of ibuprofen or placebo and were exposed to filtered air and 0.4 ppm ozone for 2-hours with intermittent exercise ($\underline{V}_E$ at ~60 L/min). Data obtained from this study show that although anti-inflammatory agents may blunt ozone-induced decrements in FEV_1 and increases in PGE_2, most inflammatory and cell injury parameters (PMNs, fibronectin, permeability, LDH, or macrophage phagocytic function) were not affected by ibuprofen in the BAL of these subjects. Ozone-induced increases of airway resistance was not affected by ibuprofen treatment either.

In agreement with the above observation, data from Liu et al. (1997a) demonstrate that pre-treatment of healthy subjects with acetylsalicylic acid (ASA, 0.975 g) did not suppress 0.4 ppm

ozone-induced increase in PMN and decrease in macrophages sputum, although the pre-treatment with ASA inhibited 0.12 ppm ozone-induced increase in airway resistance (Liu et al., 1997b).

The above evidence suggests that:

1. ozone initiates pulmonary function decrements and airway inflammation via different mechanisms;
2. spirometric changes may not reflect pulmonary tissue injury; and
3. pre-treatment with non-steroid anti-inflammatory agents, may blunt pulmonary function changes and relieve some symptoms, but may not represent a protective mechanism against ozone-induced lung injury.

11.4.5 Use of Nasal Lavage (NL) to Assess Ozone-Induced Inflammation in the Upper Respiratory Tract

Bronchoalveolar lavage, the most frequently used method for studying lower airway damage, is an invasive procedure, and may alter some endpoints due to local anaesthesia. Since nasal lavage procedure is non-invasive, it has been proposed to be a sensitive and reliable tool to detect inflammation in the upper airways of humans exposed to xenobiotics. In summary of the U.S. EPA document (1996), limited studies using nasal lavage method demonstrate elevated PMN following exposure to 0.4 to 0.5 ppm ozone. One study (Graham and Koren, 1990) demonstrated a good qualitative correlation between changes in PMN using both BAL and NL methods on the same subjects who were exposed to 0.4 ppm ozone for 2-hours (exercise $\underline{V}_E$ at 70 L/min).

Folinsbee et al. (1994) exposed 17 healthy non smoking male subjects to filtered air or 0.12 ppm ozone for 6.6-hours on 5 consecutive days with exercise ($\underline{V}_E$ 39 L/min). Nasal lavages were obtained before and after exposure, and did not reveal significant increase in PMN numbers after ozone exposure.

McBride et al. (1994) studied the inflammatory response in nasal lavage from 10 asthmatic (18 to 41 years old) and 8 non asthmatic (18 to 35 years old) subjects. Subjects were exposed to 0.12 and 0.24 ppm ozone or air for 90 minutes (exercise $\underline{V}_E$ 23 L/min). Nasal lavage was performed immediately and 24 hours after exposures. In asthmatic subjects, a significant increase in the number of white blood cells in lavage fluid was detected both immediately and 24 hours after exposure to 0.24 ppm ozone, as was a significant increase in epithelial cells immediately after exposure. No significant differences in levels of IL-8 were seen in the nasal lavage fluids after exposure to any of the three test atmospheres. A significant correlation was observed between IL-8 and white blood cell counts after exposure to 0.24 ppm ozone ($r = 0.76$). Histamine, LTB4 or platelet-activating factor were not detected in any of the nasal lavage samples. In non asthmatic subjects, no significant cellular changes were seen. No changes in pulmonary and nasal functions were detected in either group. These data are consistent with studies using BAL technique, indicating that asthmatic patients are more sensitive toward ozone-induced airway inflammation than healthy subjects (Basha et al., 1994; Scannell et al., 1996).

These studies suggest that nasal lavage may serve as a sensitive and reliable tool to detect inflammation in the upper airways of humans exposed to air pollutants, even at ozone concentrations which do not initiate spirometric responses.

11.4.6 Changes in Host Defence Capability Following Ozone Exposure

Concern about the effect of ozone on human host defence capability derives from numerous animal studies demonstrating that exposure to as little as 0.08 ppm ozone increases mortality in rodents subsequently challenged with aerosolized or instilled bacteria (Gardner et al., 1982; Van Loveren et al., 1988). In humans, Henderson et al. (1988) exposed 24 young males to inoculations of type 39 rhinovirus administered as nose drops. Half were then exposed to 0.3 ppm ozone (6-hours/day) for 5 consecutive days with intermittent light exercise and the other half were exposed to clean air under the same conditions. Nasal lavage was performed on the morning of days 1 to 5, 8, 15 and 30. No differences in virus titres, PMNs, or interferon gamma in nasal lavage fluid were noted between the groups. No difference in blood lymphocyte proliferative response to viral antigen were found in two groups. Other studies have shown that acute *in vivo* exposure of humans to ozone results in impairment of alveolar macrophage host defence capability, potentially resulting in decreased ability to phagocytose and kill inhaled microorganisms *in vivo*.

Although there are several studies in which animals have been exposed to bacteria or viruses in conjunction with ozone exposure, which provide some evidence to suggest that ozone impairs the immune system's ability to fight viral infections, there is insufficient human data to know whether ozone exposure affects viral infectivity. However, there is potential cause for concern that ozone may render humans and animals more susceptible to a subsequent bacterial challenge.

In terms of alveolar macrophage function, several researchers have observed an ozone-induced reduction of macrophage function. Torres et al. (1996) exposed healthy non smoking subjects (n = 24) to air and ozone (0.22 ppm for 4-hours with exercise). BAL was performed immediately and 18 hours after ozone exposure, and either early or late after air. Data show a progressive decrease in hydrogen peroxide production in alveolar macrophages in some subjects. Devlin et al. (1996) also found a significant decrease in the ability of alveolar macrophages to phagocytes *Candida albicans* after exposure to 0.4 ppm ozone for 2-hours (Devlin et al., 1996).

11.5 EXTRAPULMONARY SYSTEMIC EFFECTS OF OZONE

It is believed that ozone immediately reacts on contact with airway surface liquid and tissue, and is not absorbed or transported to extrapulmonary sites to any significant degree (Pryor 1992). Nevertheless, the demonstration in the previous section of an array of inflammatory modulators released at the airway surface provides a possible mechanism for effects to occur elsewhere in the body. Additional *in vivo* studies are needed, therefore, to determine if there are significant extrapulmonary effects of ozone exposure in humans and at what levels of exposure they may occur.

Liu et al. (1997b) studied the plasma concentrations of hydroxylated salicylate metabolites (an indicator for hydroxyl radical production *in vivo*) in healthy subjects exposed to 0.12 and 0.4 ppm

ozone or air for 2-hours. They found that exposure to both 0.12 and 0.4 ppm ozone was associated with a significant increase in plasma 2,3-dihydroxybenzoic acid concentration, suggesting an increased production of hydroxyl radical in the body, although most likely occurring in lungs.

Foster et al. (1996) investigated several blood borne markers for ozone exposure. Healthy male subjects (mean age of 26 years, n = 10) were exposed to air or ozone over 3 consecutive days (130 min/day) with intermittent exercise (42 L/min for air, 44 L/min for ozone). Ozone level was varied at 20 minute intervals, from 0.25, 0.35, 0.45, 0.45, 0.35, to 0.25 ppm, and concluded with 10 minutes at 0.25 ppm. Blood was obtained before exposures on day one, immediately after exposure each day, and 18 to 20 hours after the third day of exposure. Markers measured include serum antioxidant α-tocopherol as a gauge of oxidative stress, and the blastogenic activity of peripheral blood monocytes as an index of immune function in response to ozone exposure. Twenty-hours after the third day of ozone exposure, α-tocopherol levels were reduced significantly compared with the air control subjects. Mitogen-activated T lymphocytes exhibited a 61% increase in blastogenic activity after 3 days of ozone exposure in comparison with the levels in air control or before ozone. The progressive increase in the expression of these systemic biomarkers may represent essential features of oxidant injury and/or repair.

Hazbun et al. (1993) exposed 7 healthy non smoking subjects (22 to 30 years old) to air and ozone (0.25 ppm for 1-hour) with heavy exercise ($\underline{V}_E$ ~60 L/min). They did not observe significant changes in plasma levels of substance P, 8-epi-$PGF_{2\alpha}$, C3a and C5a after ozone exposure, although in airway lavage fluid there were significant increases in concentrations of these mediators and protein.

Vender et al. (1994) examined the levels of glutathione and catalase in red blood cells following exposure of 7 healthy male subjects to 0.16 ppm or air for 7.5-hours. They did not find any significant changes in glutathione or catalase levels following ozone exposure. It appears that ozone-initiated health effects are most likely localized in the respiratory system.

11.6 SYMPTOMS AND PULMONARY FUNCTION IN CONTROLLED STUDIES OF AMBIENT AIR EXPOSURES

11.6.1 Mobile Laboratory Studies

The Rancho Los Amigos Medical Centre in California used a mobile laboratory for their field studies (U.S. EPA, 1996). These studies emphasize the importance of adequate characterization of subjects and the ambient air, exercise levels, duration of exposure, and individual variations in sensitivity when interpreting observed exposure effects. Although these factors need to be investigated over a wider range of experimental conditions, the results from these field studies are, so far, consistent with those from controlled human exposure studies. The effects include pulmonary function decrements at ozone concentrations of 0.144 ppm (1-hour) in healthy exercising ($\underline{V}_E$ 0.32 L/min) adolescents, and increased respiratory symptoms and pulmonary function decrements at 0.153 ppm (1-hour) in heavily exercising ($\underline{V}_E$ 53 L/min) athletes, and at 0.174 ppm (2-hours) in lightly exercising normal and asthmatic subjects. Many of the normal subjects with a history of allergy appeared to be more responsive to ozone than ‘nonallergic’

normal subjects, although a standardized evaluation of atopic status was not performed. These short term respiratory effects of summer ambient oxidant pollution in Southern California are believed to be predominantly, if not entirely, caused by ambient ozone in typical healthy or asthmatic residents according to mobile laboratory studies (Avol et al., 1984a, 1985). However, ambient air studies in the mobile laboratory are dependent on ambient conditions, which cannot be predicted, controlled, nor completely characterized.

