



Air Resources Board  
Alan C. Lloyd, Ph.D.  
Chairman

State of California

Governor Arnold Schwarzenegger

Office of Environmental  
Health Hazard Assessment  
Joan E. Denton, Ph.D.  
Director



# **Review of the California Ambient Air Quality Standard For Ozone**

Appendix A:  
Review of the Animal Toxicological Studies  
on the Health Effects of Ozone

Appendix B:  
June 4, 2004 Letter Submitting OEHHA  
Recommendations to the ARB for  
an Ambient Air Quality Standard for Ozone

*Public Review Draft  
June 21, 2004*

***California Environmental Protection Agency***

**Air Resources Board  
and  
Office of Environmental Health and Hazard Assessment**

*The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption.  
For a list of simple ways you can reduce demand and cut your energy costs, see our Website: <http://www.arb.ca.gov>.*

---

*California Environmental Protection Agency*

Terry Tamminen, Secretary

*Printed on Recycled Paper*

## Table of Contents

|       |   |      |
|-------|---|------|
| A     | Review of Animal Toxicological Studies on the Health Effects of Ozone   | A-1  |
| A.1   | Dosimetry of Ozone in the Respiratory Tract   | A-1  |
| A.1.1 | Experimental Ozone Dosimetry Data   | A-1  |
| A.1.2 | Dosimetry Modeling  | A-3  |
| A.1.3 | Species Sensitivity   | A-4  |
| A.1.4 | Animal-to-Human Extrapolation   | A-5  |
| A.2   | Respiratory Tract Effects   | A-7  |
| A.2.1 | Inflammation and Lung Permeability Changes  | A-7  |
| A.2.2 | Lung Host Defense   | A-15 |
| A.2.3 | Biochemical Effects   | A-28 |
| A.2.4 | Mutagenic and Carcinogenic Potential of Ozone   | A-37 |
| A.3   | Systemic Effects  | A-40 |
| A.3.1 | Liver   | A-40 |
| A.3.2 | Hematopoietic System Effects  | A-41 |
| A.3.3 | Reproductive and Developmental Effects  | A-45 |
| A.3.4 | Central Nervous System and Behavioral Effects   | A-46 |
| A.3.5 | Hematology and Serum Chemistry  | A-48 |
| A.3.6 | Cardiovascular Effects  | A-49 |
| A.3.7 | Thermoregulatory Effects  | A-50 |
| A.4   | Interactions of Ozone with Other Pollutants   | A-51 |
| A.4.1 | Ozone and Sulfur Oxides   | A-51 |
| A.4.2 | Ozone and Nitrogen-Containing Pollutants  | A-54 |
| A.4.3 | Ozone and Particulate Matter including Complex Mixtures   | A-59 |
| A.5   | References  | A-68 |
| B     | Office of Environmental Health Hazard Assessment Recommendations to the ARB for an Ambient Air Quality Standard for Ozone | B-1  |
| B.1   | OEHHA Recommendation for Standard   | B-1  |
| B.2   | Defining an Adverse Effect  | B-3  |
| B.3   | Summary of the Scientific Evidence  | B-4  |
| B.3.1 | Summary of Findings from Chamber Studies  | B-4  |
| B.3.2 | Summary of Findings from Toxicological Studies  | B-10 |
| B.3.3 | Summary of Findings from Epidemiologic Studies  | B-11 |
| B.4   | Consideration of Infants and Children   | B-15 |
| B.5   | Recommended Pollutant Indicator   | B-16 |
| B.6   | Recommended Averaging Times and Forms   | B-17 |
| B.7   | Recommended Concentrations  | B-19 |
| B.7.1 | Considerations for the Margin of Safety   | B-19 |
| B.7.2 | One-hour Average  | B-20 |
| B.7.3 | Eight-hour Standard   | B-22 |
| B.7.4 | Consideration of Infants and Children in Recommending the Ozone Standards   | B-23 |
| B.8   | Summary of OEHHA Recommendation:  | B-24 |
| B.9   | References  | B-25 |

## List of Abbreviations

|                   |  |
|-------------------|--|
| ADSS              | aged and diluted sidestream cigarette smoke                        |
| AM                | alveolar macrophage  |
| ARB               | Air Resources Board  |
| BAL               | bronchoalveolar lavage fluid                                       |
| BALT              | bronchus-associated lymphoid tissue                                |
| BHPN              | N-bis(2-hydroxypropyl)nitrosamine                                  |
| BFU-E             | burst forming erythroid progenitor                                 |
| ConA              | concanavalin A   |
| C                 | concentration (in reference to concentration x time relationships) |
| CAP               | concentrated ambient particles                                     |
| Cr                | chromium   |
| Cu                | copper   |
| Cu-Zn SOD         | copper-zinc superoxide dismutase                                   |
| DNA               | deoxyribonucleic acid  |
| GSH               | glutathione  |
| HMSA              | hydroxymethanesulfonate  |
| Mn SOD            | manganese superoxide dismutase                                     |
| Mn                | manganese  |
| NADPH             | nicotinamide adenine dinucleotide phosphate                        |
| NO <sub>2</sub>   | nitrogen dioxide   |
| PHA               | phytohemagglutinin   |
| PM <sub>2.5</sub> | particulate matter with an aerodynamic size cutoff of 2.5 microns  |
| PM <sub>10</sub>  | particulate matter with an aerodynamic size cutoff of 10 microns   |
| PMN               | polymorphonuclear leukocyte  |
| Ppm               | parts per million  |
| RBC               | red blood cells  |
| RNA               | ribonucleic acid   |
| AlSi              | aluminum silica  |
| SO <sub>2</sub>   | sulfur dioxide   |
| SOD               | superoxide dismutase   |
| STM               | Salmonella typhimurium glycoprotein                                |

|     |   |
|-----|---|
| T   | time (in reference to concentration x time relationships) |
| Zn  | zinc  |
| ZnO | zinc oxide  |

### **Measurement Abbreviations**

|                   |                            |
|-------------------|----------------------------|
| hr/day            | hours per day              |
| days/wk           | days per week              |
| ppm               | parts per million          |
| mg/m <sup>3</sup> | milligrams per cubic meter |

# **Appendix A**

## **Review of Animal Toxicological Studies on the Health Effects of Ozone**

# **A Review of Animal Toxicological Studies on the Health Effects of Ozone**

## **A.1 Dosimetry of Ozone in the Respiratory Tract**

Experimental and theoretical dosimetry studies are used to estimate amount or rate of ozone absorbed by target sites within the respiratory tract. The ozone dose that lung airway regions receive has been expressed a number of ways, but often has been shown as grams of ozone per unit of airway surface area or volume which react with the tissue to produce the toxic effect. An understanding of the dosimetry of ozone can assist in estimating doses necessary to induce various toxic responses in mammalian species and reduce the uncertainty in animal-to-human extrapolation. Only a brief review of ozone dosimetry will be covered in this report. Greater detail on ozone dosimetry and related issues on which this section is based can be found in a recent review by U.S. EPA (U.S. Environmental Protection Agency, 1996).

### **A.1.1 Experimental Ozone Dosimetry Data**

Experimental ozone dosimetry studies are used to obtain direct measurements of absorbed ozone in the respiratory tract or in specific regions of the respiratory tract.

In one of the original experimental dosimetry studies, Yokoyama et al. (1972) reported up to 72% ozone uptake in beagle dogs when ozone was administered via the nose. The relative uptake of ozone was inversely related to concentration and flow rate, and was higher by nasal administration than by oral administration.

Total respiratory tract uptake of ozone was estimated at 40% in rats, based on mass balance measurements (Wiester et al., 1987). Uptake was independent of ozone concentration over a range of concentrations (0.3 – 1.0 ppm). Later work by Wiester et al. (1988) adjusted ozone uptake efficiency to an average of 47%, based on revised methods and ozone uptake efficiencies that were similar among three strains of rats and in the guinea pig.

In a study that addressed both total and regional uptake of ozone, Hatch et al. (1989) exposed rats to 1.0 ppm oxygen-18-labeled ozone for 2 hours and assayed excess <sup>18</sup>O in bronchoalveolar lavage fluid (BAL) and respiratory tract tissue. Total uptake efficiency was estimated at 54.3%. Of ozone absorbed by the rats, 49.3% was taken up in the head (nasopharynx), 6.5% by the larynx/trachea, and 44.0% by the lungs. In another experiment using oxygen-18-labeled ozone, detection of accumulated <sup>18</sup>O in BAL cells and extracellular material lavaged from the lung of rats and humans was used to estimate dose of ozone to the lung (Hatch et al., 1994). Exercising humans had four- to five-fold greater <sup>18</sup>O concentration in their BAL constituents after a 2-hour exposure to 0.4 ppm ozone than rats exposed at rest to an identical ozone concentration. Rats exposed to 2.0 ppm ozone at rest had levels of <sup>18</sup>O in BAL that were comparable to but still lower than those of exercising humans. The data suggest that activity level, because of its influence on ventilation rate and mode of breathing, may be

more important than species in determining dose of ozone to the lung. The researchers also noted that  $^{18}\text{O}$  was also found in the surfactant-containing and soluble protein fractions of the supernatant of humans and rats, which confirms that ozone reaches alveolar regions of the lung.

In *in vitro* studies, Ben-Jebria et al. (1991) excised the tracheae of sheep and pigs to investigate mass transfer coefficients of ozone. Uptake efficiencies in both pigs and sheep decreased with increasing flow (0.50 to 0.15 at increasing airflows from 50 to 200 ml/sec) but mass transfer coefficients were generally independent of flow (i.e., the overall mass transfer coefficient, a useful parameter for characterizing ozone absorption, is insensitive to ozone flow rate). Postlethwait et al. (1994) used an isolated rats lung to investigate several parameters that could affect ozone absorption in the lung. His observations noted that vascular perfusion had little or no effect on uptake efficiency, that lowering lung temperature decreased uptake efficiency (suggesting a chemical reaction dependence), and that ozone uptake is virtually complete by the time ozone reaches the alveolar spaces of the lung. Pryor et al. (1991) and Pryor (1992) investigated the formation of toxic reaction products (i.e., hydrogen peroxide, aldehydes) following contact of ozone with the liquid lining of the lung. The results indicated a large fraction of ozone reacts in the liquid lining and that only lung regions with a fluid layer less than 0.1 microns thick (i.e., central acinar regions) will have significant penetration of ozone to lung tissue.

Utilizing  $^{18}\text{O}$ -labeled ozone, Slade et al. (1997) investigated strain differences in ozone dosimetry in mice. Following exposure (2.0 ppm for 2-3 hours), the less ozone-sensitive mouse strain (C3H/HEJ strain) had 46% less  $^{18}\text{O}$  in lungs and 61% less in tracheas than the more sensitive strain (C57BL/6J strain). The less sensitive strain also had a greater decrease in core body temperature during exposure than the more sensitive strain. Hypothermia in response to ozone exposure may be related to oxygen consumption, pulmonary ventilation, and ozone dose to the lung. These results suggest that the strain differences in ozone susceptibility may be due to differences in ozone dose to the lung, which may be related to differences in the hypothermic response of the mice to ozone exposure. An implication is that humans, which do not have labile thermoregulatory abilities as found in rodents, would be more akin to the ozone-sensitive mouse strain in terms of ozone dosimetry.

Plopper et al. (1998) measured site-specific ozone dose in various airway branches of monkeys exposed to 0.4 or 1.0 ppm ozone for 2 hours utilizing  $^{18}\text{O}$ -labeled ozone. In monkeys exposed to 1.0 ppm ozone, local ozone dose varied by as much as a factor of three with respiratory bronchioles having the highest concentration (excess  $^{18}\text{O}$  of 32.2  $\mu\text{g/g}$  dry weight) and the parenchyma the lowest concentration (excess  $^{18}\text{O}$  of 7.8  $\mu\text{g/g}$  dry weight). In monkeys exposed to 0.4 ppm, the ozone dose was 60% to 70% less than in the same site in monkeys exposed to 1.0 ppm. When the mass of necrotic cells identified at a specific airway level was analyzed by regression against the  $^{18}\text{O}$  content at that airway level, there was a significant correlation at most branch levels, including trachea,

distal bronchioles, and respiratory bronchioles. This finding suggests that  $^{18}\text{O}$  content by airway level can predict airways that will exhibit oxidant injury.

In rats and guinea pigs exposed to 1 ppm  $^{18}\text{O}$ -labeled ozone for 2 hours, the content of  $^{18}\text{O}$  in lavage fluid samples suggests that dose is greater in nose-only exposures than whole-body exposures, and that guinea pigs received higher doses than rats (Campen et al., 2000). It was suggested that the rats' hypothermic response to ozone exposure was responsible for the lower ozone dose compared to guinea pigs (which do not have a hypothermic response to ozone at this dose level), but ventilatory and oxygen consumption parameters were not collected for verification.

### **A.1.2 Dosimetry Modeling**

Dosimetry modeling is based on theoretical studies that use mathematical models to simulate uptake and distribution of the gas in fluids and tissues of the respiratory tract. Ideally, ozone dosimetry modeling can be used to make interspecies and intraspecies dose comparisons, to compare and reconcile data from different experiments, to predict dose in conditions not feasible to examine experimentally, and to better understand the processes involved in toxicity.

For ozone, the only significant route of exposure is inhalation, and exposure can be defined as the concentration at the nose and mouth. However, ozone exposure is only one determinant of ozone dose. The volumes of air inhaled and the pattern of uptake of ozone molecules along the respiratory tract also determine dose. Factors that mathematical models take into account with inhalation of this relatively insoluble but highly reactive gas include respiratory tract geometry, fluid mechanics and ozone solubility, and assumptions about the thickness of mucus in the airways and the reactivity with and diffusion through surface components. Taking all these factors into account, models show that the tissue dose of inhaled ozone is greatest at the bronchoalveolar junction, or central acinus. Many histopathological studies also confirm this conclusion.

In the original ozone uptake models developed by Miller et al. (1978b; 1985), guinea pigs, rabbits, and humans received the highest local dose from inhaled ozone in the central acini (the airway region from the terminal bronchioles to the alveolar ducts). Ozone tissue dose was predicted to be relatively low in the trachea and increased to a maximum in the central acini, and then decreased distally. Tissue dose in these models was defined as the ozone flux to the liquid-tissue interface. Though quantitative differences exist among various models with regard to regional uptake of ozone, the findings of Miller et al. (1978b; 1985) are in general agreement with other models of regional ozone uptake (Overton et al., 1987, 1989; 1989; Grotberg et al., 1990; Hu et al., 1992).

Other similarities among dosimetry models concern increasing physical exertion or increasing ventilation rate (Miller et al., 1985; Overton et al., 1987, 1989; 1989; Grotberg et al., 1990; Hanna et al., 1989). Under these conditions, the contribution of ozone concentration to total dose of ozone becomes a much greater determinant of total ozone dose. The dose to target tissues in the central acini increases even more with physical exertion, since ozone penetration to the

deep lung increases with both tidal volume and flow rate. In other words, increasing inspiratory flow rates displaces ozone absorption from the upper airways to more distal sites.

The models also predict that the longer the airway path length from trachea to central acini, the lower the tissue dose of ozone in the central acini. Overton et al. (1989) predicted a threefold greater proximal alveolar region dose for the shortest path relative to the longest path in rats. Mercer et al. (1991) also noted that path distance and ventilatory unit size affect dose, with proximal portions of larger ventilatory units absorbing more ozone than in smaller units. Together, these data suggest that variations in dose between ventilatory units is one of the mechanisms leading to focal areas of injury often seen in histopathological studies of ozone exposure.

Cohen Hubal et al. (1996) refined existing dosimetry models to account for regional differences in quantity of mucosubstances lining the nasal epithelium of the rat to address ozone uptake in the nose, or upper respiratory tract. Comparison of the model with experimental data in rats were within the range of measured uptake and indicated that regional differences in mucus thickness play a role in observed patterns of ozone-induced toxicity in the nose.

### **A.1.3 Species Sensitivity**

The issue of species sensitivity refers to the relative susceptibility to ozone-related injury for a given delivered dose. A related issue is tissue sensitivity in which species comparisons of protective mechanisms (i.e., antioxidants, etc.) to tissue oxidant injury are made. Endpoints such as pulmonary inflammation and lung function are often used for comparisons, due to qualitative homology of these responses among mammalian species. The following is a brief overview of various studies/factors that have an impact on species sensitivity.

With regard to acute ozone exposure and responses among animal species, the tachypneic response between animals and humans is similar, with rodents appearing to be slightly more responsive and initiated at lower concentrations when compared to humans (Tepper et al., 1990; DeLucia and Adams, 1977). Airway or lung resistance effects are not a particularly sensitive measure of ozone exposure in either animals or humans (Tepper et al., 1990; Hackney et al., 1975). Animals may need special preparations that bypass nasal scrubbing in order to exhibit pulmonary resistance effects. Functional responses to acute ozone exposure are similar between rodents and humans, with functional responsiveness in rodents appearing to be half that of humans. However, confounding factors when conducting spirometric measurements in animals (anesthetic effects, hyperventilation caused by CO<sub>2</sub>) and humans (exercise) likely alter the sensitivity of the functional response to ozone. Species sensitivity differences to ozone have been observed with regard to the recovery of inflammatory cells and protein in BAL following exposure (Hotchkiss et al., 1989b; Devlin et al., 1991). However, ventilatory differences and tissue sensitivity (i.e., antioxidant status) likely influence the apparent species

differences in inflammatory response (Slade et al., 1993; Slade et al., 1989; Koren et al., 1989a; Kodavanti et al., 1995a).

With repeated exposures to ozone, there is full or partial attenuation of functional and inflammatory effects in rodents that is similar to that seen in humans (Tepper et al., 1989; van Bree et al., 2002; Devlin et al., 1997). With regard to chronic effects at or near ambient levels of ozone, the limited functional data available in monkeys generally agree with the pattern of distal lung pathophysiology reported in rats (Tyler et al., 1988). However, some lung function deficits (i.e., elasticity) observed in monkeys have not been shown in controlled human studies. While the animal data demonstrate that chronic ozone exposure can induce changes in the structure and function of the lung, similar changes occurring in humans as a result of prolonged ozone exposure have not been well-established yet (U.S. Environmental Protection Agency, 1996). One would, however, expect qualitatively similar responses in humans chronically exposed to ambient ozone.

#### **A.1.4 Animal-to-Human Extrapolation**

The definitive goal of many animal toxicology studies is extrapolation of animal toxicity data to humans. Qualitatively, a large array of experimental animal data and human data has shown that the toxic endpoints of ozone exposure and the regions of the lung airways most affected are similar among species. The dosimetry experiments in animals and theoretical dosimetry models described above provide the basis on which responses may be examined as a function of delivered dose. The result is that better quantitative extrapolations from animal to human can be made with reduced uncertainty.

Quantitative extrapolations may include intraspecies and interspecies comparisons. Intraspecies comparisons are the examination of a delivered dose versus response within a given species. For example, Miller et al. (1995) compared the distribution of predicted ozone tissue dose to a ventilatory unit in a rat as a function of distance from the bronchoalveolar duct junction, with the distribution of alveolar wall thickening as a function of the same distance measure. A strong positive correlation was found between the predicted dose distribution and the response distribution.

In interspecies comparisons of delivered dose versus response, the tachypneic response to ozone was compared in rats and humans (Miller et al., 1985; Miller et al., 1988; Overton et al., 1987). At comparable ozone exposures, this response in rats greatly exceeded that of humans and was initiated at lower doses. The tachypneic response between rats and humans was magnified when dose-response comparisons (measured as ng ozone/cm<sup>3</sup>/min in the proximal alveolar region) were used rather than concentration-response comparisons. In a model by Overton et al. (1987), a given exposure concentration of ozone was determined to produce an injury to the respiratory acinus that was approximately twice as high in humans and monkeys as compared with rats. Miller et al. (1988) compared inflammatory responses (protein in BAL) among rats, guinea pig, rabbit, and humans, as a function of ozone dose delivered to the pulmonary region. Protein recovered in BAL among all species followed a log-linear

relationship, suggesting consistency of response across species. However, the species data clustered together, which suggests a species-specific sensitivity factor is involved. This finding suggests that species-specific issues, such as pharmacokinetics, oxidant-injury repair processes, metabolic rates, antioxidant protection mechanisms, and other factors, are important in animal-to-human extrapolations and may not be as well defined as specific dosimetric animal-to-human extrapolation determinations.

To address effects resulting from long-term exposure, an interspecies extrapolation from animals to humans was made based on long-term exposure studies in rats and monkeys (U.S. Environmental Protection Agency, 1996). The animal studies chosen used the same chronic ozone response parameters: the altered interstitial thickness in the proximal alveolar regions (PAR) of the lung. Because the PAR is considered the primary site of ozone injury and represents that region of the lung from which most chronic lung diseases originate, it was selected as the most appropriate target to develop cross-species dose-response extrapolations. The model simulations for extrapolation to humans used an urban profile of ozone exposure in children and adults and the assumption was that the rate of change of interstitial thickness is related to the rate of ozone uptake. Using dosimetry assumptions needed for model prediction, a linear relationship within species for rat and monkey was observed with high correlation coefficients (0.80 to 0.98, depending on species and effect). The predicted dose for the hypothetical humans indicated a seasonal response for the child of a 20 to 75% increase in PAR tissue thickness and, for the adult, a 15 to 70% increase, depending on the animal species used for the prediction. The interpretation is that human exposure to an urban profile of ozone could impart a chronic injury-repair process that leads to potentially irreversible changes in the lung.

In summary, experimental and theoretical dosimetry studies have been developed to estimate amount or rate of ozone absorbed by target sites within the respiratory tract. An understanding of the dosimetry of ozone can assist in estimating animal-to-human extrapolation for effective ozone dose. Mathematical models have incorporated species differences in airway anatomy, regional airway differences in ozone dose, and physiochemical interactions with the liquid lining layer of the upper and lower respiratory tracts. These models support the experimental animal studies in that the primary site of lung damage due to inhalation of ozone is in the centriacinar region. Experimental dosimetry studies with <sup>18</sup>O-labeled ozone indicate exercising humans had four- to five-fold greater ozone dose to BAL constituents than rats exposed at rest to an identical ozone concentration. Differences in exertion level between species are likely a more important determinant than species differences. However, theoretical models predict greater sensitivity of humans compared to rodents, in that a given exposure concentration of ozone results in an injury to the respiratory acinus roughly half that in rats compared to humans. While knowledge of dosimetry has allowed quantitative animal-to-human extrapolation for effective ozone doses, species sensitivity issues, such as antioxidant status, metabolic rates, and repair/defense mechanisms, is also an important determinant of effective ozone dose and are not as well defined.

## A.2 Respiratory Tract Effects

### A.2.1 Inflammation and Lung Permeability Changes

Two interrelated consequences of exposure to toxic levels of ozone in both experimental animals and controlled human studies are lung inflammation and disruption of the pulmonary epithelial barrier, resulting in increased transmucosal permeability. The deleterious effects of ozone-caused lung inflammation include recruitment of inflammatory cells and stimulation of epithelial cells and macrophages resulting in the release of prostaglandins, leukotrienes, and other inflammatory mediators. The release of proteolytic enzymes and reactive oxygen species from inflammatory cells are thought to further enhance injury to cell membranes and intracellular components by their adverse effects on membrane lipids and proteins.

Under normal conditions, the airway epithelia restrict the penetration of foreign particles and macromolecules from the lumen into the interstitium and blood. The tight junctions between epithelial cells are thought to be a major factor in providing barrier properties to airway epithelia (Bhalla, 1999). Disruption of this barrier by ozone increases permeability of serum proteins and fluid into the air spaces while also allowing transport of exogenous material from the air spaces into the blood. Therefore, permeability is generally detected by the transport of an introduced tracer between airway spaces and blood or measurement of total protein and albumin collected by bronchoalveolar lavage (BAL).

The last ozone review (ARB, 1987) included a report describing the passage of blood proteins into the alveoli and/or airways of experimental animals after ozone exposure. Injection of radiolabelled albumin into the bloodstream of rats resulted in increased levels of the tracer in BAL after continuous exposure to 0.2 ppm ozone for 2 days and 0.4 ppm for 6 hours (Guth et al., 1986). Recent reports have expanded on this observation. Using isotope and non-isotope tracers, tracheal and bronchoalveolar permeability was increased following 2-3 hour exposures of rats to 0.8 ppm ozone (Bhalla and Crocker, 1986; Bhalla et al., 1986; Bhalla and Crocker, 1987; Bhalla et al., 1987; Young and Bhalla, 1992). Tracer transport was observed to be bidirectional, moving from airspaces to blood and vice versa. The changes in permeability are transient in nature, returning to baseline levels within 24-48 hours postexposure. In guinea pigs exposed to 1.0 ppm ozone for 1 hour, levels of horseradish peroxidase tracer instilled intratracheally was observed to increase in blood at 2 and 8 hrs postexposure, but had returned to baseline levels by 24 hours (Miller et al., 1986). An *in vitro* model employing rat alveolar epithelial monolayers has shown that acute exposure to a range of ozone concentrations (0.1-1.0 ppm) results in a dose-dependent increase in monolayer permeability, which resulted from damage to intercellular junctions and/or loss of epithelial integrity (Cheek et al., 1994).

Even though permeability changes are transient following ozone exposure, Bhalla et al. (1986) have shown that large protein tracers can become lodged in the interstitium as a result of increased permeability. Sequestration of tracers in

this compartment has much slower removal by blood. This suggests that combined exposure to ozone and very small toxic particles may result in particle accumulation in interstitial lung tissue. Other supporting studies have observed increased retention of mineral fibers *in vivo* and enhanced uptake of mineral fibers *in vitro* following ozone exposure (Pinkerton et al., 1989; Churg et al., 1996).

Ozone exposure induces a pulmonary inflammatory response that is often estimated by measuring total protein and albumin leaking into airways and/or the number of inflammatory cells (i.e., alveolar macrophages (AMs) and polymorphonucleated (PMN) cells) in the airways and alveoli. Measurement of total protein in BAL fluid following ozone exposure is one of the more sensitive indicators of pulmonary airway inflammation. These measurements are performed by analysis of BAL fluid or by morphometric techniques.

Guth et al. (1986) observed increased levels of total protein in BAL of rats after continuous exposure to ozone concentrations as low as 0.4 ppm for 6 hours and 0.12 ppm for 1 or 2 days. Increased total BAL protein occurred in guinea pigs exposed to ozone concentrations as low as 0.2 ppm for 4 hours (Hatch et al., 1986). Mice, hamsters, rats and rabbits did not exhibit this inflammatory effect until 4-hour ozone exposures of 1.0 ppm or higher were reached.

In recent acute exposure studies, increases in macrophage numbers were observed in rabbits 7 days following exposure to 0.1 ppm ozone for 2 hours (Driscoll et al., 1987). However, higher ozone concentrations (1.2 ppm) did not result in increased macrophage numbers on day 7, suggesting the results of exposure to 0.1 ppm ozone could have been a false-positive. In rats, exposure to 0.4 ppm ozone for 4 hours increased total protein in BAL fluid at 23-48 hours postexposure and produced lung parenchymal injury (Mautz et al., 1991). However, 4-hour exposure to 0.2 ppm ozone did not result in measurable lung parenchymal injury in the rats (total protein in BAL fluid was not measured). Using the same acute exposure regimen, Kleinman et al. (1999) obtained similar results, in that inflammatory injury was seen following 0.4 ppm ozone, but not following 0.2 ppm ozone. Bhalla et al. (1997) observed increases in protein and albumin levels and PMNs in BAL fluid in rats following 3-hour exposure to 0.5 ppm, but not 0.15 or 0.3 ppm ozone. In a study of ozone-induced inflammatory cell infiltration, a single exposure of 0.4 or 0.8 ppm ozone for up to 12 hours in both rats and mice did not affect the number of AMs isolated from BAL fluid immediately after exposure (Oosting et al., 1991). However, rats exposed to 0.8 ppm ozone for 6 hours showed increased macrophage number in BAL fluid at 42 hours post-exposure and was still significantly elevated at 66 hours post-exposure (Hotchkiss et al., 1989a). An increase in neutrophils was observed 42 hours following exposure but had returned to control levels by 66 hours post-exposure. Exposure to 0.12 ppm ozone for 6 hours had no effect on AM or neutrophil numbers up to 66 hours post-exposure.