11.7 SUMMARY AND CONCLUSIONS

Pulmonary responses observed during acute exposure to ozone at concentrations ranging from 0.10 to 0.50 ppm in healthy human subjects include decreases in TLC, IC, FVC, FEV_1, $FEF_{25-75\%}$, and V_T; and increases in SR_{aw}, breathing frequency, and airway responsiveness to methacholine challenge. Symptoms observed include cough, pain on deep inspiration, shortness of breath, throat irritation and wheezing. The physiological and symptom responses observed in the above studies can be separated in 5 different categories:

- symptoms,
- spirometric changes,
- airway resistance, and
- bronchial responsiveness to methacholine or antigen.
- airway inflammation

Inflammatory response detected in body fluids (BAL, nasal lavage, sputum) can also be observed directly. From the data collected, it appears that there is no correlation between spirometric changes and either airway resistance, bronchial responsiveness, or inflammation. Attenuation of airway resistance by atropine with no effects on spirometry (Beckett et al., 1985; Adams, 1986) coupled with the attenuation of spirometry response by indomethacin, Ibuprofen or ASA with no effect on airway resistance or responsiveness (Schelegle et al., 1987; Eschenbacher et al., 1989; Ying et al., 1990; Hazucha, 1996; Liu et al., 1997a and 1997b) support both this categorization and the notion of independent mechanisms.

The symptoms are often subjective measurements or simple reflex responses (cough), and are therefore difficult to correlate with objective physiological responses. However, cough and pain on deep inspiration have been related temporally to spirometry and breathing pattern responses.

11.7.1 Effects on Pulmonary Function

Healthy Subjects

Controlled human ozone exposure studies have provided strong and quantifiable exposure-response data on the health effects of ozone. The section on healthy subjects reviewed principally young adult subjects (18 to 35 years) exposed to ozone concentrations ranging from 0.08 to 0.75 ppm ozone either at rest or during continuous or intermittent exercise (20 to 90 L/min) protocols for durations of up to 8 hours. The major drawback of these studies is the small sample size. As a result, the conclusions derived from such studies may underestimate the actual response to low ozone concentrations in healthy young adults.

For resting exposures of a 2-hour duration, the effects noted include decreased FEV_1 and FVC at doses of 0.50 ppm or above.

For exercising exposures of a 1 to 4-hour duration, decreases in FEV_1 and FVC are observed at ozone concentration of 0.2 to 0.30 ppm for $\underline{V}_E$ of 30 to 50 L/min using intermittent exercise protocol (Aris et al., 1993; Hazbun et al., 1993; Fox et al., 1993; Aris et al., 1995; Balmes et al., 1996; Foster and Stetkiewicz 1996; Jorres et al., 1996; McKittrick and Adams, 1995; Frampton et al., 1997). At 0.12 ppm of ozone and $\underline{V}_E$ of ≥65 L/min using the same protocol, decrements in FVC, FEV_1 and pulmonary symptoms are observed (McDonnell et al., 1983). One study showed that exposure to 0.12 ppm ozone with a 2-hour intermittent exercise at $\underline{V}_E$ 45 L/min caused a significant increase in airway resistance (Liu et al., 1997b). Continuous heavy exercise ($\underline{V}_E$ = 55 to 90 L/min) for one hour has resulted in similar effects at 0.16 to 0.3 ppm ozone (McKittrick and Adams 1995).

Prolonged exposure (6 to 8-hours) has resulted in responses being observed at lower ozone concentration and lower ventilation rates. Responses are observed with ozone concentrations as low as 0.08 ppm using moderate intermittent exercise protocols ($\underline{V}_E$~ 35-40 L/min) (Folinsbee et al., 1988, 1991; Horstman et al., 1990; McDonnell et al., 1995). The symptoms and spirometry responses increase in a time, ozone concentration and $\underline{V}_E$ dependent pattern. Airway resistance is not always a sensitive parameter for exposure to ozone at concentrations up to 0.50 ppm with moderate or heavy exercise protocol.

Differences in responses between individuals, as well as the reproducibility of a response for each individual, have been documented. Healthy 'responders' have shown FEV_1 decrements at 0.12 ppm ozone during 2-hour intermittent exercise ($\underline{V}_E$ = 45 L/min), while 'non-responders' at this exposure regimen did not show any effects (Liu et al., 1997b). This suggests that some individuals are consistently more susceptible than others in their physiological response.

Group mean decrements in pulmonary function can be roughly estimated when expressed as a nonlinear (sigmoid) function of effective dose of ozone (ozone concentration x duration x $\underline{V}_E$) (McDonnell et al., 1993; McDonnell et al., 1995; Seal et al., 1996). The ozone concentration appears to have a greater impact on the pulmonary function response than does $\underline{V}_E$ or exposure duration. The exponent of approximately 4/3 suggests that doubling ozone concentration would be similar to increasing exposure duration by about 2.5 times (Larsen et al., 1991). Since at a given ozone concentration, the major external variables of spirometric response are $\underline{V}_E$ and exposure duration, and there is an intersubject variability in response, the models have not been able to estimate individual responses. Nevertheless, prediction of group mean FEV_1 responses using the variables of ozone concentration, duration and $\underline{V}_E$ can be successful. When taking into consideration duration and $\underline{V}_E$, a threshold ozone concentration below which no pulmonary function response would be elicited does not seem to exist.

Nevertheless, data from prolonged exposure (6 to 8-hours) at low ozone concentration (0.08 - 0.12 ppm) demonstrate that there is a plateau of FEV1 response to ozone for exposure duration. FEV1 response tends not to increase further with increasing duration of exposure, though the lung function decrements are in a ozone concentration-dependent linear fashion. The level of the

response plateau is dependent upon ozone concentration and ventilation rate, and increasing ozone concentration can still enchance the response to beyond the existing plateau. Prolonged exposure studies at low dose suggest that ozone-induced spirometry responses depend upon the immediate exposure history.

Data from repeated exposure studies at higher concentrations (0.35 to 0.45 ppm) typically show that the spirometric response to ozone is enhanced on the second day of several consecutive days of exposure. At lower dose (0.12 ppm for 6.6-hours), this enhancement is observed on the first day and subsides on the second day, which may be explained by a more complete recovery and/or less damage to pulmonary tissues. The same studies have shown an attenuation of effects of ozone ('adaptation' or 'tolerance') after 3 to 5 days of exposure regardless of ozone dose rate, although attenuation occurs earlier at lower dose rates. Attenuation is partially reversed after 4 to 7 days post-exposure and normal responsiveness is returned within 1 to 2 weeks. The attenuation of bronchial responsiveness to methacholine challenge may occur somewhat more slowly than that of symptoms and spirometric responses.

In a study designed to compare the effects of varying and constant levels of ozone exposure (Hazucha et al., 1992), subjects were exposed to either air, a constant concentration of 0.12 ppm ozone, or a linearly variable concentration of ozone that ranged from 0 at start to 0.24 ppm at 4-hours and back down to 0 ppm at 8-hours (end of experiment). The workload of intermittent exercise was the same ($\underline{V}_E$ 40 L/min). At a constant 0.12 ppm of ozone, FEV_1 was decreased by 5% at the fifth hour until the end of the exposure period. In the variable concentration group, the FEV_1 decreased almost twice as much as the constant dose regimen, though the total concentration of ozone in two groups was the same. In essence, the results show that the average dose value calculated as a mean over an 8-hour exposure may underestimate the effect of ozone on FEV_1 and possibly other parameters (e.g., inflammatory response), when peaks are much higher than the averaged value over 8 hours. Although it is difficult to quantitatively determine spirometric responses to peak values in a epidemiological field study, the information obtained from the controlled human exposure studies is indicative of a potentially substantial health effect. The above data raise a concern about exercising people with increased $\underline{V}_E$. When taking into consideration the potential for exercising people to be exposed during a peak, it may increase their spirometric (e.g., FEV_1) and biological response (such as pulmonary injury). As such, they should be identified as a population sub-group more at risk.

The data from other authors (McDonnell et al., 1983) appear to show that a plateau is reached at a certain dose/time; FEV_1 decrements reach a plateau of 15-17% between 0.24 to 0.4 ppm exposure concentrations after 2.5-hour exposure. This may in fact mean that high peaks may not be able to produce effects larger than this. Since conditions of exposure are different in these studies, one must be careful when estimating the potential outcome of such peaks.

It has been hypothesized that there may be an overall sequence of events influencing the outcomes of ozone exposure on lung toxicity. Initially, ozone is delivered to the lung tissue where it then reacts with components in airway surface liquid and/or epithelial cell membranes. This then triggers local tissue responses including injury and inflammation. Finally, there is a stimulation of

the neural afferent (bronchial C fibres) which results in a reflex response. The stimulation of C fibres, possibly through an increased sensitization of the cholinergic receptors, results in the contraction of the lung smooth muscle fibres which increases airway resistance. This is followed by rapid shallow breathing and increased frequency. These effects contribute to maintain a constant $\underline{V}_E$.

It is noteworthy that there exists evidence that airway inflammatory responses are not correlated with decrements in FEV_1 (Aris et al., 1993; Balmes et al., 1996; Hazucha et al., 1996). The inflammatory effects observed in 'responders' (substantial decrement in FEV_1) versus 'non-responders' (little decrement in FEV_1) are inconsistent. One study (Balmes et al., 1996) shows that there were similar increases in inflammatory cells and mediators in airway lavage fluids in responders and non-responders after exposure to 0.2 ppm ozone for 4-hours. Another study (Torres et al., 1996) indicates that responders had significantly increased superoxide anion production in lavage cells and reduced hydrogen peroxide production in alveolar macrophages after exposure to 0.22 ppm ozone (4-hours), whereas non-responders did not have significant changes in inflammatory cell function. Nevertheless, the number of PMN in BAL increased to a similar extent in responders and non-responders (Torres et al., 1996). Since ozone-induced FEV_1 and FVC decrements are thought to involve neurally mediated involuntary inhibition of inspiration and reduction of vital capacity (Hazucha et al., 1989), changes in spirometric parameters may not necessarily reflect cell injury. Since the US National Ambient Air Quality Standard (NAAQS) for ozone was established largely based on spirometric responses to ozone inhalation, some researchers have suggested rethinking the NAAQS-setting process for ozone.

Subjects with pre-existing disease

Of the subpopulations studied, those with pre-existing limitations in pulmonary function and exercise capacity are of primary concern in evaluating the health effects of ozone. Most of the studies available to date have definite limitations (e.g., subject selection, non-standardized method of characterization, limited dose-range) that preclude any final conclusion from being drawn.

For patients with COPD performing light to moderate intermittent exercise, early studies showed no decrements in pulmonary function after 1- and 2-hour exposures to ≤0.3 ppm ozone. A more recent study, however, shows that COPD patients had significantly greater loss of FEV_1 than their same age healthy counterparts (-19% versus -2%, respectively), when exposed to 0.24 ppm ozone for 4-hours during intermittent exercise ($\underline{V}_E$ = 20 L/min) (Gong et al., 1997b). COPD patients also showed moderate increase in symptoms and blood oxygenation which were not seen in healthy subjects (Gong et al., 1997b). This study suggests that COPD patients may be more sensitive to ozone exposure than healthy subjects.

For patients with asthma, data consistently show that no significant decrements in group pulmonary functions could be elicited with 40 minute to 2-hour ozone exposures ranging from 0.12 to 0.25 ppm, at rest or using light to moderate intermittent exercise ($\underline{V}_E$ 30 to 33 L/min) protocols, in both adults and adolescents. However, although the percent decrease in spirometry values was similar for healthy and asthmatic subjects after an acute exposure to an even higher effective dose of ozone (0.25 ppm for 3-hours, or 0.4 ppm for 2-hours), the asthmatic group had a pre-existing

decrement of pulmonary function in comparison with healthy subjects (Kreit et al., 1989; Hiltermann et al., 1995; Jorres et al., 1996). Studies using a prolonged (6 to 8-hours, $\underline{V}_E$ approximately 30 L/min) exposure protocol have demonstrated that asthmatic subjects had more pronounced decrements in FEV1 than did healthy subjects in response to 0.12 to 0.16 ppm ozone (Linn et al., 1994; Horstman et al., 1995). Thus, if the total inhaled dose (or effective dose) is increased significantly by either increasing exposure duration or $\underline{V}_E$ during exposure, or ozone concentration, mild to moderate asthmatics will respond with a greater obstructive response than will normal subjects. Therefore, asthmatic subjects may be considered more susceptible and the average response of these subjects is probably more important, since their baseline values are already significantly depressed due to the underlying illness. Moreover, asthmatic subjects appear to have higher airway cellular responses (McBride et al., 1994) and higher responsiveness to allergens (Jorres et al., 1996) than healthy subjects, suggesting that exposure to ozone may facilitate the onset of asthma.

For patients with allergic rhinitis, data suggest that they have a greater rise in airway resistance than healthy subjects when exposed to 0.18 to 0.25 ppm ozone for 2 to 3-hours with intermittent exercise ($\underline{V}_E$ ~ 30 L/min). Allergen treatment substantiated ozone-induced FEV_1 decrement in these patients (Jorres et al., 1996).

11.7.2 Effects on exercise performance

A reduction of $\underline{V}O_{2max}$ has been observed after exposure to ozone (0.75 ppm for 2-hours) as well as a reduction in endurance. The ozone concentration that induces decrements in performance time during vigorous exercise can be as low as 0.06 ppm (duration 16 to 28 min) (Linder et al., 1988; Avol et al., 1984a). The actual mechanisms that lead to effects on exercise performance are not clear. Reports indicate that breathing discomfort associated with maximal ventilation may be an important factor in limiting exercise performance. This does not exclude the potential for a physiological mechanism to be responsible for these effects.

11.7.3 Effects on airway responsiveness

Ozone causes an increase in non-specific airway responsiveness which predisposes the airway to narrowing upon inhalation of a variety of stimuli. For young healthy subjects, increased airway responsiveness to histamine and methacholine has been observed following exposure to 0.20 ppm ozone for 1-hour (vigorous exercise) (Gong et al., 1986), and 0.08 to 0.12 ppm ozone for 6.6-hours with intermittent exercise ($\underline{V}_E$ 39 to 50 L/min) (Folinsbee et al., 1988; Horstman et al., 1990). In view of effective dose, elderly subjects appear to be less sensitive to ozone-induced airway responsiveness in comparison with young people.

Ozone exposure (0.12 ppm for 6.6-hours with exercise ~ $\underline{V}_E$ 29 L/min) of asthmatic subjects, who have increased baseline airway responsiveness, can cause further increases in responsiveness. The difference in baseline airway responsiveness between healthy and mild asthmatic subjects may be as much as 1000 fold, whereas the changes in airway responsiveness induced by ozone are typically 2 to 4 fold. Similar relative changes in airway responsiveness are seen in asthmatics exposed to ozone despite their markedly different baseline airway responsiveness. One of the important aspects of this observation of increase in airway responsiveness after ozone exposure is

that this may represent a plausible link between ambient ozone exposure and increased hospital admissions for asthma.

The increases in airway responsiveness initiated by ozone exposure tend to resolve within 24 hours after exposure but may persist longer in some individuals. Changes in airway responsiveness after ozone exposure appear to be resolved more slowly than changes in FEV_1 or respiratory symptoms. In studies of repeated exposure to ozone, changes in airway responsiveness tend to be somewhat less susceptible to attenuation with consecutive exposures than changes in FEV_1. The question of whether chronic ozone exposure can induce a persistent increase (or decrease) in airway responsiveness has not been adequately studied.

Increases in airway responsiveness do not appear to be strongly associated with decrements in lung function or increases in symptoms. The mechanism of ozone-induced increases in airway responsiveness appears to be a consequence of cellular and biochemical changes in airway tissue.

11.7.4 Inflammation and host defence effects

Exposure to ozone (0.2 to 0.25 ppm for 1 to 4-hours, at $\underline{V}_E$ 40 to 60 L/min; 0.35 to 0.6 ppm, for 2 to 4-hours, at $\underline{V}_E$ 50 to 60 L/min) results in an inflammatory response characterized by increased numbers of PMNs, increased permeability of the epithelial cells lining the respiratory tract, cell damage, and production of proinflammatory cytokines and prostaglandins. This response can be detected as early as 1 hour after exposure and persists for at least 18 hours. The response profile of these mediators is not adequately defined, although it is clear that the time course of response varies for different mediators and cells.

Limited data have demonstrated that exposure to ambient levels of ozone (0.08 to 0.12 ppm) for 6.6-hours with exercise (40 - 45 L/min) can cause increases in inflammatory cells and mediators in upper and lower airways (Devlin et al., 1991). There is a considerable interindividual variation of inflammatory responses to ozone. These data suggest that although population as a whole may have a small inflammatory response to low levels of ozone, there may be significant subpopulation that is very sensitive to the low levels of ozone.

Asthmatic patients reveal more sensitive toward ozone-induced airway inflammation than healthy subjects. At concentrations of 0.12 to 0.24 ppm (90 minutes at $\underline{V}_E$ ~ 50 L/min, and 6-hours at $\underline{V}_E$ ~ 25 L/min), ozone induced higher inflammatory responses in asthmatics than in healthy subjects (Basha et al., 1994; Scannell et al., 1996).

The ‘adaptation’ phenomenon has been observed in inflammatory responses during repeated ozone exposure studies. Limited data show that after 5 consecutive days exposure to 0.4 ppm ozone, ozone-induced increase in cellular and biochemical parameters in BAL are attenuated, which can be partially reversed within 10 to 20 days. However, some cell injury markers did not

show adaptation, implying a continuing tissue damage during repeated exposure, although spirometric parameters may return to a normal level.

In vitro studies suggest that epithelial cells are the primary target of ozone in the lung, and that ozone may induce them to produce many of the mediators found in human BAL fluid. Moreover, exposure to ozone at 0.2 to 0.4 ppm for 2 to 4-hours (with moderate to heavy exercise at $\underline{V}_E$ up to 66 L/min) has been found to cause the impairment of macrophage phagocytosis and hydrogen peroxide production, and hence may impair the endogenous antibiotic system.

11.7.5 Factors modifying responsiveness to ozone

None of the potential influences on ozone responsiveness (age, gender, race, hormonal fluctuations, smoking, seasonal variations in responsiveness, and ambient environmental factors) have been thoroughly investigated. However, the observation that healthy elderly adults appear to be less responsive to ozone exposure than young adults in spirometric parameters should be taken into consideration in risk assessment. No data have yet been reported on ozone-induced airway inflammatory changes in elderly subjects. In addition, responses of children and adolescents to ozone remain inadequately studied.

11.7.6 Extrapulmonary systemic effects of ozone

It is still believed that ozone immediately reacts on contact with respiratory system tissue and is not absorbed or transported to extrapulmonary sites to any significant degree.

11.7.7 Effects of ozone mixed with other pollutants

For simultaneous exposure to mixtures of ozone (0.12 to 0.6 ppm) with SO_2, NO_2, H_2SO_4, HNO_3, or particulate aerosols for 1 or 2-hours ($\underline{V}_E$ 25 - 40 L/min), or with multiple combinations of these pollutants, no significant enhancement of respiratory effects have been consistently demonstrated for these chemicals, i.e. response is not more than additive. Exposure to PAN at high levels (0.30 ppm) combined with ozone exposure has resulted in greater pulmonary function responses than ozone alone (Horvath et al., 1986). At ambient concentration, the effect of PAN appears negligible (Dreschler-Parks et al., 1989).

However, a study of exposure to ozone followed by SO_2 exposure has revealed an interaction between the two pollutants (Koenig et al.1990). The study shows that pre-exposure to ozone (0.12 ppm for 45 min, $\underline{V}_E$ 30 L/min) followed by exposure to a low concentration of SO_2 (0.1 ppm for 15 min) elicited a greater degree of bronchial hyperreactivity than did pure air followed by SO_2 or by two exposures to ozone alone; indicating some synergism between the effects of SO_2 and ozone. These results also can be interpreted, in view of the fact that ozone enhances non-specific bronchial responsiveness, to mean that the elevated SO_2 responses may simply reflect this increased responsiveness. Two studies investigating the effect of exposure to co-pollutants in a sequential pattern also show an additive effect of NO_2 (0.6 ppm for 2-hours) (Hazucha et al., 1994) and sulphuric acid (100 $\mu g/m^3$, for 3-hours) (Utell et al., 1994) on ozone (0.18 to 0.3 ppm, 45 minutes to 3-hours) induced spirometric responses. The exercise levels were at $\underline{V}_E$ 30 - 40 L/min.