In acute exposure studies with non-rodent species, 6-hour exposure of dogs to 0.2 ppm ozone increased the total number of cells recovered in BAL fluid immediately after exposure and increased the number of PMNs 18 hours after

exposure (Freed et al., 1999). Morphometric analysis of pulmonary airways of monkeys exposed to 0.4 or 1.0 ppm ozone for 2 hours revealed increased density of inflammatory cells in the alveolar spaces and along the bronchiolar epithelial surface at both ozone concentrations (Plopper et al., 1998). There was also an increase in necrotic epithelial cells found on the respiratory bronchiolar surface and in larger airways at both ozone concentrations. However, cellular content and total protein of BAL fluid were unchanged in monkeys exposed to 0.4 ppm ozone, suggesting that morphometric methods are more sensitive than BAL fluid examination for evaluating inflammatory effects following low-level ozone exposure. In monkeys exposed to 1.0 ppm ozone, total protein in BAL fluid was increased while total cells in BAL decreased (Plopper et al., 1998). A comparison of the inflammatory response in rats, monkeys and ferrets was performed following exposure to 1 ppm ozone for 8 hours (Sterner-Kock et al., 2000). BAL fluid analysis revealed 3- to 4-fold more PMNs per milliliter fluid and more severe epithelial injury in the centriacinar region in monkeys and ferrets than in rats. Based on these parameters of inflammation and the pulmonary structure similarities with humans, ferrets were considered a better model of humans for ozone-induced effects than rodents.

Rombout et al. (1989) examined concentration-time relationships of pulmonary inflammation in rodents due to acute ozone exposure in relation to likely scenarios of acute human exposure in urban settings. Daytime ozone exposure of rats was for 1, 2, 4, or 8 hours to 0.38, 0.76, 1.28, or 2.04 ppm, irrespectively (sixteen concentration (C) x time (T) products). Nighttime exposures, when rats are more active, were also conducted for 4, 8, or 12 hours to 0.13, 0.25, or 0.38 ppm ozone (nine C x T products). Total protein in BAL fluid was increased at 4 and 8 hours for all C x T products, including daytime exposure to 0.38 ppm and nighttime exposure to 0.13 ppm. Elevated levels of protein or albumin at these two exposure durations generally peaked 22-36 hours from the start of exposure and were still increased over controls at 54 hours. Nighttime exposure to ozone exhibited roughly a twofold increase in effect compared to daytime exposure and was similar to the ozone exposure-response dynamics for exercising humans presented by Koren et al. (1989b;1991) and Horstman et al. (1990).

In another investigation of C x T relationships, Gelzleichter et al. (1992a) exposed rats to 0.2, 0.4, 0.6, or 0.8 ppm ozone for 24, 12, 8, or 6 hr/day, respectively, over three days. All exposures occurred during the nighttime cycle, with the exception of the 0.2 ppm group, which had continuous exposure for three days. At the three highest concentrations, increased levels of total protein in BAL fluid and lavageable epithelial cells were proportionally similar indicating that the product of C x T remained constant. The lowest concentration (0.2 ppm) showed significantly less toxicity, likely due to significant exposure during daytime when rats are less active and have lower ventilation rates. In another ozone C X T study, Highfill et al. (1992) varied C (0.1, 0.2, 0.4, and 0.8 ppm) and T (2, 4, and 8 hours) in both rats and guinea pigs and measured total protein in BAL fluid. The lowest measurable increased protein in BAL fluid of both species occurred at 0.4 ppm for the 8-hour exposure groups. The results also indicated that protein in BAL fluid was not linearly related to C x T, that C had an influence

on T and, conversely, that T had an influence on C. Therefore, mathematically, both C and T are important in predicting protein in BAL fluid after ozone exposure. When comparing these data to BAL protein changes in exercising humans exposed to ozone (Koren et al., 1989b), the authors noted that rats and guinea pigs are less sensitive to the effects of ozone. Whether these species differences are due to exercise-enhanced deposition of ozone or whether humans are simply more responsive to ozone, as measured by protein BAL content, was unclear.

With exposure of rats to ozone during exercise (0.6 ppm for 3 hours), Mautz et al. (1988) observed a three-fold enhancement of focal lung lesions over resting exposures (0.6 ppm for 4 hours). In addition, it was found that exercise exposure to 0.35 ppm ozone for 3 hours produced a focal lung lesion response similar to the resting exposure of 0.6 ppm for 4 hours. Exercise exposures were conducted using a rodent treadmill and raised metabolic gas exchange by a factor of about two over resting metabolism.

Kleeberger et al. (1993) noted strain differences in mice in that total BAL protein in 'sensitive' C57BL/6J mice was increased following continuous exposure to 0.12 ppm ozone for 2 days or continuous exposure to 0.3 ppm for 1 day. The more resistant C3H/HeJ mice did not exhibit increased total protein in BAL fluid until one additional day of continuous ozone exposure at each ozone concentration and the inflammatory response to 0.3 ppm ozone was significantly less in the resistant strain compared to the sensitive strain. Genetic variation in the pulmonary membrane lipid composition of these two murine strains was thought to contribute to differences in peroxidative capacity of ozone on airway membranes, resulting in differential inflammatory responses (Kleeberger et al., 1993). More recent reports suggest ozone susceptibility among mouse strain may also be related to a gene encoding the proinflammatory cytokine tumor necrosis factor-alpha (Kleeberger et al., 1997). Together, these results suggest that the response to ozone is complex and determinants of susceptibility may occur at several different genetic foci.

In repeated exposure studies, exposure to 0.1 ppm ozone (2 hr/day) resulted in increased macrophages and neutrophils in BAL fluid on days 7 and 14 (Driscoll et al., 1987). While single exposure of 0.4 or 0.8 ppm ozone for up to 12 hours did not alter AM number in rats and mice, repeated exposure to 0.4 ppm ozone (12 hr/day for up to 7 days) in rats increased number of AM's in BAL fluid by day 3 and was still elevated on day 7 (Oosting et al., 1991). In the mice, repeated exposure increased number of AM's in BAL fluid at a later time point (day 7) and was less pronounced compared to rats (Oosting et al., 1991). Similar findings were observed by Mautz et al. (Mautz and Nadziejko, 2000), in that a single 4-hour exposure to 0.4 ppm ozone did not result in increased neutrophil cell count or increased total protein in BAL fluid, but repeated exposure (4 hr/day) to 0.4 ppm ozone for 3 days did increase these inflammatory parameters in BAL fluid. Repeated exposure of rats to 0.4 ppm (4 hr/day) ozone, but not 0.2 ppm, for 5 days resulted in increased numbers of inflammatory cells in alveolar lumens and increased interstitial hyperplasia of alveolar septa (Kleinman et al., 1999).

However, the inflammatory response was also observed after 1 day of exposure and was more severe compared to 5 days of exposure. Continuous exposure of rats to 0.1 ppm ozone for one week or 0.2 ppm ozone for 11 weeks resulted in increased levels of protein and AMs in BAL fluid (Mochitate et al., 1992). Dormans et al. (1990) observed similar findings utilizing morphometric methods, in that continuous 7-day exposure to 0.13 ppm ozone in rats resulted in increased AMs in centriacinar regions that was still elevated 5 days after the end of exposure.

Dormans et al. (1999) morphometrically compared the extent and time course of pulmonary injury and repair in rats, mice and guinea pigs continuously exposed to 0.2 or 0.4 ppm ozone for 3 to 56 days. In all three species, a concentration-related centriacinar inflammation (i.e., number of alveolar macrophages (AM) and the pulmonary cell density) occurred that was statistically significant at 0.2 ppm and maximum after three days of exposure. Only a slight or no decrease in these inflammatory effects occurred up to day 56 of exposure, with the extent of the inflammatory response in guinea pigs being about two-fold greater than that of rats and mice. Recovery from the inflammatory response in all animals exposed for 28 days took only 3 days. A similar study provided a detailed time study on development and repair of lung injury in rats exposed continuously to 0.4 ppm ozone for up to 56 days (van Bree et al., 2001). The acute inflammatory response, as measured by an increase of PMN cells, albumin and total protein in BAL fluid, reached a maximum at day 1 and resolved largely within 6 days during ongoing exposure. However, numbers of AM in BAL fluid increased progressively up to day 56, and slowly returned to near control levels during the post-exposure period. Morphometry of the AM population in the centriacinar region revealed a 10-fold increase in rats exposed for 7, 28, and 56 days versus controls. Pulmonary cell density in centriacinar regions was also increased at 7, 28 and 56 days of exposure.

Exposure of rats to 8-week and 26-week episodic exposures (4-hour exposures, 3 consecutive days/wk) to 0.3 ppm ozone had no effect on neutrophil count or total protein of rat lung lavage fluid, even though acute, 4-hour exposure to 0.4 ppm for 3 days resulted in increased levels of these inflammatory parameters (Mautz and Nadziejko, 2000). These findings indicated that adaptation to ozone occurred with longer exposures. Similar episodic exposures to 0.15 ppm ozone for 12 and 40 weeks in rats and rabbits also had no effect on neutrophil count or total protein in BAL fluid.

In rats exposed to an urban diurnal pattern of ozone (13-hour background of 0.06 ppm with an exposure peak rising to 0.25 ppm, and declining to background over a 9-hour period, with 2-hour downtime for maintenance) for 78 weeks, acute tissue reactions after 1 week of exposure included epithelial inflammation, interstitial edema, interstitial cell hypertrophy, and influx of macrophages (Chang et al., 1992). However, these inflammatory responses subsided after 3 weeks of exposure and were not significantly different from controls at 78 weeks of exposure.

A study by Cheng et al. (1995) noted the differential effects of ozone on lung epithelial lining fluid volume and protein content. Exposure of rats to 1 ppm ozone for 6 hours resulted in only a modest increase (21%) in lung lining fluid volume, while protein and albumin concentrations were 2.3- and 4.5-fold of control values, respectively. Similar exposure to 0.5 ppm ozone had no effect on these factors. These results imply that movement of water and protein into the airspaces due to ozone exposure is not strictly coupled, and that protein recovery by BAL should be used cautiously to indicate airspace edema as a result of ozone injury.

Measures of ozone-induced inflammation obtained by BAL were shown to increase with decreasing temperature in rats exposed to 0.5 ppm ozone for either 6 or 23 hr/day over 5 days while maintained at an ambient temperature of either 10, 22, or 34°C (Wiester et al., 1996b). The magnification of ozone toxicity with cold temperatures was demonstrated with increases in lavageable protein, percent PMN, lysozyme and alkaline phosphatase activity in continuously (23 hr/day) exposed rats. Daily 6-hour exposures resulted in relatively marginal, but significant, increases in percent of PMNs and alkaline phosphatase activity at 22°C. These effects were largely attenuated by the fifth day of exposure. Levels of urea, creatinine, glucose, and potassium in BAL fluid, (used as indicators of increased permeability and cell injury) appeared to be unaffected by temperature during ozone exposure.

Dormans et al. (1996) carried out experiments to investigate age-related inflammatory responses to ozone in 1, 3, 9, and 18 month-old rats. Exposure to 0.8 ppm ozone for 12 hours resulted in highest levels of protein and albumin in BAL fluid from one month old rats. Lesser increases occurred in older rats. A decrease in the net percentage of PMN influx in BAL fluid was also observed in older rats. These data indicate that younger rats are more sensitive to the inflammatory effects of ozone.

Studies in both animals and humans have demonstrated that repeated exposure to ozone results in a lessening of the effects as exposure progresses. This reduction in response has historically been referred to as tolerance or adaptation. However, although some responses such as lung function, airway reactivity, airway inflammation, and permeability of airway epithelium decrease with continued exposure to ozone, other responses such as morphological and biochemical effects appear to progress with ongoing exposure. The scientific literature often refers to this reduction in some ozone-induced responses as attenuation. Since the first Ozone Review (ARB, 1987), some detailed studies have been published that investigated the phenomenon of attenuation.

Tepper et al. (1989) utilized a short repeated exposure regimen to determine if attenuated pulmonary function reflects histopathologic and biochemical changes in the lung. In rats exposed to 0.35 or 0.5 ppm ozone for 2.25 hr/day for 5 consecutive days, initial alterations in breathing response to ozone had diminished by day 5. However, a group exposed to 1.0 ppm ozone still showed altered breathing patterns. Early flow limitations in smaller airways of the 0.5 ppm group had recovered by day 5. Initial increases in lung glutathione were

within the control range by day 4. In contrast, lung ascorbate was elevated by the end of exposure. In addition, elevated BAL fluid protein and a progressive pattern of epithelial damage and inflammation in the central acinus region was apparent in the 0.5 ppm group over the course of the 5-day exposure. The findings suggested that some biochemical and morphologic aspects of lung tissue response do not attenuate with repeated exposures to ozone.

In a study investigating attenuation and the subsequent time course of recovery of pulmonary injury, van Bree et al. (2002) exposed rats for 5 consecutive days to 0.4 ppm ozone for 12 hr/night and then administered a single challenge of 0.4 ppm ozone for 12 hours at various time points over a 20-day recovery period. Five-day exposure to ozone resulted in attenuation of permeability and inflammatory responses. With respect to BAL fluid levels of albumin, interleukin (IL)-6, and the numbers of AMs and PMNs, the period for lung tissue to regain its full susceptibility and responsiveness to ozone following the 5-day preexposure period was about 15-20 days. However, total protein and fibronectin responses in BAL still exhibited an attenuated response to ozone challenge at 30 days postexposure. Morphometry (number of bromodeoxyuridine-labeled epithelial cells in terminal bronchioles, and number of AMs) showed that after a recovery of 5-10 days following a 5-day preexposure the response to a challenge was identical to that after a single exposure. These results suggest that complete repair from lower airway inflammation caused by short-term, repeated exposure to ozone may take longer than previously assumed. Remarkably, the permeability and inflammatory findings of the rat data (van Bree et al., 2002) show a marked correlation with the data from a study in humans (Devlin et al., 1997), in which generally similar exposure protocols and effect parameters were used. The similar findings aid not only the extrapolation of ozone data from rats to humans but suggest that the morphological effects observed in the rat study may very well occur in humans exposed to ozone.

In rats exposed to episodes of ozone (1 ppm, 8 hr/day for 5 days) followed by 9 days of filtered air for four cycles, each 5-day episode induced a characteristic pattern of rapid shallow breathing (days 1 and 2), epithelial injury, and interstitial and intraluminal inflammation (Schelegle et al., 2003). In contrast, the neutrophil component of inflammation, tracheal substance P release, and cell proliferation became attenuated with each consecutive episode of exposure. Over the four exposure episodes, terminal bronchiolar remodeling (hypercellularity and thickening of the centriacinar airway epithelium) was cumulative and was not dependent upon an increase in cell proliferation. The findings suggested that the cumulative distal airway lesion is at least in part the result of a depressed cell proliferative response to injury. The depressed cell proliferative response, in turn, may be in part the result of diminished neutrophil inflammation and/or release of mitogenic neuropeptides (i.e., substance P) in response to ozone-induced injury. Attenuation of airway neuropeptide levels induced by repeated ozone exposure may play a role in the adaptation of functional processes and epithelial injury/repair.