The assessment of such mixtures is far from complete and much work remains to be done. Few studies have included more than two chemicals, and the endpoints measured are often limited to pulmonary function variables.

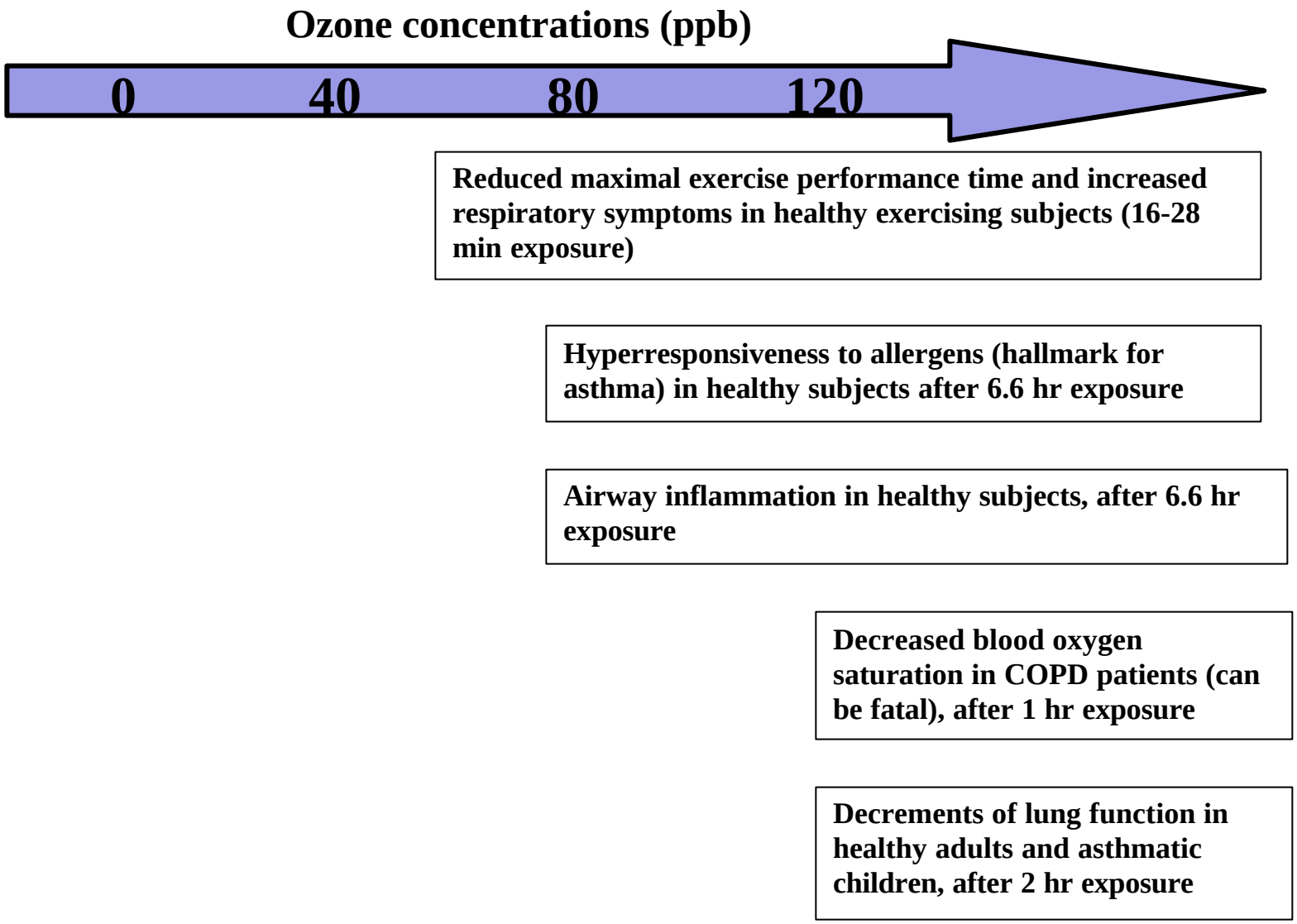
11.7.8 Conclusions

Overall, the responses observed in humans exposed to ozone can be separated into five different categories:

1. symptoms: cough, pain on deep inspiration, shortness of breath, throat irritation, and wheezing;
2. spirometric (spirographic) changes: decrements in FEV_1, FVC, and peak expiratory flow rate (PEFR);
3. airway resistance: increases in airway resistance (R_{aw});
4. bronchial responsiveness: to methacholine, allergens, sulphur dioxide, cold air, etc.; and
5. airway inflammation: changes in numbers and functions of inflammatory cells, and changes in mediator levels, measured in BAL and nasal lavage fluids and sputum.

The lowest doses reported in clinical studies that have induced health effects are summarized in Figure 11.3. It must be noted that no study on tissue injury, such as pulmonary inflammation, has been carried out at ozone concentrations below 80 ppb for healthy subjects, or below 120 ppb for asthmatics. These drawbacks limit the use of clinical data for predicting the health effects from ambient ozone exposure in a general population, or for establishing a lowest observed effect level (LOAEL) for ozone.

Figure 11.3 Summary of lowest doses of ozone reported to have induced health effects in human clinical studies.



An important finding of the human clinical studies is that at a given ozone concentration, the increase in ventilation results in elevated pulmonary function responses and inflammation, and that increased ventilation lowers the concentration of ozone required for a given pulmonary response. Thus the concept of 'effective dose' is introduced, namely, a product of the ventilation rate, the concentration of ozone and the exposure duration ($\underline{V}_E$ x ozone concentration x duration). This concept allows us the opportunity to further establish models and to investigate the sensitivity of different ages, genders, and disease status in response to ozone exposure. This also implies that persons doing outdoor exercise during an ozone episode may be at higher risk due to their higher intake of ozone. This assumption has been confirmed by observations that exposure to ozone as low as 60 ppb for only 16 to 28 min caused a significant decrease in endurance to heavy exercise and a significant increase in symptoms.

Dose-response curves have been derived from several studies using different models (logistic model, exponential model, quadratic model, and the log regression model), all for spirometric responses. An analysis of all these models was performed by Highfill and Costa (1995) which shows the limitations of each model. In general, the models that include variables for concentration (C) and duration (T) of exposure show a better fit to measured values. Attempts by these authors to include the ventilation rate ($\underline{V}_E$) in the models are stated to have improved the models. The log regression model proposed by Larsen et al. (1991) appears to predict quite well the FEV_1 decrements under very specific conditions. More importantly, on the grounds of the 'effective dose', there does not appear to be a threshold for ozone-induced spirometric responses; a prolonged ozone exposure at a concentration ≤0.08 ppm (the current Canadian National Ambient Air Quality Objective for ozone) may still exhibit an adverse health effect.

In respect to the sensitivity of biological markers (parameters) for ozone-induced adverse health effects, data have demonstrated that prolonged exposure (6 to 8-hours) of healthy subjects to ambient levels of ozone (0.08 to 0.12 ppm) with intermittent exercise at $\underline{V}_E$ of 35 to 50 L/min may cause increased bronchial responsiveness to methacholine, increased inflammation in airway (BAL), and decreased FEV_1. For shorter exposure duration (1 to 4-hours) to ≤0.12 ppm ozone, a significant increase in bronchial responsiveness has been observed, while little or no response in pulmonary function is seen, and no data have been reported on inflammatory response. The symptoms are often subjective measurements or simple reflex responses (cough), and are therefore difficult to correlate with injury. Airway resistance is not always sensitive in response to up to 0.5 ppm ozone exposure. Compared with measurements of bronchial responsiveness and inflammation parameters in BAL, FEV_1 appears to be a most convenient, non-invasive, and relatively sensitive marker for ozone-induced response. There are several observations which support this assumption, and may assist us in determining the best approach to regulating ozone in Canada. The exposure levels of ozone associated with increases in the hospitalization rates due to asthma are in the same range as those associated with the FEV_1 decrements observed in chamber studies. A dose-response curve can be applied to FEV_1 decrements. Asthmatic subjects who have higher inflammatory responses and bronchial reactivity also have higher decrements in FEV_1 in comparison with healthy subjects.

However, numerous studies have shown that changes of FEV_1 do not correlate with cell injury endpoints and bronchial hyperresponsiveness in response to ozone exposure. 'Responders' to ozone-induced decrements in FEV_1 do not necessarily have higher level of pulmonary tissue injury in comparison with 'non-responders'. This hinders the evaluation of the toxicity of ozone using spirometric parameters (i.e., at which point does a decrement in FEV_1 reflect injury). Therefore, when assessing the risks associated with ozone exposure, it is preferable to consider incorporating all the data on inflammation, bronchial hyperresponsiveness and spirometric responses.

In terms of sensitive subpopulations, only asthmatic subjects have constantly revealed higher alterations in spirometric and inflammatory parameters, and bronchial reactivity than their healthy counterparts. Since asthmatic patients have lower baseline values for most of the spirometric parameters than healthy subjects, a further decrease in FEV_1 induced by ozone may carry the value below the threshold which is associated with asthma attacks. In light of the epidemiological data indicating an increase in hospitalization due to asthma during high ozone days in Canada (Burnett et al., 1994; Thurston et al., 1994a,b), we may conclude that asthmatic subjects are a sensitive subgroup for ozone exposure. Data for COPD patients and subjects with allergic rhinitis are scarce and often inconsistent, and hence preclude any final conclusions.

A limited number of clinical studies have investigated the age difference in response to ozone. It appears that adolescents are more sensitive to ozone-induced pulmonary function decrements when compared with adults using the same dose regimen. However, older adults (60 years or older) have not been demonstrated to be particularly more susceptible to ozone in pulmonary function changes than their younger counterparts when given the same dose, although no study has been done on age differences in inflammatory responses. At present, based on limited studies, age does not appear to be a major determinant of response to ozone exposure, however, more research needed to elucidate this aspects of the issue.