In summary, consequences of ozone-induced lung inflammation include disruption of the pulmonary epithelial barrier, resulting in increased transmucosal permeability, and recruitment of inflammatory cells to lung airways. In addition, ozone-induced inflammation and increased permeability can enhance the accumulation of inhaled particles in interstitial lung tissue, where clearance to blood is very slow. Even though rodents appear to be more resistant to the inflammatory effects of ozone compared to humans, the permeability and inflammatory findings of the rodent data parallel the data from counterpart studies in humans using similar exposure protocol and effect parameters. Recent work reported here support studies from the previous ozone review, in that measures of inflammatory and permeability changes in the lungs of experimental animals occur at ozone concentrations as low as 0.1-0.13 ppm. Minimal inflammatory effects with acute ozone exposure were observed with 4-hour exposure to 0.13 ppm, while repeated daily ozone exposure (2-hr/day for 7 days) has resulted in minimal inflammatory effects at 0.1 ppm.

Some key findings regarding acute ozone exposure include quantitative influences of time-of-day of exposure and activity level on pulmonary inflammation. Concentration x time relationships for ozone-induced inflammatory responses provided a comparison of nighttime and daytime exposures and minimal exposure levels that resulted in pulmonary inflammation. Minimal inflammatory effects were noted with nighttime exposure, when rats are most active, to 0.13 ppm for 4 hours, while minimal inflammatory effects for daytime exposure was 0.38 ppm for 4 hours, roughly a 3x difference. Other daytime acute exposure studies in rats support a minimal inflammatory effect at 0.4 ppm with 4-hour exposures. Increasing the activity level through exercise, resulting in an increased metabolic gas exchange by a factor of about two over resting metabolism, reduced the ozone dose necessary to cause inflammatory lung lesions by about 2x. In other words, exercise exposure to 0.35 ppm ozone for 3 hours produced a focal lung lesion response similar to the resting exposure of 0.6 ppm for 4 hours. While quantitative comparisons suggest that rodents are more resistant to the inflammatory effects of ozone relative to humans, time-of-day of exposure and activity level effects may, in part, explain some of these sensitivity differences between species.

Other key findings indicate that morphological analysis of inflammatory changes resulting from ozone exposure may be more sensitive than analysis of BAL fluid for inflammatory cells and protein content. Exposure of monkeys to 0.4 ppm ozone for 2 hours resulted in clear evidence of inflammatory effect by morphometric techniques. However, changes in BAL fluid protein and inflammatory cells could not be measured at this level. Finally, prolonged exposure of rats to an urban profile of ozone that reached a daily peak concentration of 0.25 ppm resulted in pulmonary inflammation the first week of exposure, but became attenuated with continued exposure. Other studies investigating ozone attenuation noted that the inflammatory effects can become attenuated with continued exposure, but other aspects of ozone exposure, including biochemical and morphological effects, may not. Time to recovery from ozone attenuation also varies depending on the endpoint measured.

## **A.2.2 Lung Host Defense**

The host defense system in the respiratory tract of humans and animals protects against infectious and particulate deposition primarily by utilizing two well-coordinated systems, the mucociliary system and the immune system. The animal data provides a basis for comparison relevant to humans because the pulmonary defense systems function similarly in both animals and humans. Although the respiratory defense mechanisms act in concert to protect the lung, various aspects of the integrated system are discussed separately below. The clearance section discusses the effect of ozone on removal of inhaled particles. The section on alveolar macrophages discusses the effects of ozone exposure on the functions of these cells that help to clear the lungs of debris and particles. The section on other immune system cells covers the effect of ozone on other immune cells present or recruited in the lungs other than alveolar macrophages. The section on interaction with infectious microorganisms discusses the effect of ozone exposure on defense against viral or bacterial exposure.

### *A.2.2.1 Clearance*

The muciliary transport mechanism is one of the primary defense mechanisms against inhaled particles. The mucociliary escalator clears the airways of their own secreted mucus, together with inhaled substances that became trapped in it. Clearance of alveoli and conducting airways depends on the function of alveolar macrophages (AMs), ciliated cells, and secretory cells, and on the physical and chemical properties of fluids lining the alveoli and airways. Impairment of clearance mechanisms by ozone could produce accumulation of secretions in airways or result in longer residence times for toxic, particulate, and infectious agents.

Previous studies reviewed in the prior Ozone Review (ARB, 1987) suggested that acute and prolonged ozone exposures in the range of 0.4-0.6 ppm reduces the mucociliary clearance rate in experimental animals. However, alteration of alveolar clearance was dependent on ozone concentration; levels as low as 0.1 ppm increased alveolar clearance while concentrations above 0.6 ppm may reduce it.

In a recent long-term study, exposure of rabbits to 0.1 ppm ozone (2 hours/day, 5 days/wk) for up to one year did not affect mucociliary clearance (Schlesinger et al., 1992a). However, clearance had become slower following a six-month post-exposure period. The slower post-exposure clearance suggests an attempt to reach a new level of homeostasis during prolonged irritant exposures. Maintenance of the new clearance rate might have been dependent on the continuation of such exposures. However, it is unclear if this represents a permanent alteration.

Similar to humans, the sheep mucociliary system is incompletely developed at birth and undergoes postnatal maturation during the first weeks of life. Exposure of lambs to a high ozone concentration (1 ppm, 4 hr/day for 5 days) during the first week of life retarded the normal development of the mucociliary system by reducing tracheal mucus velocity, increasing tracheal mucus cell populations and

total mucus load, and reducing tracheal ciliated cell populations (Mariassy et al., 1990). Lower tracheal mucus velocity was still apparent in ozone-exposed lambs 24 weeks later, suggesting that early impairment of the natural development of the mucociliary system can lead to a prolonged decrement of function. However, 4-hour exposure of adult sheep to 1 ppm ozone did not alter lung clearance of a radiolabeled tracer that was instilled in the lungs (Hornof et al., 1989).

A radiolabeled tracer was also used in dogs to measure regional clearance rates following 6-hour exposure to 0.4 ppm ozone delivered directly onto sublobar segments via a bronchoscope (Foster and Freed, 1999). Clearance halftime in sublobar bronchi was decreased by 50% at one day postexposure, and was still reduced (28.8%) at 7 days post-exposure. The clearance rate was the same as the baseline mean at 14 days post-exposure. It was hypothesized that the increased clearance at 1 day post-exposure was partly the result of airway injury leading to increased permeability to the tracer. However, clearance was still accelerated at 7 days post-exposure when airway inflammation had apparently subsided.

In adult sheep, both short- and medium-term exposure (4 hr/day for 2 days or 6 weeks) to 0.5 ppm ozone resulted in tracheal mucus hypersecretion, which has been associated with a slowing of mucus transport (Phipps et al., 1986). In a similar experiment on ferret tracheal glands, continuous exposure to 1 ppm ozone *in vivo* for 3 or 7 days resulted in *in vitro* increases of basal secretion of respiratory glycoconjugates and increased tracheal gland sensitivity to the cholinergic agonist carbachol (McBride et al., 1991). Ferret airways, like human airways, have large numbers of mucus glands that are under autonomic control. In addition to potential slowing of mucus transport, increased mucus secretion in conjunction with ozone-induced smooth muscle hyper-responsiveness may adversely affect airway conductance and contribute to exacerbation of asthma in humans.

To examine alveolar duct clearance of inhaled fibers resulting from exposure to environmentally relevant concentrations of ozone, Pinkerton et al. (1989) continuously exposed rats to 0.06 ppm ozone 7 days a week with a slow rise in ozone to a peak of 0.25 ppm and subsequent decrease to 0.06 ppm over a 9-hour period five times each week for 6 weeks. The rats were then exposed to aerosolized asbestos fibers for 5 hours. Immediately after exposure to asbestos, lung asbestos fiber burden was similar in both control and ozone-exposed animals but the ozone-exposed rats had significantly less clearance of fiber mass and fiber number from the bronchiolar-alveolar duct region 30 days later. The reduced clearance in ozone-exposed rats was speculated to be the result of greater movement of fibers into the bronchiolar wall due to increased permeability of airway epithelium and/or reduced function of AMs.

To examine whether the retention and distribution of chromium (Cr) compounds within the deep lung were affected by coexposure with ozone, rats were exposed nose-only to soluble potassium chromate or insoluble barium chromate (0.360 mg Cr/m<sup>3</sup>), either alone or in combination with 0.3 ppm ozone (Cohen et al., 1997). Exposures were for 5 hr/day, 5 days/wk for 2 or 4 weeks. Coexposure to

soluble Cr and ozone caused a decrease in Cr retention relative to that of rats breathing soluble Cr alone. Conversely, insoluble Cr/ozone mixtures resulted in significant increases in relative burdens over exposure to insoluble Cr alone. The presence of ozone itself had no effect upon lavageable cell Cr levels when either compound was used, although ozone did lead to reductions in acellular lavage fluid Cr levels compared to those in rats inhaling either Cr agent alone. Cohen et al (Cohen et al., 2003) conducted a similar experiment with calcium chromate, in which rats were exposed nose-only, 5 hr/day, 5 days/wk for 4, 8, 12, 24, or 48 weeks to ozone only (0.3 ppm), calcium chromate-only (0.360 mg Cr/m<sup>3</sup>) or their mixture. The majority of the Cr (>94%) was in nonlavageable sites corresponding to the epithelium and interstitium. Coinhalation of ozone initially caused an increase in percentages of the Cr present to be localized in those cells recoverable by lavage. But the absolute amounts of Cr found in all lavaged cells and recovered fluids did not differ as a result of copresence of ozone. In addition, coexposure with ozone did not affect the numbers of cells recoverable from the lavaged lung tissues or their relative cellular Cr burdens. While calcium chromate is not considered highly soluble, it was suggested that the lack of increased lung Cr burdens in rats exposed to the mixture was related to its solubility, which is 40x more soluble than barium chromate, but is less than potassium chromate (Cohen et al., 2003). Thus, the potential for ozone to affect Cr retention is apparently closely related to the solubility of Cr agents.

#### *A.2.2.2 Alveolar Macrophages*

AMs are the primary cellular defense system in the lower lung. Following exposure to inhaled or blood-borne antigens, AMs phagocytize foreign antigens and secrete mediators that recruit and activate inflammatory cells in the lung, thus amplifying their role in host defense. Impairment of AM's by ozone or other toxic agents can have a significant effect on host defense by affecting their phagocytic abilities, membrane integrity, mobility, and enzymatic capacity. Previous studies reviewed in the Ozone Review (ARB, 1987) found that ozone concentrations in the range of about 0.1-0.5 ppm can affect AM abilities in host defense in all these areas. Recent reports have greatly expanded our knowledge of ozone's effect on AM function.

Because phagocytosis of foreign particles is one of the major roles of AMs, inhibition of this function by ozone may increase the spread of infection and disease in the respiratory tract. AMs recovered from BAL fluid immediately and 24 hours after a single exposure of rabbits to 0.1 ppm ozone for 2 hours had reduced phagocytic capacity (Driscoll et al., 1987). Exposure of rabbits to 0.1, 0.3, or 0.6 ppm ozone for 3 hours resulted in a dose-dependent reduction of phagocytic activity that was significant at the lowest exposure (Schlesinger et al., 1992b). Repeated exposure of rabbits to 0.1 ppm ozone (2 hr/day for 13 days) produced reductions in the numbers of phagocytically active AMs when measured on days 3 and 7 (Driscoll et al., 1987). However, phagocytic activity had returned to control levels by day 14, suggesting that adaptation to repeated ozone exposures had occurred. In a similar study, AMs in mice exposed continuously to 0.5 ppm ozone for 14 days displayed suppressed phagocytic

activity when measured on days 1, 3 and 7 of exposure (Gilmour et al., 1991). However, phagocytic activity had returned to control levels by day 14. These results reflected the findings of intrapulmonary bacterial killing of *Staphylococcus aureus* in the mice (see section A.2.2.4), in that there was initial suppression of bacterial killing followed by recovery of bacterial killing activity by day 14.

In other studies investigating the effects of ozone on phagocytosis of infectious bacteria, mice infected with *S. zooepidemicus* following exposure to 0.4 or 0.8 ppm ozone for 3 hours resulted in decreased AM phagocytosis, impaired intrapulmonary bacterial killing and increased mortality at both ozone concentrations (Gilmour and Selgrade, 1993; 1993a). Rats exposed under the same conditions also exhibited decreased AM phagocytosis and impaired intrapulmonary bacterial killing but experienced no mortality (Gilmour and Selgrade, 1993). At exposures to 0.4 ppm ozone for 3 hours, the suppression of AM phagocytic activity was greater in two strains of mice (80-100%) compared to similarly treated rats (about 50%).

Pretreatment with indomethacin, a cyclooxygenase inhibitor, partially inhibited ozone-induced (0.5 ppm, 1-14 days) suppression of AM phagocytic activity in mice (Canning et al., 1991). Indomethacin pretreatment also inhibited ozone-induced increases in prostaglandin E<sub>2</sub>, which likely plays a role in immunity changes and AM phagocytic suppression following ozone exposure. AMs lavaged from rabbits exposed to 1 ppm ozone intermittently (2 hr/day) for 3 days showed substantial depression of cytotoxicity towards xenogeneic tumor cells immediately and 24 hours after exposure (Zelikoff et al., 1991). The number of AM's in BAL fluid did not change but cell viability was significantly depressed immediately after exposure.

With longer exposures, Christman et al. (1982) observed increased AM phagocytosis of inert carbon-coated latex microspheres following continuous exposure of rats to 0.8 ppm ozone for 20 days. Creutzenberg et al. (1995) noted increased phagocytic capacity of polystyrene beads per AM in rats exposed to 0.5 ppm ozone, 6 hr/day, 5 days/wk for 2 months, but not in rats exposed under the same conditions for only 7 days. Kleinman et al. (2000) did not measure a difference in AM phagocytosis of polystyrene latex microspheres following exposure of aged rats (22-24 months old) to 0.2 ppm ozone, nose-only, 4 hr/day, 3 consecutive days per week, for 4 weeks.