Studies using varying doses (0 to 0.24 ppm, back to 0 ppm) over an 8-hour exposure have demonstrated that FEV_1 decrements are almost twice as much compared with a constant dose of 0.12 ppm for the same duration. Hence, using average dose value may underestimate the effects of ozone on FEV_1, and likely on other parameters, such as inflammation. The above data raise a concern about exercising people with increased $\underline{V}_E$. When taking into consideration the potential for exercising people to be exposed during a peak, it may increase their spirometric (e.g., FEV_1) and biological response (such as pulmonary injury). As such, they should be identified as a population sub-group more at risk.

11.8 RESEARCH NEEDS

- Data on ambient-levels of ozone-induced pulmonary injury are very limited, and need to be expanded, to corroborate the findings in epidemiological studies. A majority of the BAL analyses so far have been carried out at ozone concentrations of 0.4 ppm or higher.

- More systemic studies, such as ones investigating cardiovascular effects, should be carried out to elucidate the mechanisms of ozone-induced cardiovascular mortality observed in epidemiological studies.

- More sensitive biological markers of tissue injury should be developed to link ambient levels of ozone exposure to adverse health effects, in order to enhance the sensitivity of human clinical studies.

- Much work needs to be carried out to assess the effects of air pollutant mixtures. So far, few studies have included more than two chemicals, and the endpoints measured are often limited to pulmonary function variables.

REFERENCES

Adams, W.C. (1986) Physiological responses of healthy human subjects consequent to inhalation of NO_2, O_3, and NO_2 plus O_3 during heavy, sustained exercise. Sacramento, CA: California State Air Resources Board; report no. ARB-R-87/294. Available from: NTIS, Springfield, VA; PB87-165106.

Adams, W. C., Schelegle E.S., Shaffrath J.D. (1989). Oral and orinasal breathing during continuous exercise produce similar responses to ozone inhalation. Arch. Environ. Health 44: 311-316.

Aris, R., Christian, D., Tager, I., Ngo, L., Finkbeiner, W.E., Balmes, J.R. (1993). Effects of nitric acid gas alone or in combination with ozone on healthy volunteers. Am Rev Respir Dis, 148, 965-73.

Aris R.M., Christian D., Hearne P.Q., Kerr K., Finkbeiner W.E., and Balmes J.R. (1993). Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am J Respir Crit Care Med, 148, 1363-72.

Aris, R.M., Tager, I., Christian, D., Kelly, T., Balmes, J.R. (1995). Methacholine responsiveness is not associated with O_3-induced decreases in FEV_1. Chest, 107, 621-8.

Avol, E.L., Linn, W.S., Venet, T.G., Shamoo, D.A., & Hackney, J.D. (1984a). Comparative respiratory effects of ozone and ambient oxidant pollution exposure during heavy exercise. J Air Pollut Control Assoc 34: 804-809.

Avol, E.L., Linn, W.S., Venet, T.G., Shamoo, D.A., Spier, C.E., & Hackney, J.D. (1984b). Short-term health effects of ambient air pollution in adolescents. In: Evaluation of the scientific basis for ozone/oxidants standards: Transactions of an APCA international specialty conference, 1984. Edited by Lee, S.D. Air Pollut Contr Assoc, Pittsburgh, PA.

Avol, E.L., Linn, W.S., Venet, T.G., Shamoo, D.A., Spier, C.E., Hackney, J.D. (1985). Comparative effects of laboratory generated ozone and ambient oxidant exposure in continuously exercising subjects. In: Lee, S.D., ed. Evaluation of the scientific basis for ozone/oxidants standards: proceedings of an APCA international specialty conference; November 1989; Houston, TX. Pittsburgh, PA: Air Pollution Control Association; pp. 216-225. (APCA international specialty conference transaction: TR-4).

Ball, B.A., Folinsbee, L.J., Peden, D.B., Kehrl, H.R. (1996). Allergen bronchoprovocation of patients with mild allergic asthma after the exposure. J Allergy Clin Immunol, 98, 563-72.

Balmes, J.R., Chen, L.L., Scannell, C., Tager, I., Christian, D., Hearne, P.Q., Kelly, T., Aris, R.M. (1996). Ozone-induced decrements in FEV_1 and FVC do not correlate with measures of inflammation. Am J Respir Crit Care Med, 153, 904-9.

Bascom, R., Naclerio, R.M., Fitzgerald, T.K., Kagey-Sobokta, A., Proud, D. (1990). Effect of ozone inhalation on the response to nasal challenge with antigen of allergic subjects. Am. Rev. Respir. Dis. 142: 594-601.

Basha, M.A., Gross, K.B., Gwizdala, C.J., Haidar, A.H., Popovich, Jr. (1994). Bronchoalveolar lavage neutrophilia in asthmatic and healthy volunteers after controlled exposure to ozone and filtered purified air. Chest, 106, 1757-65.

Beckett, W.S., McDonnell, W.F., Horstman, D.H., House, D.E. (1985) Role of the parasympathetic nervous system in acute lung response to ozone. J. Appl. Physiol. 59:1879-85.

Bedi, J.F., Drechsler-Parks, D.M., Horvath, S.M. (1985). Duration of increased pulmonary function sensitivity to an initial ozone exposure. Am. Ind. Hyg. Assoc. J. 46: 731-734.

Brookes, K.A., Adams, W.C., Schelegle, E.S. (1989). 0.35 ppm O_3 exposure induces hyperresponsiveness on 24-hour reexposure to 0.20 pp, O_3. J. Appl. Physiol. 66: 2756-2762.

Burnett, R.T., Dales, R.E., Raizenne, M.E., Krewski, D, Summers, P.W., Roberts, G.R., Raad-Young, M., Dann, T., and Brook, J. (1994). Effect of low ambient levels of ozone and sulfates on the frequency of respiratory admissions to Ontario hospitals. Environ. Res. 65: 172-179.

Bush, M.L., Asplund, P.T., Miles, K.A., Ben-Jebria, A., Ultman, J.S. (1996). Longitudinal distribution of O_3 absorption in the lung: gender differences and intersubject variability. J Appl Physiol, 81, 1651-7.

Coffey, M.J., Wheeler, C.S., Gross, K.B., Eschenbacher, W.L., Sporn, P.H.S., Peters-Golden, M. (1996). Increased 5-lipoxygenase metabolism in the lungs of human subjects exposed to ozone. Toxicology, 114, 187-97.

Coleridge, H.M., Coleridge, J.C.G., Baker, D.G., Ginzel, K.H., Morrison, M.A. (1978). Comparison of the effects of histamine and prostaglandin on afferent C-fiber endings and irritant receptors in the intrapulmonary airways. Adv Exp Med Biol, 99, 291-305.

Coleridge, H.M., Coleridge, J.C.G., Ginzel, K.H., Baker, D.G., Banzett, R.B., Morrison, M.A. (1976). Stimulation of 'irritant' receptors and afferent C-fibers in the lungs by prostaglandins. Nature, 264, 451-3.

Devlin, R.B., McDonnell, W.F., Koren, H.S., Becker, S. (1990). Prolonged exposure of humans to 0.10 and 0.08 ppm ozone results in inflammation in the lung. Presented at: 83 annual meeting of the Air and Waste Management Association; June; Pittsburgh, PA. Pittsburgh, PA: Air & Waste Management Association; paper no. 90-150.2.

Devlin, R.B., McDonnell, W.F., Mann, R., Becker, S., House, D.E., Schreinemachers, D., Koren, H.S. (1991). Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am. J. Reso. Cell. Mol. Biol. 4: 72-81.

Devlin, R.B., McDonnell, W.F., Becker, S., Madden, M.C., McGee, M.P., Perez, R., Hatch, G., House, D.E., Koren, H.S. (1996). Time-dependent changes of inflammatory mediators in the lungs of humans exposed to 0.4 ppm ozone for 2 hr: A comparison of mediators found in bronchoalveolar lavage fluid 1 and 18 hr after exposure. Toxicol Appl Pharmacol, 138, 176-85.

Devlin, R.B., Folinsbee, L.J., Biscardi, F., Hatch, G., Becker, S., Madden, M.C., Robbins, M., Koren, H.S. (1997). Inflammation and cell damage induced by repeated exposure of humans to ozone. Inhalation Toxicology, 9, 211-35.

Dimeo, M.J., Glenn, M.G., Holtzman, M.J., Sheller, J.R., Nadel, J.A., Boushey, H.A. (1981). Threshold concentration of ozone causing an increase in bronchial reactivity in humans and adaptation with repeated exposures. Am. Rev. Respir. Dis. 124: 245-248.

Drechsler-Parks, D.M. (1995a). Cardiac output effects of O_3 and NO_2 exposure in healthy older adults. Toxicol Ind Health, 11, 99-109.

Drechsler-Parks, D.M. (1995b). The dose-response relationship in older men exposed to ozone. Experimental Gerontology, 30, 65-75.

Drechsler-Parks, D.M., Bedi, J.F., Horvath, S.M. (1989) Pulmonary function responses of young and older adults to mixtures of O_3, NO_2 and PAN. Toxicol. Ind. Health 5: 505-517.

Emmons, K., Foster, W.M. (1991). Smoking cessation and acute airway response to ozone. Arch. Environ. Health 46: 288-295.

Eschenbacher, W.L., Ying, R.L., Kreit, J.W., Gross, K.B. (1989). Ozone-induced lung function changes in normal and asthmatic subjects and the effect of indomethacin. In: Schneider, T., Lee, S.D., Wolters, G.J.R., Grant, L.D., eds. Atmospheric ozone research and its policy implications: proceedings of the 3rdUS-Dutch international symposium; May 1988; Nijmegen, The Netherlands. Amsterdam, The Netherlands: Elsevier Science Publishers; pp. 493-499. (Studies in environmental science 35).

Fahy, J.V., Wong, H.H., Liu, J.T., Boushey, H.A. (1995). Analysis of induced sputum after air and ozone exposures in healthy subjects. Environ Res, 70, 77-83.