In a comparison study of AM phagocytic function in normal and ozone-containing atmospheres, Selgrade et al. (1995) demonstrated that the immune system of mice are accurate predictors of effects in humans. *In vitro*, the phagocytic capability of macrophages removed from humans and mice and exposed to comparable doses of ozone (0.8 ppm for 3 hours) was similar as measured by the phagocytic index (number of fluorescent particles ingested per 100 macrophages). *In vivo* exposure resulted in a significant drop in the phagocytic index of both murine and human macrophages. Mice exposed to 0.8 ppm ozone for 3 hours had a 42 percent drop in phagocytic index, while humans exposed to 0.08 ppm ozone for 6.6 hours while exercising had a 25 percent drop in phagocytic index. When the *in vivo* results are corrected for dosimetric

differences, the phagocytic indices for mice and humans are similar (28 percent for mice, 25 percent for humans). In a comparison study of AM function in rats and mice following ozone exposure, Oosting et al. (1991) exposed the rodents to 0.4 ppm ozone for 3, 6, or 12 hours. In rats, six-hour exposure to ozone resulted in suppressed phagocytosis of AMs followed by recovery above control levels with 12-hour exposure. In mice, suppressed AM phagocytosis occurred only after 12-hour exposure. With repeated daily exposure of rats and mice to 0.4 ppm ozone (12 hr/day for 7 days), AM phagocytosis in rats was unaffected with the exception of the day 1 increase (Oosting et al., 1991). However, suppression of AM phagocytosis in mice occurred out to day seven of exposure. Speculation as to which animal species best reflects human AM function following ozone exposure was not discussed.

Kleinman et al. (1999) measured the ability of rat AMs to bind sheep red blood cells to Fc receptors (Fc-receptor binding), which had been previously activated with anti-sheep red blood cell antibody, following exposure of rats to ozone (nose-only, 0.2 or 0.4 ppm, 4 hr/day for 1 or 5 days). Exposure to 0.2 and 0.4 ppm ozone caused a significant decrease in Fc-receptor activity, relative to control, after both 1 and 5 days of exposure. With longer exposure, Kleinman et al. (2000) did not measure a difference in AM Fc-receptor binding activity following exposure of aged rats (22-24 months old) to 0.2 ppm ozone (nose-only), 4 hr/day, 3 consecutive days per week, for 4 weeks.

Inhibition of the mobility of AMs by ozone could also have implications for increased susceptibility to infection. AM mobility was unaffected by single (0.1 and 1.2 ppm) or repeated (0.1 ppm only) 2-hour ozone exposures in rabbits (Driscoll et al., 1987). In rabbits exposed to 1 ppm ozone intermittently (2 hr/day) for 3 days, random migration of AM's was depressed immediately after exposure, but had returned to control levels by 24 hours after end of exposure (Zelikoff et al., 1991). However, stimulus-directed movement of AMs by a chemotactic agent was unaffected immediately after exposure, and showed significant enhancement at 24 hours following exposure. One hypothesis for differences in ozone-induced random migration and stimulus-directed movement following ozone exposure is that an influx into the lung of chemotactically activated mononuclear cells occurred 24 hours after ozone injury. However, Bhalla (1996) noted that AMs isolated from rats only 12 hours after ozone exposure (0.8 ppm, 3 hours) also exhibited greater motility in response to a chemotactic stimulus. Chemotactic migration of lavaged AMs from rats was marginally, but not significantly, stimulated following exposure to 0.5 ppm ozone 5 hr/day for 2 days (Creutzenberg et al., 1995). Exposure of rats to 0.5 ppm, 6 hr/day, 5 days/wk for either 7 days or 2 months had no effect on chemotactic migration of lavaged AMs.

The adhesive capability of AMs is considered an important factor for defense functions and inflammatory responses. AM functions critical to the release of proinflammatory cytokines and development of inflammation are stimulated as the macrophages adhere to various surfaces. Substrate attachment by AMs collected from BAL fluid of rabbits exposed to 1.2 ppm ozone for 2 hours was

impaired immediately after exposure, but not at 24 hours post-exposure (Driscoll et al., 1987). Single and daily repeated exposures (2 hr/day) to 0.1 ppm ozone did not result in a statistically significant reduction in AM attachment. However, Pearson et al. (1997) observed that 3-hour exposure of rats to 0.8 ppm ozone increased adherence of AMs to cultured lung epithelial cells 8-12 hours post-exposure. AMs isolated from ozone-exposed rats (0.8 ppm, 3 hours) exhibited greater adhesion when placed in culture with epithelial cells isolated from adult rat lung (Bhalla, 1996). A modest increase in expression of one adhesion molecule (CD11b) but not another (ICAM-1) was observed from AMs of ozone-exposed rats. In seeming contrast, Hoffer et al. (1999) found that exposure to 1 ppm ozone for 2 hours resulted in lowered expression of an integrin adhesion molecule (CD18) on AMs. Differences between these two studies suggest that adhesive behavior might depend on factors other than changes in regulation of cell adhesion molecules. Increased adherence of AM's following ozone exposure could explain why fewer AMs are collected from BAL fluid at certain time points following ozone exposure. For example, Pino et al. (1992a) observed fewer AMs in BAL of rats following acute exposure to 1.0 ppm ozone but morphometric analysis of AMs in airways found no change in AM volume.

The release of oxidant species (superoxide anion; hydrogen peroxide) by AMs on a target cell, such as bacteria or tumor cells, is an important factor in the cytotoxic action of AMs. Hydrogen peroxide production by zymosan-stimulated AMs from rats exposed to ozone (0.1 or 0.3 ppm, 4 hr/day, for 1 or 3 weeks) was dose-dependently reduced and significantly different from controls at the lowest exposure concentration (Cohen et al., 2002). This has importance, in that hydrogen peroxide is one of the primary reactive oxygen intermediates involved in the intracellular killing of bacteria such as *Listeria*. In contrast, 4-week exposure to 0.3 ppm ozone (5 hr/day, 5 days/wk) did not impair zymosan-stimulated or spontaneous production of hydrogen peroxide by rat AMs (Cohen et al., 1998). In rabbits, 3-day ozone exposure to 1 ppm, 2 hr/day had no effect on hydrogen peroxide production by zymosan-stimulated AMs compared to a zymosan-stimulated control group (Zelikoff et al., 1991). The same exposure protocol also had effect on hydrogen peroxide production by unstimulated rabbit AMs compared to air-exposed controls (Zelikoff et al., 1991).

A number of studies investigated the alteration of superoxide anion radical production by AMs as a result of ozone exposure. Both unstimulated and zymogen-stimulated AM superoxide production remained unchanged in rabbits exposed to 0.1, 0.3, or 0.6 ppm ozone for 3 hours (Schlesinger et al., 1992b). Ryer-Powder et al. (1988) reported that mouse macrophage production of superoxide was depressed following exposure to 0.11 ppm ozone for 3 hours. However, in rats similarly exposed, superoxide production was not depressed until a concentration of 1.6 ppm ozone was reached (Ryer-Powder et al., 1988). In rabbits exposed to 1 ppm ozone, 2 hr/day for 3 days, zymosan-stimulated production of superoxide anion in AMs was depressed immediately after exposure, but was increased significantly above control levels 24 hour following exposure (Zelikoff et al., 1991). In the same study, superoxide anion production by resting (unstimulated) AMs was unchanged in ozone-exposed rabbits

immediately after exposure, but increased significantly above control levels 24 hours following exposure. Other than modulation of AM superoxide dismutase (SOD) production by various cytokines and arachidonic acid metabolites, alteration of superoxide anion production by AMs following ozone exposure could be due to an influx of a large number of not fully matured, and hence not fully functional, AMs (Oosting et al., 1991).

In other studies that included repeated ozone exposure, mice exposed to 0.4 ppm one for up to 12 hours did not result in an alteration of AM superoxide production, while repeated exposure to 0.4 ppm ozone (12 hr/day for 7 days) led to a maximal 50% inhibition of AM superoxide production in the mice (Oosting et al., 1991). Concurrent acute exposure (0.4 ppm up to 12 hours) in rats showed a tendency towards an initial decrease in superoxide production by AMs with 6-hour exposure, but was followed by recovery above control levels after 12 hours exposure (Oosting et al., 1991). Repeated exposure of rats to 0.4 ppm (12 hr/day for up to 7 days) resulted in impaired production of superoxide by AMs at day 3, but was not different from control values at Day 7. In contrast, exposure of rats to 0.2-0.8 ppm ozone for 7 hr/day for up to 4 days did not result in consistent evidence of altered spontaneous or phorbol-stimulated release of superoxide anion from bronchiolar leukocytes (primarily AMs and PMNs) obtained from BAL fluid (Donaldson et al., 1993).

With prolonged exposures, the formation of superoxide anion was increased in lavaged AMs from rats after a 2-month exposure to 0.5 ppm ozone (6 hr/day, 5 days/wk), but superoxide production was unaffected after a 7-day exposure under the same exposure conditions (Creutzenberg et al., 1995). In a repeated 4-week ozone exposure study (0.3 ppm ozone, 5 hr/day, 5 days/wk), Cohen et al. (1998) observed no change in spontaneous production of superoxide in rat AMs compared to control values, but observed depressed superoxide formation in zymosan-stimulated rat AMs compared to zymosan-stimulated controls. Kleinman et al. (2000) did not observe a difference in superoxide production in zymosan-stimulated AMs from aged rats (22-24 months old) exposed nose-only to 0.2 ppm ozone, 4 hr/day, three consecutive days per week, for 4 weeks.

The enzyme lysozyme is important in AM host defense, in that lysozyme is released by AMs to chemically cleave cell walls of some invading microorganisms. Morphometric examination of pulmonary centriacinar regions of rats that were continuously exposed to 0.13 ppm ozone for seven days revealed increased levels of lysozyme-positive AMs, which was associated with an overall increase of AMs (Dormans et al., 1990). Numbers of lysozyme-positive AMs were still elevated five days post-exposure.

Optimal intracellular pH of AMs is critical for the maintenance of normal function and is regulated within a narrow physiological range. Exposure of rabbits to 0.1, 0.3, or 0.6 ppm ozone for 3 hours resulted in a concentration-dependent reduction of intracellular pH in AMs, which was significant at the 0.6 ppm level (Chen et al., 1995).

Morphological differences in the appearance of AMs following ozone exposure have been reported. Donaldson et al. (1993) noted that AMs lavaged from rats exposed to 0.8 ppm ozone were larger and vacuolated on the first day of exposure, with significant recovery by day 4. Hotchkiss et al. (1989a) described a similar increase in size and vacuolation of AMs of rats following 6-hour exposure to 1.5 ppm ozone, but not 6-hour exposure 0.8 ppm ozone.

AMs have also been implicated in additional lung tissue injury following ozone exposure due to amplification of the oxidant insult. Various actions that may be involved in this process include the release of direct-acting cytotoxic compounds (i.e., hydrogen peroxide, nitric oxide, peroxyxynitrite) and mediators that degrade the extracellular matrix (collagenase, elastase) and/or promote inflammatory cell infiltration, proliferation, and activation (i.e., cytokines, eicosanoids). A review of mechanistic studies that investigate these potential pro-inflammatory processes is not directly relevant to the setting of an ambient air quality standard for ozone and is generally beyond the scope of this report. However, a recent report of note investigated AM-mediated immunosuppressive activity. AMs play an important immunomodulatory role in the lung via suppression of lymphocyte proliferation, thus limiting the magnitude and duration of local immune response. Koike et al. (1998) observed that AM-mediated suppression of lymph node cell proliferation was markedly inhibited by BAL fluid from ozone-exposed rats (1 ppm for 3 days), which may then result in excessive T-cell activation and immunoinflammatory responses. It was indicated that the inhibition of AM-mediated immunosuppressive activity was caused by ozone-induced release of soluble factors, which inhibit nitric oxide production by AMs (nitric oxide is known to play a crucial role in the immunosuppressive activity of AMs).

#### *A.2.2.3 Other Immune System Cells*

Ozone has been shown to alter the function of PMNs, also known as neutrophils or polymorphonuclear leukocytes, which may play a role in augmenting ozone-induced lung injury. PMNs migrate to lung airways as a result of pulmonary oxidant injury. The chemoattraction of PMNs to the airways is part of a stereotypical inflammatory response to airway injury. Another cell type that appears in the lung following ozone exposure is lymphocytes. Lymphocytes generate, regulate and carry out immune and non-immune inflammatory reactions. Because lymphocytes are closely associated with non-pulmonary lymphoid tissues, such as the thymus and spleen, review of ozone-induced lymphocyte function alterations will be largely covered in section A.4 (systemic effects).

Repeated exposure to 0.4 ppm ozone (12 hr/day for up to 7 days) induced a strong rise in the number of PMNs in BAL fluid of rats out to Day 3, which declined considerably by Day 7 (Oosting et al., 1991). In comparison to rats, repeated exposure of mice showed a continuous rise in the number of PMNs in BAL fluid that was less pronounced compared to rats on Day 3 and not significant until Day 7. Six-hour exposure of rats to 0.8 ppm ozone resulted in increased numbers of PMNs recovered from BAL fluid by 42 hours post-exposure (Hotchkiss et al., 1989a). However, 6-hour exposure to 0.12 ppm

ozone did not elicit an effect on PMN number in BAL fluid. Exposure of rats to 0.2, 0.4, 0.6, and 0.8 ppm ozone intermittently (7 hr/day) for four days resulted in an increased proportion of PMNs in BAL at the two highest concentrations on days 1 and 2, but not day 4 (Donaldson et al., 1993). Overall levels of bronchiolar leukocytes, primarily AMs with occasional PMNs and lymphocytes, were unchanged. Histological examination noted an increase of inflammatory cells in distal air spaces, including PMNs, at 0.6 and 0.8 ppm ozone on days 1 and 2 as well. In an acute exposure study in monkeys, 2-hour exposure to 1.0 ppm, but not 0.4 ppm ozone, increased the percentage of PMNs and eosinophils in BAL fluid (Plopper et al., 1998).

PMNs isolated from blood of rats exposed to 0.8 ppm ozone for 2 hours showed increased adhesion and motility when incubated with an epithelial cell line derived from rat lung or with primary alveolar Type II cell cultures (Bhalla et al., 1993). These results suggest extrapulmonary effects of ozone, presumably through the release of chemotactic agents and oxygen metabolites, which cause a modification of PMN function.

Similar to AMs, PMNs are thought to amplify the tissue injury due to ozone exposure. While studies investigating pro-inflammatory actions of PMN's following ozone exposure are generally beyond the scope of this review, several studies are worth noting. An isolated perfused rat lung model has shown that neutrophils introduced during 3 hour exposure to 1 ppm ozone had a synergistic action on ozone-induced airway epithelial injury and were primarily responsible for the resulting increase in transmucosal permeability (Joad et al., 1993). Thus, pulmonary toxicants that enhance migration of neutrophils to lung airways may lead to further injury. Pino et al. (1992b) depleted rats of neutrophils with anti-neutrophil serum and exposed the animals to 1.0 ppm ozone for 8 hours. Contrary to the findings of Joad et al. (1993), no differences in inflammatory measures (BAL protein, airway epithelial cell damage) were seen when compared to rats treated with normal rabbit serum (to enhance neutrophil influx) and exposed to the same ozone regimen. In addition, Reinhart et al. (1998) observed that recruitment of PMNs into rat airways using intratracheally instilled rabbit serum did not amplify lung injury with subsequent exposure to 0.8 ppm ozone for 3 hours. In a study by Donaldson et al. (1993), bronchiolar leukocytes from rats exposed to ozone (0.2-0.8 ppm, 7 hr/day for up to 4 days) showed no increased ability to damage epithelial cells *in vitro* compared to controls. The combined data currently suggests that the inflammatory response to ozone is complex, and that the ozone-induced influx of PMNs into lung airways do not mediate further injury to epithelial cells under various experimental conditions.

Recent work has also explored the mechanism of neutrophil influx resulting from ozone inhalation. In Rhesus monkeys exposed to 0.96 ppm ozone for 8 hours, epithelial necrosis and repair were associated with the presence of granulocytes (including neutrophils and eosinophils) in the epithelium and interstitium of the tracheobronchial airways during the week-long postexposure period (Hyde et al., 1992). In similarly exposed monkeys, the appearance of the chemokine interleukin (IL)-8 in airway epithelium cells correlated well with neutrophil influx

into airway epithelium and lumens (Chang et al., 1998). IL-8 is known to be the principal chemoattractant for PMNs. *In vitro* neutrophil chemotaxis showed a parallel dose and time profile to epithelial cell secretion of IL-8 in human and monkey tracheobronchial epithelium.

Lymphocyte numbers in BAL fluid have also been shown to increase following ozone exposure. For example, Bassett et al. (1988) noted increased lymphocyte numbers in BAL fluid of rats following 3 days of continuous exposure to 0.75 ppm ozone that were still elevated over controls at 4 days post-exposure. However, continuous exposure of rats to 0.35 ppm ozone for 3 days had no effect on lymphocyte number in BAL fluid, though increased numbers of AM's in BAL fluid were noted (Bassett et al., 1988). Use of an indirect immunofluorescence technique showed that T lymphocytes infiltrate the pulmonary centriacinar regions of mice exposed to 0.7 ppm (20 hours/day) for 4 days (Blevins and Dziedzic, 1990). T lymphocyte numbers had increased by 14 days of exposure and tended to occur in clusters within ozone-induced lesions. As expected, B lymphocyte infiltration (IgM-positive cells) was found to be virtually nonexistent during ozone exposure. In contrast to the immunofluorescence findings, Donaldson et al. (1991) did not find altered proportions of lymphocytes among lavaged leukocytes of rats intermittently exposed to a range of ozone concentrations (0.2-0.8 ppm, 7 hours/day) for up to 4 days.

Mast cell density in lung airways may be an important factor in understanding why persons with asthma are most susceptible to inhaled pollutants. Exposure of mast cell-deficient mice to subchronic and chronic levels of ozone (0.26 ppm) significantly reduced the inflammatory response and bronchiolar epithelial injury compared to mast cell-sufficient mice similarly exposed (Kleeberger et al., 1999).

Sielczak et al. (1983) exposed sheep to 0.5 ppm for 2 hours and then performed a tracheal lavage 24 hours post-exposure. Lymphocyte and mast cell numbers were both increased in tracheal lavage fluid, leading the authors to speculate that the presence of these cell types following ozone exposure could contribute to ozone-induced increased nonspecific airway hyperresponsiveness and susceptibility to allergic IgE-mediated reactions.

#### *A.2.2.4 Interaction with Infectious Microorganisms*

Previous studies described in the last Ozone Review (ARB, 1987) show increased susceptibility to bacterial infection in mice following exposure to ozone in the range of 0.08-0.10 ppm for single 3-hour exposures (Miller et al., 1978a) and 0.10 ppm for long-term exposure (Aranyi et al., 1983). However, acute and sub-chronic ozone exposure in the range of 0.16-0.5 ppm was found to diminish the severity of viral infections.

More recent studies support these findings, in that acute and repeated exposures to low concentrations of ozone result in increased susceptibility to bacterial infection. In rats continuously exposed to various ozone concentrations (0.13 to 1.0 ppm for seven days) and then infected with *Listeria monocytogenes*, reduced clearance of viable *Listeria* from the lungs was related to both a reduced uptake and killing of the bacteria by AM's and a depression in T-/B-lymphocyte ratios

within bronchial lymph nodes (Van Loveren et al., 1988). Defense against *Listeria* respiratory infections depends on natural nonspecific defense mechanisms (AM) and acquired specific cellular immune responses involving lymphocytes. Suppressed ingestion and killing of the bacteria by AM's appeared to be the most sensitive indicator of ozone exposure, occurring at the lowest concentration (0.13 ppm). In rats continuously exposed to 0.75 ppm ozone for seven days and then infected with *Listeria monocytogenes*, pathological alterations in the lungs due to infection were greatly enhanced (Van Loveren et al., 1988). Cohen et al. (2001; 2002) noted no effect in cumulative mortality or lung weights of rats intermittently exposed (4-5 hr/day) to 0.1 or 0.3 ppm ozone for 5 days and subsequently infected with *Listeria monocytogenes*. However, concentration-related effects upon morbidity onset and persistence were induced in the form of a greater degree of disease symptoms (i.e., breathing difficulty, body shivers, encrustation of eyes, diarrhea, and nasal discharge) and a greater bacterial burden in ozone-exposed rats, which was significantly greater compared to controls at 0.1 ppm (Cohen et al., 2001). At 96 hours post-infection, lung burdens of *Listeria* were diminishing in the 0.1 ppm group but not the 0.3 ppm group. The *Listeria*:AM ratios in the 0.3 ppm ozone-exposed rats were increased 96 hours post-infection, indicating that those AM's that were present at the time of infection were either unable to ingest bacteria or may have been incapable of killing bacteria that had been ingested. Examination of AM's recovered from infected hosts indicated that, as a result of exposure to 0.3 ppm ozone, the number of cells actively phagocytizing *Listeria in situ* was decreased in the early stages of infection. Three-week exposure of rats to 0.1 or 0.3 ppm ozone, followed by infection by *Listeria monocytogenes*, had no effect on mortality, lung weights, or *Listeria* lung burdens in the 0.1 ppm group but resulted in increased *Listeria* burdens in the 0.3 ppm group 48 hours post-infection (Cohen et al., 2001; 2002). This finding suggested that adaptation to longer ozone exposures occurred with lower concentrations (0.1 ppm) but that adaptation to higher levels of ozone (0.3 ppm) may not occur as readily.

Gilmour and colleagues (Gilmour et al. 1991, 1993a, 1993b, 1993c) performed a series of experiments in which rodents were exposed to ozone and subsequently infected with bacteria. Continued exposure to 0.5 ppm ozone in mice for 1 or 3 days impaired the intrapulmonary killing of *Staphylococcus aureus* (Gilmour et al., 1991). But with continued exposure for 7 or 14 days, intrapulmonary killing was similar to controls. This trend of an initial suppression followed by recovery has also been reflected in the phagocytic capacity of the AMs (see section A..2.2). In contrast, when *Proteus mirabilis* was used as the challenge organism, ozone exposure had no suppressive effect on pulmonary bactericidal activity (Gilmour et al., 1991). Unlike *S. aureus*, the gram-negative bacteria *P. mirabilis* causes a massive influx of PMNs that provided an auxiliary phagocytic defense to the lungs.

In a comparative study of mice and rats, exposure to 0.4 and 0.8 ppm ozone for 3 hours caused decrements in AM phagocytosis and impaired intrapulmonary bacterial killing of *Streptococcus zooepidemicus* in rats and two strains of mice (Gilmour and Selgrade, 1993). However, fatal infections occurred only in mice.

Exposure of rats to 1 ppm ozone under the same protocol did not result in any fatal infections. The authors also noted that PMN infiltration occurred sooner after infection in rats than in mice and that disappearance of the bacteria in rats corresponded with the PMN influx into the lung. In addition, pretreatment of rats with antineutrophil serum prevented the PMN influx and impaired the inactivation of pulmonary bacteria to a greater extent than did ozone exposure alone. The suppressed phagocytic activity of AMs in ozone-exposed mice was accompanied by increased proliferation of capsulated *S. zooepidemicus*, which prevents the ingestion of the bacteria by AMs and leads to increased severity of infection (Gilmour et al., 1993a).

In addition to these experiments, exposure to 0.4 and 0.8 ppm ozone followed by infection with *S. zooepidemicus* produced greater mortality in 5-week old mice compared to 9-week old mice (Gilmour et al., 1993b). Ingestion and intrapulmonary killing of the bacteria by AMs were reduced in all ozone-exposed mice, but the apparent reduction of AM phagocytosis in younger mice was more marked suggesting they may be more susceptible to pulmonary bacterial infection following ozone exposure. Dormans et al. (1996) carried out experiments to investigate possible age-related effects of ozone in 1, 3, 9, and 18 month-old rats on host resistance to pulmonary *Listeria* infection. While ozone exposed (0.8 ppm for 12 hr, or 7 days for 12 hr/day) groups at 1, 9, and 18 months of age had decreased clearance to *Listeria*, no effect of age on the clearance of the bacteria was observed in control or in ozone-exposed animals.

In a bacterial susceptibility study using a simulated daily diurnal cycle of ozone found in urban regions, mice were exposed to basal levels of ozone for 15 days on which were superimposed 2 daily 1-hour peaks for 5 days/wk and then challenged with *Streptococcus zooepidemicus* (Graham et al., 1987). Increased mortality occurred in one out of two experiments in which mice were exposed to a basal level of 0.05 ppm and spikes of 0.1 ppm. Exposure to a basal level of 0.1 ppm with spikes of 0.3 or 0.5 ppm resulted in greater mortality following infection.

Rats were infected with *Pseudomonas aeruginosa* prior to exposure to 0.64 ppm ozone for 4 weeks to test the effect of ozone on lysosomal levels in AMs (Sherwood et al., 1986). Measurement of lysozyme in AMs *in situ* showed decreased enzyme content in relation to non-infected, ozone-exposed controls, which in turn had less lysozyme content than clean air controls. The authors speculated that ozone elicited an influx of new macrophages that contained lower amounts of lysozyme.

Recent work investigating the effects of ozone on pulmonary viral infections has improved the understanding of the seemingly counterintuitive action of ozone exposure reducing viral infection severity. Mice infected with influenza virus and exposed continuously to 0.5 ppm ozone during the acute phase of infectious lung damage had reduced lung inflammation that was independent of pulmonary virus titers (Jakab and Hmieleski, 1988). It was found that ozone exposure resulted in less widespread infection of the lung parenchyma, concomitant with a reduced antiviral immune response, as shown by reduced numbers of pulmonary T- and B-lymphocytes. This study suggested that redistribution of virus growth in the

lungs and immunosuppressive mechanisms are factors in the reduced viral disease severity in ozone-exposed mice. In a follow-up study, mice infected with influenza virus, then exposed continuously to 0.5 ppm ozone for 30 days did not exhibit an alteration of virus proliferation in the lungs, but experienced about 50 percent less virus-induced alveolitis during ozone exposure (Jakab and Bassett, 1990). However, continued exposure for up to 3 months resulted in a potentiation of post-influenzal alveolitis, which led to greater long-term residual lung damage in exposed mice. It was postulated that the mechanism for the enhanced post-influenzal lung damage was related to impairment of the repair process by ozone following the acute phase of infectious lung injury.

In another related study, Selgrade et al. (1988) exposed mice to a range of ozone concentrations for 3 hr/day for 5 days. Separate groups of mice were infected with influenza virus following each of the individual exposures. Lung wet weights were increased in mice infected after the second ozone exposure at both 1 and 0.5 ppm but not at 0.25 ppm. Increased mortality and reduced survival times occurred only in mice infected on the second day of exposure to 1 ppm ozone. In addition, histopathological and pulmonary function changes were enhanced by 1 ppm ozone exposure in mice infected after the second day of exposure but not after the fifth day of exposure. Virus titers in the lungs of mice infected either after the second or fifth exposure was unchanged compared to controls. It was suggested that lung pathology produced by infection could be enhanced by 1 ppm ozone exposure and that daily repeated exposures beyond the second day may attenuate lung injury due to an adaptation response.

In summary, ozone exposure has been shown to induce changes in all areas of lung host defense, including airway clearance, functions of alveolar macrophages and other immune system cells, and defense against infectious microorganisms. Alveolar, or deep lung airway, clearance of insoluble particles and fibers is reduced by prolonged exposures to an urban pattern of ozone (background of 0.06 ppm with daily peaks of 0.25 ppm) or repeated exposure to 0.3 ppm ozone. Normal development of the mucociliary system was retarded in newborn sheep by short-term, repeated exposure to 1.0 ppm ozone. Whether this developmental effect is permanent and can occur at near-ambient ozone levels is unknown.

The previous ozone review noted that AMs are one of the most sensitive indicators of ozone exposure, showing deficits in host defense capabilities with acute exposures as low as 0.1 ppm. Studies reported in this review have supported these earlier findings. AM phagocytic capacity of inert beads and bacteria, and AM production of superoxide anion and hydrogen peroxide, have been reduced by acute or short-term repeated exposure to concentrations as low as 0.1 ppm. Moreover, recent evidence shows that certain human and murine AM function alterations resulting from ozone exposure are similar.

In addition to reduced phagocytic capacity of bacteria, short-term repeated exposure resulted in greater bacterial burden and increased morbidity and mortality in infected animals at ozone concentrations as low as 0.1 ppm. The lung response to viral infection challenge appears dependent on the timing of

exposure to ozone. The acute phase of viral infection in mice is reduced by subsequent exposure to ozone (0.5 ppm), but continued ozone exposure for up to 3 months resulted in greater long-term lung damage.