Fernandes A.L.G., Molfino N.A., McClean P.A., Silverman F., Tarlo S., Raizenne M., Slutsky A.S., and Zamel N. (1994). The effect of pre-exposure to 0.12 ppm of ozone on exercise-induced asthma. Chest, 106, 1077-1082

Folinsbee, L.J., Hazucha, M.J. (1989). Persistence of ozone-induced changes in lung function and airway responsiveness. In: Schneider, T., Lee, S.D., Wolters, G.J.R., Grant, L.D., eds. Atmospheric ozone research and its policy implications: proceedings of the 3rd US-Dutch international symposium, May 1988, Nijmegen, The Netherlands. Amsterdam, the Netherlands: Elsevier Science Publishers, pp. 483-492. (Studies in environmental science 35).

Folinsbee, L.J., Silverman, F., Shephard, R.J. (1977). Decrease of maximum performance following ozone exposure. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 42: 531-536.

Folinsbee, L.J., Drinkwater, B.L., Bedi, J.F., Horvath, S.M. (1978). The influence of exercise on the pulmonary function changes due to exposure to low concentrations of ozone. In: Folinsbee, L.J., Wagner, J.A., Borgia, J.F., Drinkwater, B.L., Gliner, J.A., Bedi, J.F., eds. Environmental stress: individual human adaptations. New York, NY: Academic Press; pp. 125-145.

Folinsbee, L.J., Horstman, D.H., Vorona, R.D., Prince, J.M. (1986). Determinants of endurance performance during ozone inhalation. Presented: 1986 IUPS meeting; July; Vancouver. Canada. Proc. Int. Union Physiol. Sci. 16: 176. Paper no. P217.04.

Folinsbee, L.J., McDonnell, W.F., Horstman, D.H. (1988). Pulmonary function and symptom responses after 6.6-hour exposure to 0.12 ppm ozone with moderate exercise. JAPCA 38: 28-35.

Folinsbee, L.J., Horstman, D.H., Kehrl, H.R., McDonnell, W.F., Gerrity, T.R., Seal, E., Larson, R., Hazucha, M.J., Abdul-Salaam, S., Faucette, B., Ives, P.J. (1991). Effects of single and repeated prolonged low-level ozone exposure in man. Presented at: annual meeting of the Society for Occupational and Environmental Health; March; Washington, DC.

Folinsbee L.J., Horstman D.H., Kehrl H.R., Harder S., Abdul-Salaam S., and Ives P.J. (1994). Respiratory responses to repeated prolonged exposure to 0.12 ppm ozone. Am J Respir Crit Care Med, 149, 98-105.

Foster, W.M., Stetkiewicz, P.T. (1996). Regional clearance of solute from the respiratory epithelia: 18-20 h postexposure to ozone. J Appl Physiol, 81, 1143-9.

Foster, W.M., Wills-Karp, M., Tankersley, C.G., Chen, X., Paquette, N.C. (1996). Bloodborne markers in humans during multiday exposure to ozone. J Appl Physiol, 81, 794-800.

Fox, S.D., Adams, W.C., Brookes, K.A., Lasley, B.L. (1993). Enhanced response to ozone exposure during the follicular phase of the menstrual cycle. Environ. Health Perspect. 101: 242-244.

Frampton, M.W., Morrow, P.E., Gibb, F.R., Speers, D.M., Gavras, J.B. Utell, M.J. (1993). Airways responsiveness to ozone in smokers and nonsmokers. Am. Rev. Respir. Dis. 147: A636.

Frampton M.W., Morrow P.E., Cox C., Levy P.C., Condemi J.J., Speers D., Gibb F.R., Utell M.J. (1995). Sulfuric acid aerosol followed by ozone exposure in healthy and asthmatic subjects. Environ. Res. 69(1):1-14

Frampton, M.W., Morrow, P.E., Torres, A., Cox, C., Voter, K.Z., Utell, M.J. (1997). Ozone responsiveness in smokers and nonsmokers. Am J Respir Crit Care Med, 155, 116-21.

Gardner, D.E., Miller, F.J., Illing, J.W., Graham, J.A. (1982). Non-respiratory function of the lungs: host defences against infection. In: Schneider, T., Grant, L., eds. Air pollution by nitrogen oxides: proceedings of the US-Dutch international symposium; May; Maastricht, The Netherlands. Amsterdam. The Netherlands; Elsevier Scientific Publishing Company; pp. 401-415. (Studies in environmental science 21).

Gerbase, M.W., Foster, W.M., Thomas, K., Zacur, H., Weinmann, G.G. (1993). Effect of menstrual cycle on ozone responsiveness. Am. Rev. Respir. Dis. 147: A484.

Gerrity T.R., McDonnell W.F., and House D.E. (1994). The relationship between delivered ozone dose and functional responses in humans. Toxicol Appl Pharmacol, 124, 275-83.

Gliner, J.A., Horvath, S.M., Folinsbee, L.J. (1983). Preexposure to low ozone concentrations does not diminish the pulmonary function response on exposure to higher ozone concentrations. Am. Rev. Respir. Dis. 127: 51-55.

Golden, J.A., Nadel, J.A., Boushey, H.A. (1978). Bronchial hyperirritability in healthy subjects after exposure to ozone. Am. Rev. Respir. Dis. 118: 287-294.

Gong, H. Jr., Bradley, P.W., Simmons, M.S., Tashkin, D.P. (1986). Impaired exercise performance and pulmonary function in elite cyclists during low-level exposure in a hot environment. Am. Rev. Respir. Dis. 134: 726-733.

Gong, H., Jr., McManus, M.S., Linn, W.S. (1997a). Attenuated response to repeated daily ozone exposures in asthmatic subjects. Arch Environ Health, 52, 34-41.

Gong, H., Jr., Shamoo, D.A., Anderson, K.R., Linn, W.S. (1997b). Responses of older men with and without chronic obstructive pulmonary disease to prolonged ozone exposure. Arch Environ Health, 52, 18-25.

Graham D.E., and Koren H.S. (1990). Biomarkers of inflammation in ozone-exposed humans: comparison of the nasal and bronchoalveolar lavage. Am. Rev. Resp. Dis. 142:152-156.

Hackney, J.D., Linn, W. S., Buckley, R.D., Hislop, H.J. (1976). Studies in adaptation to ambient oxidant air pollution: effects of ozone exposure in Los Angeles residents vs. new arrivals. Environ. Health Perspect. 18: 141-146.

Hackney, J.D., Linn, W.S., Karuza, S.K., Buckley, R.D., Law, D.C., Bates, D.V., Hazucha, M., Pengelly, L.D., Silverman, F. (1977). Effects of ozone exposure in Canadians and southern Californians: evidence for adaptation? Arch. Environ. Health 32: 110-116.

Hatch, G.E., Slade, R., Harris, L.P., McDonald, W.F., Devlin, R.B., Koren, H.S., Costa, D.L., McKee, J. (1994). Ozone dose and effect in humans and rats: A comparison using oxygen-18 labelling and bronchoalveolar lavage. Am Rev Respir Cell Mol Biol, 150, 676-83.

Hazbun M.E., Hamilton R., Holian A., and Eschenbacher W.L. (1993). Ozone-induced increases in substance P and 8-epi-prostglandin $F_{2\alpha}$ in the airway of human subjects. Am J Respir Cell Mol Biol, 9, 568-72.

Hazucha, M.J., Silverman, F., Parent, C., Field, S., Bates, D.V. (1973). Pulmonary function in man after short-term exposure to ozone. Arch Environ Health, 27, 183-8.

Hazucha, M.J. (1987). Relationship between ozone exposure and pulmonary function changes. J. Appl. Physiol. 62(4): 1671-1680.

Hazucha, M.J., Bates, D.V., Bromberg, P.A. (1989). Mechanism of action of ozone on the human lung. J Appl Physiol, 67, 1535-41.

Hazucha, M.J., Folinsbee, L.J., Seal, E., Jr. (1992). Effects of steady-state and variable ozone concentration profiles on pulmonary function. Am. Rev. Respir. Dis. 146: 1487-1493.

Hazucha, M.J., Folinsbee, L.J., Seal, E., Bromberg, P.A. (1994). Lung function response of healthy women after sequential exposures to NO_2 and O_3. Am J Respir Crit Care Med, 150, 642-7.

Hazucha, M.J., Madden, M., Pape, G., Becker, S., Devlin, R., Koren, H.S., Bromberg, P.A. (1996). Effects of cyclo-oxygenase inhibition on ozone-induced respiratory inflammation and lung function changes. Eur J Appl Physiol, 73, 17-27.

Henderson, F.W., Dubovi, E.J., Harder, S., Seal, E., Jr., Graham, D. (1988). Experimental rhinovirus infection in human volunteers exposed to ozone. Am. Rev. Respir. Dis. 137: 1124-1128.

Highfill J.W., and Costa D.L. (1995). Statistical response models for ozone exposure: their generality when applied to human spirometric and animal permeability functions of the lung. J. Air Waste Manag. Assoc. 45(2):95-102.

Hiltermann, T.J.N., Stolk, J., Hiemstra, P.S., Fokkens, P.H.B., Rombout, P.J., Sont, J.K., Sterk, P.J., Dijkman, J.H. (1995). Effect of ozone exposure on maximal airway narrowing in non-asthmatic and asthmatic subjects. Clin Sci, 89, 619-24.

Holtzman, M.J., Cunningham, J.H., Sheller, J.R., Irsigler, G.B., Nadel, J.A., Boushey, H.A. (1979). Effect of ozone on bronchial reactivity in atopic and nonatopic subjects. Am. Rev. Respir. Dis. 120: 1059-1067.

Horstman, D.H., Folinsbee, L.J., Ives, P.J., Abdul-Salaam, S., McDonnell, W.F. (1990). Ozone concentration and pulmonary response relationships for 6.6-hour exposures with five hours of moderate exercise to 0.08, 0.10, and 0.12 ppm. Am. Rev. Respir. Dis. 142: 1158-1163.