As with the inflammatory and permeability effects, continued, repeated ozone exposure results in an attenuation of the ozone-induced effects on host defense. Attenuation of deleterious responses on AM phagocytic function, intrapulmonary bacterial killing, and viral-induced lung injury have been observed with continued ozone exposure. It is also noteworthy that some studies have observed a normal or increased production of superoxide anion by AM's with prolonged exposures to ozone, whereas acute exposure studies have often reported reduced abilities of AM's to produce superoxide anion.

### **A.2.3 Biochemical Effects**

The changes in pulmonary biochemistry observed after ozone exposure are associated with cell damage and death and with increased activities of protective and repair mechanisms. The major types of biochemical changes observed include: changes in the synthesis and content of structural proteins; changes in anti-oxidant enzymes and substances; and changes associated with cell death and inflammation. Such changes can be sensitive indicators of the occurrence, as well as the mechanism, of ozone-associated toxicity.

Studies previously reported in the Ozone Review (ARB, 1987) indicated that ozone exposure increases collagen synthesis rate and collagen content in the lung. Excess accumulation of lung collagen in exposed animals is a hallmark of pulmonary fibrosis and can lead to impairment of lung function. Changes in the collagen synthesis rate were reported to result from acute exposures as low as 0.125 ppm, while morphological changes related to collagen lung content were observed at concentrations as low as 0.25 ppm ozone. However, a few reports employing prolonged exposures to ozone concentrations near ambient levels have not produced changes as severe as would be predicted by simple linear extrapolation from the acute studies reviewed. Thus, a number of recent reports since the first ozone review focused on changes in lung collagen content following long-term ozone exposure.

Using techniques to quantify mRNA concentrations for the major collagen isotypes (type I and type III), Armstrong et al. (1994) observed preferential increased synthesis of type I collagen in rats exposed continuously for 7 days to 1.2 ppm ozone. *In situ* hybridization techniques showed increased  $\alpha_1(I)$  procollagen mRNA in septal tips and at the bronchiolar-alveolar duct junctions of ozone-exposed rats, suggesting that ozone exposure could result in fibrosis in this region of the lung.

In rats exposed to 0.57 ppm ozone for 19 hr/day for 11 days, there was little or no indication of increased proteinolysis or increased collagen production in the lungs (Pickrell et al., 1987a). However, exposure to 1.1 ppm ozone using the same exposure protocol led to increased total lung collagen production one day after exposure but only mild fibrosis in the alveolar duct regions by 2 months after exposure. Rats exposed intermittently (12 hr/day) or continuously (23.5 hr/day)

to either 0.12 or 0.20 ppm ozone were analyzed at 30 and 90 days of exposure for biochemical markers of excess accumulation of lung collagen (Last and Pinkerton, 1997). Assays were performed to test for accumulation of excess 4-hydroxyproline content, a marker for lung collagen, and accumulation of hydroxyypyridinium, a trifunctional collagen crosslink that is a marker for fibrotic collagen. While a trend towards increased lung 4-hydroxyproline content was noted in exposed rats, the increase was not significant at any exposure concentration or any time point. Morphometric analysis of the centriacinar region found significantly increased alveolar tissue density in both 0.12 and 0.20 ppm exposure groups, which was suggestive of increased deposition of lung collagen in this region of the lung. Intermittent exposure to ozone was found to elicit greater lung changes, interpreted as a mild fibrotic response, than did continuous exposure.

Dormans et al. (1999) compared the extent and time course of alveolar duct fibrosis by histochemical staining methods for collagen in rats, mice and guinea pigs continuously exposed to 0.2 or 0.4 ppm ozone for up to 56 days. Exposures to 0.2 ppm ozone for 56 days resulted in alveolar duct fibrosis only in rats and the guinea pigs. However, mice were affected as well after 56 days of exposure to 0.4 ppm ozone. In a follow-up report, continuous exposure of rats to 0.4 ppm ozone resulted in increased collagen content, measured as hydroxyproline concentration in whole lungs, only after 56 days of exposure (van Bree et al., 2001). However, hydroxyypyridinium content was not different from control values. Histological staining for collagen revealed collagen content in ductular septa increasing progressively up to day 56.

A 52-week exposure of rats to 0.50 ppm ozone (20 hr/day) caused mild inflammatory and fibrotic changes in the central acini as well as restrictive changes in ventilatory function parameters (Gross and White, 1987). Functional and inflammatory changes had resolved during a 3-6 month post-exposure period, but some histologic evidence of minimal fibrosis remained. It was suggested that the functional changes immediately after exposure resulted from the underlying inflammatory response rather than from connective tissue deposition. In rats exposed to 0.12, 0.25, or 0.50 ppm ozone 20 hr/day for 18 months, total lung hydroxyproline increased with age in all groups but no dose-related changes were observed (Wright et al., 1988). Lung collagen was about 28% greater in the 0.5 ppm group but was not statistically different from controls.

In long-term exposures, collagen deposited in the lungs of monkeys exposed for up to 1 year to ozone (0.61 ppm, 8 hr/day) was structurally abnormal and characteristic of collagen deposited in fibrotic lungs (Reiser et al., 1987). The abnormal collagen deposition included elevated levels and ratios of difunctional collagen crosslinks immediately after exposure. Lung hydroxyypyridinium was also increased in ozone-exposed monkeys and was still elevated following a 6-month post-exposure period, even though levels of difunctional crosslinks had returned to control levels. This may indicate that collagen synthesis had returned to control levels but the high levels of difunctional crosslinks had matured into

hydroxypyridinium. The results suggest that ozone exposure led to irreversible changes in lung collagen structure.

In rats exposed to an urban diurnal pattern of ozone (13-hour background of 0.06 ppm with an exposure peak rising to 0.25 ppm, returning to background over a 9-hour period, and 2-hour downtime for maintenance) for 78 weeks, electron microscopic morphometry revealed both increased amounts of basement membrane and collagen fibers in proximal alveolar regions (Chang et al., 1992). After a 4-month post-exposure period, the interstitial matrix accumulation of collagen had resolved but the thickening of the basement membrane had not.

Excess stainable collagen was observed in the centriacinar region of rats intermittently exposed (6 hr/day, 5 days/wk) to 0.5 and 1.0 ppm ozone for 20 months (Last et al., 1993b). Rats exposed to 0.12 ppm ozone displayed no detectable centriacinar fibrotic lesions. Measures of increased lung collagen deposition were evident only in females at the two higher exposures. Active synthesis of collagen, as measured by *in situ* hybridization for type I procollagen mRNA, were negative suggesting that the rats were not actively synthesizing and depositing new collagen in their lungs after 20 months of exposure. However, younger rats exposed to 1 ppm ozone for two months did show active synthesis of lung collagen, indicating that attenuation of collagen synthesis occurs in lungs of older rats exposed to ozone (Last et al., 1993b). Boorman et al. (1995) examined other animals in the same study following lifetime and two-year ozone exposure. In agreement with Last et al. (1993b), exposure to 0.5 and 1.0 ppm ozone resulted in evidence of extensive but mild progressive fibrosis in the centriacinar regions, featuring alveolar septa occasionally thickened by eosinophilic fibers (a characteristic of collagen). In contrast, only a few central acini of rats exposed to 0.12 ppm ozone exhibited similar lesions. The degree of fibrosis found in the centriacinar regions following lifetime exposure was more severe than that found at two years. Using the same long-term exposure protocol, Herbert et al. (1996), noted similar lesions in the centriacinar region of mice, with the exception that lifetime exposure did not appreciably increase the severity of the lesions over two-year exposure.

In mice continuously exposed to 0.5 ppm ozone for 3 months, lung hydroxyproline content was elevated only at the 60-day assay period (Jakab and Bassett, 1990). However, ozone exposure of mice infected with influenza A virus showed increased hydroxyproline values at day 30, which continued to increase until the end of exposure at day 120. In a related experiment, mice were infected with influenza A virus and exposed to 0.5 ppm ozone at various times following infection (Jakab and Bassett, 1990). The greatest increase in hydroxyproline content was observed in mice breathing clean air during the acute phase of infection (day 1 to day 9) followed by continuous ozone exposure to day 30. Reversing the treatment, with ozone exposure only during the acute phase of infection, resulted in no increase in lung hydroxyproline content compared to virus-infected mice breathing clean air. It was postulated that ozone-induced potentiation of postinfluenzal fibrogenesis may be due to impairment of the normal repair process following the acute phases of infectious lung injury.

Mautz et al. (2000) performed a detailed analysis of the biochemical events that are believed to precede connective tissue disruption, including changes in connective tissue proteases and protease inhibitors, in the BAL fluid of animals exposed to ozone. Four-hour exposures of rats to 0.4 ppm ozone for one or three days resulted in a substantial increase in the elastase inhibitory capacity of lavage fluid. Moreover, ozone exposure did not result in increased levels of free neutrophil elastase or collagenase in BAL fluid even though exposure was associated with increased numbers of neutrophils. Although this result suggests acute ozone exposure has a beneficial effect on the protease/antiprotease balance, the increase in elastase inhibitory capacity was attributed to increased lung permeability and serum transudation (i.e., increased total protein in BAL fluid) and thus is indicative of lung injury. However, 8-week and 26-week episodic exposures (4-hour exposures, 3 consecutive days/wk) to 0.3 ppm ozone had no effect on the elastase inhibitory capacity, neutrophil count, or total protein of rat lung lavage fluid, indicating adaptation with longer exposures. Similar episodic exposures to 0.15 ppm ozone for 12 and 40 weeks in rats and rabbits also had no effect on these measures of inflammation. Concurrent acute exposures in humans by Mautz et al. (2000) resulted in similar biochemical findings to those of the animal exposure studies, suggesting that humans and animals have similar inflammatory responses to ozone. In an earlier study, Pickrell et al. (1987b) also observed increased antiproteinase activity (trypsin inhibitory capacity and elastase inhibitory capacity) in BAL fluid of rats exposed to 0.5, 1.0, or 1.5 ppm ozone for 48 hours. However, decreased antiproteinase activities occurred in serum and lung tissue at the lower ozone concentrations.

The long-term ozone studies indicate that significant effects related to collagen deposition in the lung does not occur below an ozone concentration of about 0.40 ppm. However, this may be due to the regional or focal nature of the lung injury that may not be detected with biochemical analysis of the whole lung. Therefore, when morphometric findings are considered along with biochemical studies, changes related to lung collagen content may occur with prolonged ozone exposures at concentrations as low as 0.12 ppm. While it is unclear whether functional consequences result from deposition of excess collagen at ambient levels of ozone, irreversible changes in lung collagen structure have been shown in monkeys with prolonged exposure to moderately high levels of ozone (0.61 ppm).

The lung has defenses against oxidant damage, which include the antioxidant enzymes catalase, glutathione peroxidase, and the manganese and copper-zinc superoxide dismutases. These enzymes act in concert by converting superoxide anion to hydrogen peroxide (primarily by the superoxide dismutases), and hydrogen peroxide to water and oxygen (primarily by catalase and glutathione peroxidase). Other enzymes that aid in antioxidant defense are glutathione S-transferase and thioredoxin. A number of antioxidant substances also have roles in lung antioxidant protection; the most commonly studied in association with ozone exposure research include ascorbate (vitamin C), alpha-tocopherol (vitamin E), uric acid, and reduced glutathione.

Short-term acute ozone exposure increases SOD levels in the lung. Rivas-Arancibia et al. (1998) exposed rats to a range of ozone concentrations for 4 hours and measured increased lung copper-zinc SOD (Cu/Zn SOD) levels, at 0.1, 0.2, or 0.5 ppm ozone but not at 1.0 ppm ozone. The lack of a dose-response for SOD induction by ozone was interpreted to be the result of inhibitory actions on enzyme levels at high ozone concentrations.

As reported in the Ozone Review (ARB, 1987), intermittent exposure to 0.45 ppm ozone in rats over several days results in increased SOD activity, suggesting a method of adaptation to oxidant exposure. More recently, Lee et al. (1989, 1990) observed increased SOD activity in whole lung homogenate from rats exposed to 0.45 ppm ozone, but not 0.30 ppm ozone. However, other studies noted that when activity is expressed per gram of lung or per milligram DNA, there is no overall increase in lung SOD activity resulting from ozone exposure (Dubick and Keen, 1983; Jackson and Frank, 1984). These findings have been interpreted to mean that the increased SOD level in the lung of ozone-exposed animals is due to increased number of cells containing SOD, and not due to increased enzyme activity per cell. However, in rats exposed to 0.7 ppm ozone continuously for up to 5 days, total Cu-Zn and Mn SOD lung activity were increased by day 5 and total lung mRNA for Cu-Zn SOD was increased by day 3 (Rahman et al., 1991). The greater concentration of Cu-Zn SOD mRNA suggested that a faster rate of synthesis of Cu-Zn SOD might partly explain the higher anti-oxidant activity in ozone-exposed rats.

In the trachea of rats, intermittent exposure (8 hr/night) to 0.96 ppm ozone for 60 days did not result in altered SOD activity when expressed per gram of lung tissue or per gram of lung protein (Nikula et al., 1988). This finding supports the evidence in the lung that increased SOD activity per cell is not a mechanism of tracheal adaptation to ozone exposure.

However, site-specific studies have shown that intermittent exposure (6 hr/day, 5 days/wk) of rats to both 0.12 and 1.0 ppm ozone for 3 months was associated with an increase in total superoxide dismutase (SOD) activity per mg DNA in the distal bronchioles and in the centriacinar regions of the lung (Plopper et al., 1994). The increased activity was dose-related and resulted in a doubling of SOD activity in rats exposed to 1.0 ppm ozone. Immunolabeling and morphometric techniques revealed that manganese SOD (Mn SOD) increased significantly in AM and epithelial type II cells in centriacinar regions of rats exposed to 1.0 ppm ozone for up to 3 months (Weller et al., 1997). Mn SOD activity in other epithelial cell types was unaltered by prolonged ozone exposure. In contrast, Cu-Zn SOD was markedly reduced in epithelial cells within airways and parenchyma.

Two reports looked at SOD levels in lung homogenates following long-term ozone exposure in rats. Exposure to a daily average concentration of 0.021 ppm ozone (concentration altered between 0 and 0.1 ppm with a mathematic Sin curve for 10 hr/day) for 22 months did not result in changes in SOD levels at termination of the experiment or at intermediate time points (5, 9, 13, and 18 months) (Sagai and Ichinose, 1991). In rats exposed to an urban pattern of

ozone (13-hour background of 0.06 ppm with an exposure peak rising to 0.25 ppm, returning to background over a 9-hour period, and 2-hour downtime for maintenance) for 12 months, SOD activity from whole lung homogenate was unchanged compared to controls (Grose et al., 1989).