Horstman, D.H., Ball, B.A., Brown, J., Gerrity, T., Folinsbee, L.J. (1995). Comparison of pulmonary responses of asthmatic and nonasthmatic subjects performing light exercise while exposed to a low level of ozone. Toxicol Ind Health, 11, 369-85.

Horvath, S.M., Gliner, J.A., Matsen-Twisdale, J.A. (1979). Pulmonary function and maximum exercise responses following acute ozone exposure. Aviat. Space Environ. Med. 50: 901-905.

Horvath, S.M., Gliner, J.A., Folinsbee, L.J. (1981). Adaptation to ozone: duration of effect. Am. Rev. Respir. Dis. 123: 496-499.

Horvath, S.M., Bedi, J.F., Drechsler-Parks, D.M. (1986) Effects of peroxyacetyl nitrate alone and in combination with ozone in healthy young women. J. Air Pollut. Control Assoc. 36: 265-270.

Horvath, S.M., Bedi, J.F., Drechsler-Parks, D.M., Williams, R.E. (1991). Alterations in pulmonary function parameters during exposure to 80 ppb ozone for 6.6 hours in healthy middle aged individuals. In: Berglund, R.L., Lawson, D.R., McKee, D.J., eds. Tropospheric ozone and the environment: papers from an international conference; March (1990); Los Angeles, CA. Pittsburgh, PA: Air & Waste Management Association; pp. 59-70. (A&WMA transaction series no. TR-19).

Hynes, B., Silverman, F., Cole, P., Corey, P. (1988). Effects of ozone exposure: a comparison between oral and nasal breathing. Arch. Environ. Health 43: 357-360.

Joad, J.P., Kott, K.S., Bric, J.M. (1996). The local C-fiber contribution to ozone-induced effects on the isolated guinea pig lung. Toxicol Appl Pharmacol, 141, 561-7.

Jorres, R., Nowak, D., Magnussen, H. (1996). The effect of ozone exposure on allergen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med, 153, 56-64.

Kehrl, H.R., Hazucha, M.J., Solic, J.J., Bromberg, P.A. (1985). Responses of subjects with chronic obstructive pulmonary disease after exposures to 0.3 ppm ozone. Am. Rev. Respir. Dis. 131: 719-724.

Kehrl, H.R., Vincent, L.M., Kowalsky, R.J., Horstman, D.H., O'Neil, J.J., McCartney, W.H., Bromberg, P.A. (1987). Ozone exposure increases respiratory epithelial permeability in humans. Am. Rev. Respir. Dis. 135: 1124-1128.

Kehrl, H.R., Vincent, L.M., Kowalsky, R.J., Horstman, D.H., O'Neil, J.J., McCartney, W.H., Bromberg, P.A. (1989). Ozone-induced changes in the pulmonary clearance of ^{99m}Tc-DTPA in man. In: Schneider, T., Lee, S.D., Wolters, G.J.R., Grant, L.D., eds. Atmospheric ozone research and its policy implications: proceedings of the 3rd US-Dutch international symposium; May 1988; Nijmegen, The Netherlands. Amsterdam, The Netherlands: Elsevier Science Publishers; pp. 343-351. (Studies in environmental science 35).

Koenig, J.Q., Covert, D.S., Morgan, M.S., Horike, M., Horike, N., Marshall, S.G., Pierson, W.E. (1985). Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. Am. Rev. Respir. Dis. 132: 648-651.

Koenig, J.Q., Covert, D.S., Marshall, S.G., Van Belle, G., Pierson, W.E. (1987). The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. Am. Rev. Respir. Dis. 136: 1152-1157.

Koenig, J.Q., Covert, D.S., Smith, M.S., Van Belle, G., Pierson, W.E. (1988). The pulmonary effects of ozone and nitrogen dioxide alone and combined in healthy and asthmatic adolescent subjects. Toxicol. Ind. Health 4: 521-532.

Koenig, J.Q., Covert, D.S., Hanley, Q.S., Van Belle, G., Pierson, W.E. (1990). Prior exposure to ozone potentiates subsequent response to sulfur dioxide in adolescent asthmatic subjects. Am. Rev. Respir. Dis. 141: 377-380.

Koenig, G., Rommelt, H., Kienle, H., Dirnagl, K., Polke, H., Fruhmann, G. (1980). Aenderung der bronchomotorischen Reagibilitaet des Menschen durch Einwirkung von Ozon [Changes in the bronchial reactivity of humans caused by the influence of ozone]. Arbeitsmed. Sozialmed. Praeventivmed. 151: 261-263.

Koepchen, H.P., Kalia, M., Sommer, D., Klussen-Dorf, D. (1977). Action of type J afferents on the discharge pattern of medullary respiratory neurons. In: Paintal, A.S., Gill-Kumar, P., eds. Krogh centenary symposium on respiratory adaptations, capillary exchange, and reflex mechanism. Delhi, India: Vallabhbhai Patel Chest Institute; pp. 407-425.

Koren, H.S., Devlin, R.B., Graham, D.E., Mann, R., McGee, M.P., Horstman, D.H., Kozumbo, W.J., Becker, S., House, D.E., McDonnel, W.F., Bromberg, P.A. (1989a). Ozone-induced inflammation in the lower airways of human subjects. Am. Rev. Respir. Dis. 139: 407-415.

Koren, H.S., Devlin, R.B., Graham, D.E., Mann, R., McDonnell, W.F. (1989b). The inflammatory response in human lung exposed to ambient levels of ozone. In: Schneider, T., Lee, S.D., Wolters, G.J.R., Grant, L.D., eds. Atmospheric ozone research and its policy implications: proceedings of the 3rd US-Dutch international symposium; May 1988; Nijmegen, The Netherlands. Amsterdam, The Netherlands: Elsevier Science Publishers; pp. 745-753. (Studies in environmental science 35).

Koren, H.S., Devlin, R.B., Becker, S., Perez, R., McDonnell, W.F. (1991). Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone. Toxicol. Pathol. 19: 406-411.

Kreit, J.W., Gross, K.B., Moore, T.B., Lorenzen, T.J., D'Arcy, J., Eschenbacher, W.L. (1989). Ozone-induced changes in pulmonary function and bronchial responsiveness in asthmatics. J. Appl. Physiol. 66: 217-222.

Kulle, T.J., Sauder, L.R., Kerr, H.D., Farrell, B.P., Bermel, M.S., Smith, D.M. (1982). Duration of pulmonary function adaptation to ozone in humans. Am. Ind. Hyg. Assoc. J. 43: 832-837.

Kulle, T.J., Saunder, L.R., Hebel, J.R., Chatham, M.D. (1985). Ozone response in healthy non smokers. Am. Rev. Resp. Dis. 132: 36-41

Larsen, R.I., McDonnell, W.F., Horstman, D.H., Folinsbee, L.J. (1991). An air quality data analysis system for interrelating effects, standards, and needed source reductions: part 11, a lognormal model relating human lung function decrease to O_3 exposure. J. Air Waste Manag. Assoc. 41: 455-459.

Linder, J., Herren, D., Monn, C., Wanner, H.-U. (1988). Die Wirkung von Ozon auf die koerperliche Leistungsfaehigkeit [The effect of ozone on physical activity]. Schweiz Z Sportmed, 36, 5-10.

Linn, W.S., Buckley, R.D., Spier, C.E., Blessey, R.L., Jones, M.P., Fischer, D.A., Hackney, J.D. (1978). Health effects of ozone exposure in asthmatics. Am. Rev. Respir. Dis. 117: 835-843.

Linn, W.S., Fischer, D.A., Medway, D.A., Anzar, U.T., Spier, C.E., Balencia, L.M., Venet, T.G., Hackney, J.D. (1982a). Short-term respiratory effects of 0.12 ppm ozone exposure in volunteers with chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 125: 658-663.

Linn, W.S., Medway, D.A., Anzar, U.T., Valencia, L.M., Spier, C.E., Tsao, F.S.-D., Fischer, D.A., Hackney, J.D. (1982b). Persistence of adaptation to ozone in volunteers exposed repeatedly for six weeks. Am. Rev. Respir. Dis. 125: 491-495.

Linn, W.S., Shamoo, D.A., Venet, T.G., Spier, C.E., Valencia, L.M., Anzar, U.T., Hackney, J.D. (1983). Response to ozone in volunteers with chronic obstructive pulmonary disease. Arch. Environ. Health 38: 278-283.

Linn, W.S., Avol, E.L., Shamoo, D.A., Spier, C.E., Valencia, L.M., Venet, T.G., Fischer, D.A., Hackney, J.D. (1986). A dose-response study of healthy, heavily exercising men exposed to ozone at concentrations near the ambient air quality standard. Toxicol Ind Health, 2, 99-112.

Linn, W.S., Avol, E.L., Anderson, K.R., Shamoo, D.A., Peng, R.C., Hackney, J.D. (1991). Respiratory responses of healthy volunteers in prolonged, repeated exposures to ozone and sulfuric acid. Am. Rev. Respir. Dis. (suppl.) 143: A97.

Linn, W.S., Shamoo, D.A., Anderson, K.R., Peng, R.C., Avol, E.L., Hackney, J.D. (1992). Responses of asthmatics in prolonged, repeated exposures to ozone and sulfuric acid; comparison with healthy subjects. Am. Rev. Respir. Dis. (suppl.) 145: A428.

Linn, W.S., Shamoo, D.A., Anderson, K.R., Peng, R.-C., Avol, E.L., Hackney, J.D. (1994). Effects of prolonged, repeated exposure to ozone, sulfuric acid, and their combination in healthy and asthmatic volunteers. Am J Respir Crit Care Med, 150, 431-40.

Linn, W.S., Anderson, K.R., Shamoo, D.A., Edwards, S.A., Webb, T.L., Hackney, J.D., Gong, H., Jr. (1995). Controlled exposures of young asthmatics to mixed oxidant gases and acid aerosol. Am J Respir Crit Care Med, 152, 885-91.

Liu, L., Leech, J., Silverman, F. (1997a). Potential non-invasive biomarkers assessing inflammatory effects of ozone in the upper and lower airways of humans. Proc Can Fed Biol Soc, 40, 28.

Liu, L., Leech, J.A., Silverman, F.S., Urch, R.B. (1997b). *In vivo* salicylate hydroxylation: A biomarker assessing acute ozone exposure and effects in humans. Am J Respir Crit Care Med, [In Press]

Madden, M.C., Hanley, N., Harder, S., Velez, G., and Raymer, J.H. (1997). Increased amounts of hydrogen peroxide in the exhaled breath of ozone-exposed human subjects. Inhalation Toxicology, 9, 317-30.

McBride, D.E., Koenig, J.Q., Luchtel, D.L., Williams, P.V., Henderson, Jr. (1994). Inflammatory effects of ozone in the upper airways of subjects with asthma. Am J Respir Crit Care Med, 149, 1192-7.

McDonnell, W.F., III, Horstman, D.H., Milan, J. Hazucha, J., Elston, S. Jr., Edward, D.H., Sa?id, A.S., Dennis, E.H. (1983). Pulmonary effects of ozone exposure during exercise: dose-response characteristics.

McDonnell, W.F., Chapman, R.S., Leigh, M.W., Strope, G.L., and Collier, A.M. (1985a). Respiratory responses of vigorously exercising children to 0.12 ppm ozone exposure. Am. Rev. Respir. Dis. 132:875-879.

McDonnell, W.F., III, Horstman, D.H., Abdul-Salaam, S., House, D.E. (1985b). Reproducibility of individual responses to ozone exposure. Am. Rev. Respir. Dis. 131: 36-40.

McDonnell, W.F., Kehrl, H.R., Abdul-Salaam, S., Ives, P.J., Folinsbee, L.J., Devlin, R.B., O'Neil, J.J., Horstman, D.H. (1991a). Respiratory response of humans exposed to low levels of ozone for 6.6 hours. Arch. Environ. Health 46: 145-150.

McDonnell, W.F., Horstman, D.H., Folinsbee, L.J., Kehrl, H.R., O'Neil, J. (1991b). Respiratory responses to low level ozone exposure for 6.6 hours in exercising humans: a review of three clinical studies. In: Berglund, R.L., Lawson, D.R., McKee, D.J., eds. Tropospheric ozone and the environment: papers from an international conference; March 1990; Los Angeles, CA. Pittsburgh, PA: Air & Waste Management Association; pp. 52-58. (A&WMA transaction series no. TR-19).

McDonnell, W.F., Muller, K.E., Bromberg, P.A., Shy, C.M. (1993). Predicators of individual differences in acute response to ozone exposure. Am. Rev. Respir. Dis. 147: 818-825.

McDonnell, W.F., Stewart, P.W., Andreoni, S., Smith, M.V. (1995). Proportion of moderately exercising individuals responding to low-level, multi-hour ozone exposure. Am J Respir Crit Care Med, 152, 589-96.

McKittrick T., and Adams W.C. (1995). Pulmonary function response to equivalent doses of ozone consequent to intermittent and continuous exercise. Arch.Environ.Health.50(2):153-158

Molfino, N.A., Wright, S.C., Katz, I., Tarlo, S., Silverman, F., McClean, P.A., Szalai, J.P., Raizenne, M., Slutsky, A.S., Zamel, N. (1991). Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. Lancet 338 (8761): 199-203.

Osebold, J.W., Gershwin, L.J., Zee, Y.C. (1980). Studies on the enhancement of allergic lung sensitization by inhalation of ozone and sulfuric acid aerosol. J. Environ. Pathol. Toxicol. 3: 221-234.

Pryor W.A. (1992). How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? Free Radic. Biol. Med. 12(1):83-88.

Scannell, C., Chen, L., Aris, R.M., Tager, I., Christian, D., Ferrando, R., Welch, B., Kelly, T., Balmes, J.R. (1996). Greater ozone-induced inflammatory responses in subjects with asthma. Am J Respir Crit Care Med, 154, 24-9.

Schelegle, E.S., Adams, W.C. (1986). Reduced exercise time in competitive simulations consequent to low level ozone exposure. Med Sci Sports Exercise, 18, 408-14.

Schelegle, E.S., Adams, W.C., Siefkin, A.D. (1987) Indomethacin pretreatment reduces ozone-induced pulmonary function decrements in human subjects. Am. Rev. Respir. Dis. 136: 1350-1354.

Schelegle, E.S., Siefkin, A.D., McDonald, R.J. (1991). Time course of ozone-induced neutrophilia in normal humans. Am. Rev. Respir. Dis. 143: 1353-1358.

Schmidt, T., Wellh`ner, H.H. (1970). The reflex influence of a group of slowly conducting vagal afferents on " and (discharges in cat intercostal nerves. Pfluegers Arch. 318: 335-345.

Schonfeld, B.R., Adams, W.C., Schelegle, E.S. (1989). Duration of enhanced responsiveness upon re-exposure to ozone. Arch. Environ. Health 44: 229-236.

Seal, E., Jr., McDonnell, W.F., Chapman, R.S., House, D.E. (1993a). The effect of menstrual cycle and age on the pulmonary response to ozone. Am. Rev. Respir. Dis. 147: A637.

Seal, E., Jr., McDonnell, W.F., House, D.E., Salaam, S.A., Dewitt, P.J., Butler, S.O., Green, J., Raggio, L. (1993b). The pulmonary response of white and black adults to six concentrations of ozone. Am. Rev. Respir. Dis. 147: 804-810.

Seal, E., Jr., McDonnell, W.F., House, D.E. (1996). Effects of age, socioeconomic status, and menstrual cycle on pulmonary response to ozone. Arch Environ Health, 51, 132-7.

Seltzer, J., Bigby, B.G., Stulbarg, M., Holtzman, M.J., Nadel, J.A., Ueki, I.F., Leikauf, G.D., Goetzl, E.J., Boushey, H.A. (1986). O_3-induced change in bronchial reactivity to methacholine and airway inflammation in humans. J. Appl. Physiol. 60: 1321-1326.

Silverman, F., Folinsbee, L.J., Barnard, J. & Shephard, R.J. (1976). Pulmonary function changes in ozone - interaction of concentration and ventilation. J. Appl. Physiol., 29, 859-864.

Silverman, F. (1979). Asthma and respiratory irritants (ozone). Environ. Health Perspect. 29: 131-136.

Solic, J.J., Hazucha, M.J., Bromberg, P.A. (1982). The acute effects of 0.2 ppm ozone in patients with chronic obstructive pulmonary disease. Am. Rev. Respir. Dis. 125: 664-669.

Tepper, J.S., Costa, D.L., Lehmann, J.R., Weber, M.F., Hatch, G.E. (1989). Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. Am. Rev. Respir. Dis. 140: 493-501.

Thurston, G.D., Gorczynski, J.E. Jr., Currie, J.H., He, D., Ito, K., Hipfner, J., Waldman, J., Lioy, P.J. and Lippmann, M. (1994a). The nature and origins of acid summer haze air pollution in Metropolitan Toronto, Ontario. Environ. Res. 65:254-270.

Thurston, G.D., Ito, K., Hayes, C., Bates, D.V., and Lippmann, M. (1994b). Respiratory hospital admissions and summertime haze air pollution in Toronto, Ontario: consideration of the role of acid aerosols. Environ. Res. 65:271-290.

Torres A., Voter K.Z., Utell, M.J., Whitin, J.C., Morrow, P.E., and Frampton, M.W. (1996). Production of reactive oxygen intermediates following exposure to ozone. Relative contribution of alveolar macrophages. Chest, 109 (3 Suppl), 8S

U.S. Environmental Protection Agency. (1996). Air quality criteria for ozone and related photochemical oxidants. Research Triangle Park, NC: National Center for Environmental Assessment, Office of Research and Development; EPA report nos. EPA/600/P-93/004cF.

Utell, M.J., Frampton, M.W., Morrow, P.E., Cox, C., Levy, P.C., Speers, D.M., Gibb, F.R. (1994). Oxidant and acid aerosol exposure in healthy subjects and subjects with asthma. Part II: Effects of sequential sulfuric acid and ozone exposures on the pulmonary function of healthy subjects and subjects with asthma. Res Rep Health Eff Inst, 70, 37-93.

Van Bree, L., Koren, H.S., Devlin, R.B., Marra, M., Rombout, P.J.A. (1994). Recovery from attenuated inflammation in lower airways of rats following repeated exposure to ozone. Zentralblatt

fuer Hygiene und Unweltmedizin 195(2); 139. Fourth European Meeting of Environmental Hygiene, Wageningen, Netherlands, June 9-11, 1993.

Van Loveren, H., Rombout, P.J.A., Wagenaar, Sj. Sc., Walvoort, H.C., Vos, J.G. (1988). Effects of ozone on the defence to a respiratory *Listeria monocytogenes* infection in the rat: suppression of macrophage function and cellular immunity and aggravation of histopathology in lung and liver during infection. Toxicol. Appl. Pharmacol. 94: 374-393.

Vender, R.L., Horstman, D.H., Mangione, S. (1994). Red blood cell antioxidants in human volunteers exposed to ozone. J Toxicol Ind Health, 10, 53-8.

Wayne, W.S., Wehrle, P.F., Carroll, R.E. (1967). Oxidant air pollution and athletic performance. JAMA J. Am. Med. Assoc. 199: 151-154.

Weinmann, G.G., Liu, M.C., Proud, D., Weidenbach-Gerbase, M., Hubbard, W., Frank, R. (1995a). Ozone exposure in humans: Inflammatory, small and peripheral airway responses. Am J Respir Crit Care Med, 152, 1175-82.

Weinmann, G.G., Weidenbach-Gerbase, M., Foster, W.M., Zacur, H., Frank, R. (1995b). Evidence for ozone-induced small-airway dysfunction: Lack of menstrual-cycle and gender effects. Am J Respir Crit Care Med, 152, 988-96.

Weymer, A.R., Gong, H., Jr., Lyness, A., Linn, W.S. (1994). Pre-exposure to ozone does not enhance or produce exercise-induced asthma. Am J Respir Crit Care Med, 149, 1413-9.

Ying, R.L., Gross, K.B., Terzo, T.S., Eschenbacher, W.L. (1990) Indomethacin does not inhibit the ozone-induced increase in bronchial responsiveness in human subjects. Am. Rev. Respir. Dis. 142: 817-821.