One study investigated alterations in catalase activity following ozone exposure. In rats exposed to 0.7 ppm ozone continuously for 5 days, total lung catalase activity was increased on day 5 and total lung mRNA concentration of catalase was increased by day 3 (Rahman et al., 1991).

As reviewed previously (ARB, 1987), ozone-induced increases in glutathione (GSH) enzyme system activity in the lung occur at concentrations as low as 0.2 ppm in rats exposed for 7 days.

Whole lung homogenates from rats continuously exposed to 0.30 or 0.45 ppm ozone for three days showed increased enzyme activity for GSH peroxidase, GSH reductase, and GSH disulfide transhydrogenase (Lee et al. 1989, 1990). Bassett et al. (1988) found increased activity of GSH reductase and GSH peroxidase from whole lung homogenates of rats exposed continuously to 0.75 ppm ozone for 3 days. Levels of these antioxidant enzymes were still elevated on a per lung basis 4 days post-exposure. But when the activities of these enzymes were expressed per milligram DNA, no significant differences were observed immediately following exposure. These findings suggest that the ozone-induced enhancements in the whole lung activities of these antioxidant enzymes could be accounted for by an increase in cell number. Rahman et al. (1991) noted increased activity and higher mRNA concentration of GSH peroxidase in whole lung homogenates of rats exposed to 0.7 ppm ozone for 5 days. This result indicated that increased cellular rates of transcription might partly explain the higher GSH peroxidase activity.

Dormans et al. (1999) compared the extent and time course of GSH enzyme activity in whole lung homogenates of rats, mice and guinea pigs continuously exposed to 0.2 or 0.4 ppm ozone for 3 to 56 days. In all three species a gradual increase of GSH reductase and GSH peroxidase enzyme activity was observed at both ozone concentrations, until a maximum was reached at 56 days of exposure. Mice showed elevated levels of the GSH enzymes by day 3 or 7 of exposure and the highest maximum values above control levels. At both ozone concentrations, significantly increased levels of GSH enzymes in rats were apparent by day 7 or 28 of exposure and by day 56 of exposure in guinea pigs. In animals exposed for only 28 days, the recovery period for enzyme levels to get back to normal was 7 days, though GSH peroxidase levels in mice were still elevated at 28 days post-exposure.

Similar to the findings of site-specific enhancement of SOD activity, Plopper et al. (1994) observed site-specific, concentration-dependent increases in GSH peroxidase and GSH S-transferase activity (units/mg DNA) in central acini of rats exposed intermittently (6 hr/day, 5 days/wk) to ozone for 3 months (0.12 and 1.0 ppm) or 20 months (0.5 or 1.0 ppm). Significant increases in GSH S-transferase activity occurred in small airways (minor daughter bronchi) of rats exposed to

ozone levels as low as 0.12 ppm for 3 months. In another study, GSH levels were expressed as reduced GSH (a cosubstrate for GSH peroxidase and GSH S-transferase); 2-hour exposure to 0.4 ppm ozone resulted only in a airway site-specific reduction in GSH in rat trachea (Duan et al., 1996). Two-hour exposure to 1 ppm ozone resulted in increased GSH levels in distal bronchioles and lobar bronchi. Exposure of rats to 1 ppm ozone for 90 days (6 hr/day, 5 days/wk) increased GSH in most airway levels measured but was significantly increased only in distal bronchioles (164% of control value). In monkeys, microdissection and histochemical techniques showed that site-specific concentrations of reduced GSH varied throughout the airway tree, with the proximal intrapulmonary bronchus having the lowest concentration and the parenchyma having the highest concentration (Plopper et al., 1998; Duan et al., 1996). Acute exposure (2 hours) to 1.0 ppm ozone reduced GSH only in the respiratory bronchiole, whereas exposure to 0.4 ppm increased GSH only in the proximal intrapulmonary bronchus. Reduction of the GSH pool at specific airway levels with acute ozone exposure suggests that ability of epithelium at specific sites to replenish the GSH pool as it is used may be a factor in site-specific ozone-induced injury (Plopper et al., 1998). Ninety-day exposure of monkeys to 1 ppm ozone resulted in a 164% increase in GSH levels in distal bronchioles, but GSH levels were unaltered in other airway subcompartments (Duan et al., 1996). These studies in rats and monkeys indicate that GSH levels in target and nontarget areas of the lung and in susceptible versus less susceptible species are not the primary determinant in the differences observed in ozone toxicity. However, the long-term ozone exposures in the two species indicate that increased GSH levels may be one reason for adaptation of some airway epithelial cells to oxidant damage.

In long-term studies, Grose et al. (1989) measured GSH peroxidase and GSH reductase activities in whole lung homogenates of rats exposed to an urban pattern of ozone (13-hour background of 0.06 ppm with an exposure peak that rises to 0.25 ppm, and returns to the background level over a 9-hour period, and 2-hour downtime for maintenance) for 12 months. Activities of both GSH enzymes were significantly elevated in ozone-exposed rats. Rats exposed to a daily average ozone concentration of 0.021 ppm (concentration altered from 0 ppm to 0.1 ppm daily with a mathematic Sin curve over 10 hours) for up to 22 months did not show changes in GSH reductase, GSH peroxidase, or GSH S-transferase from lung homogenates at termination of the exposure or at intermediate time points (Sagai and Ichinose, 1991).

Alterations of GSH enzymes and reduced GSH levels in BAL fluid following ozone exposure have been investigated. Exposure of rats to 0.8 ppm ozone, 6 hr/day for 1, 3, or 7 days resulted in elevated GSH and GSH peroxidase levels by day 3 in the cellular fraction of BAL fluid (Boehme et al., 1992). The lavaged cells were mainly AMs and it is likely the observed changes reflect changes in AMs. After 7 days of exposure, levels of cellular GSH had returned to control levels while levels of cellular GSH reductase had increased. The extracellular levels of GSH and GSH reductase activity in BAL fluid were elevated after day 7 of exposure. The total GSH (GSH plus oxidized GSH (GSSH), cellular and

extracellular) content of BAL fluid increased about 50% in rats exposed to ozone for 3 or 7 days. In horses, exposure to 0.5 ppm ozone for 12 hours resulted in increased GSH, GSSG, GSH redox ratio (GSSG/GSH + GSSG), and free and total iron in BAL fluid immediately after exposure (Mills et al., 1996). The GSH redox ratio is a sensitive indicator of oxidant injury and showed a significant correlation with the level of pulmonary inflammation. Free iron in the BAL fluid can catalyze the formation of hydroxyl radical and exacerbate or initiate oxidant injury.

Other studies have also shown alterations in GSH enzyme activity in AMs as a result of ozone exposure. AMs lavaged from rats continuously exposed to 0.2 ppm ozone for 11 weeks showed elevated specific activity ( $\mu\text{mol}/\text{min}/\text{g}$  supernatant protein) of GSH peroxidase over controls (Mochitate et al., 1992). The enhancement of peroxidative metabolism was considered an adaptive response to ozone exposure and persisted throughout exposure. Rietjens et al. (1985) observed similar findings, in that 4-day exposure of rats to 0.75 ppm ozone enhanced cellular activities of GSH peroxidase in isolated AMs. GSH peroxidase activity was also increased in whole lung and in isolated type II cell populations of ozone-exposed rats (Rietjens et al., 1985).

In rats exposed to 0.5 ppm ozone intermittently (2.25 hr/day) for 5 days, whole lung GSH increased initially but was within control range the last two days of exposure (Tepper et al., 1989). However, whole lung ascorbate concentrations were elevated significantly on days 3 and 5 of exposure. While the reason for the different time course of response for these antioxidants is unknown, their elevated levels may be related to the observed adaptation of lung function to repeated exposure.

Dormans et al. (1996) carried out experiments to investigate possible age-related effects of ozone on antioxidant enzymes in 1, 3, 9, and 18 month-old rats. Exposure to 0.8 ppm ozone, 12 hr/day for 7 produced no age-related effects on enzymes examined (GSH reductase, GSH peroxidase, alkaline phosphatase and glucose-6-phosphate dehydrogenase) in lung homogenates.

Levels of antioxidant substances (i.e., ascorbate, vitamin E, reduced GSH, etc.) located in the lining layer of the lung airways have shown large species differences that could affect species susceptibility to ozone. For example, BAL fluid ascorbate/protein ratios in rats were 7- to 9- fold higher than in humans and guinea pigs (Slade et al., 1993). However, human BAL fluid had 2- to 8-fold higher GSH/protein and vitamin E/protein ratios than those in BAL fluid from rats and guinea pigs.

Exposure of dogs to 0.2 ppm ozone for 6 hours did not alter ascorbate levels in BAL fluid during exposure or up to 18 hours after exposure (Freed et al., 1999). In guinea pigs exposed to 0.12 or 1.0 ppm ozone for six hours, or 1.0 ppm ozone for 1 hour while exercising, levels of ascorbate and uric acid in BAL fluid and plasma was not altered (Long et al., 2001). In guinea pigs exposed to 0.2, 0.4, or 0.8 ppm ozone (23 hr/day) for 7 days, cells in BAL fluid appear to increase their load of ascorbate, uric acid and GSH following exposure (Kodavanti et al.,

1995b; 1996). Although the increase in GSH and uric acid occurred at all dose levels in an ozone-concentration dependent manner, ascorbate levels were increased only in the 0.2 ppm group (Kodavanti et al., 1996). It was postulated that cellular mechanisms that increase ascorbate levels in response to ozone may have been induced at all concentrations, but at 0.2 ppm, ozone did not react with all the ascorbate, allowing the latter to accumulate. Unlike uric acid and GSH, vitamin E levels were decreased in BAL cells in an ozone-dose-dependent manner.

Levels of ascorbate in BAL fluid increased in rats exposed to 0.5 ppm ozone for either 6 or 23 hr/day over 5 days, but ambient temperature differences did not affect ascorbate levels (Wiester et al., 1996b). However, in rats exposed to ozone continuously or intermittently, levels of uric acid in BAL fluid decreased in a warm ambient temperature (34°C) while uric acid levels in BAL fluid increased or were similar to controls in a cold ambient temperature (22°C). Kirschvink et al. (2002) measured levels of total GSH and uric acid in BAL fluid of calves exposed to 0.75 ppm ozone, 12 hr/day for 7 days. Control levels of the antioxidants were determined prior to exposure (i.e., the calves acted as their own controls). Uric acid levels were increased ten-fold after the first exposure and decreased only slightly during the following days. Total GSH levels increased only about two-fold on day 3 of exposure and was near control levels by day 7. Because measures of ozone-induced inflammation were attenuated by days 3 and 7 of exposure, the authors suggested that increased uric acid levels in lung airways play an important role in antioxidant defense and ozone tolerance.

With prolonged exposure to 0.25 ppm ozone (12 hr/day, for 6 or 14 weeks), BAL fluid levels of ascorbate were elevated while BAL fluid levels of total protein, potassium, lysozyme, uric acid, and vitamin E were unaffected by ozone exposure (Wiester et al., 1996a). A second test measured attenuation of the ozone effect on frequency of breathing with a challenge test that re-exposed rats to 1.0 ppm ozone following the prolonged exposures (Wiester et al., 1996a). A significant correlation was found between ascorbate concentration and the magnitude of adaptation, suggesting ascorbate may play an important role in mechanisms associated with ozone adaptation in rats. Wiester et al. (2000) performed a related adaptation study in mice, exposing the animals to 0.25 ppm ozone (6 hr/day) for 10 days, then challenging them with 1.0 ppm ozone at 2 days post-exposure. Adaptation to ozone's inflammatory effects corresponded with high levels of ascorbate in BAL fluid without significant effects on other antioxidants (i.e., GSH or uric acid). It was proposed that the upward adjustment in the transport of ascorbate into the luminal lining fluid may act as an important first line of defense against ozone exposure (Wiester et al., 2000).

Grose et al. (1989) measured levels of ascorbate and vitamin E in BAL fluid of rats exposed to an urban pattern of ozone for 12 months (13-hour background of 0.06 ppm with an exposure peak rising to 0.25 ppm, returning to background over a 9-hour period, and 2-hour downtime for maintenance). Vitamin E levels were decreased in lung lavage supernatant and unchanged in lavaged cells. However, ascorbate levels in lavaged cells increased by 99%.

Supplementation and deprivation studies with ascorbate and vitamin E have also shown that these antioxidant substances likely have a role in protecting against the effects of ozone in animals (Slade et al., 1989; Kodavanti et al., 1996; Elsayed et al., 1988). In general, the studies indicate that the absence of dietary levels of these antioxidant substances may exacerbate lung injury from ozone inhalation while dietary supplementation of the antioxidant substances has a protective effect against injury from ozone exposure.

Taken together, recent histopathological investigations show that increased levels of anti-oxidant enzyme activity (i.e., SOD and GSH) in response to ozone exposure are site-specific in lung airway epithelium with prolonged exposure, occurring chiefly in regions that are most susceptible to ozone-induced injury. Site-specific increases in anti-oxidant activity have occurred with prolonged exposures of ozone at levels as low as 0.12 ppm. Acute ozone exposure may deplete or enhance airway epithelium of anti-oxidant enzyme activity, depending on ozone concentration and airway level. With regard to GSH levels in airway epithelium, acute exposure to ozone can reduce the GSH pool at specific airway levels, suggesting that the ability of epithelium at specific sites to replenish the GSH pool as it is used may be factor in site-specific ozone-induced injury. This finding may also apply to other antioxidant enzymes as well. Anti-oxidant enzyme activity from whole lung homogenates has been shown to be altered with acute exposure as low as 0.1 ppm ozone, but anti-oxidant activity may be diluted with this method of analysis. In addition, ascorbate levels increase in BAL cells and fluid in response to repeated and prolonged exposures to ozone concentrations as low as 0.2-0.25 ppm and correlate with onset of attenuation to ozone injury. Other results suggest alterations of GSH and uric acid levels in lung lining fluid and cells also play a role in protection from, and adaptation to, ozone-induced injury.

#### **A.2.4 Mutagenic and Carcinogenic Potential of Ozone**

Ozone has been shown to be genotoxic and mutagenic in a variety of *in vitro* and *in vivo* bacterial and animal test systems (Victorin, 1996). However, there are also many published results that are negative for these effects. The extreme reactivity, gaseous nature, and toxicity of ozone present methodological difficulties in many genotoxicity and mutagenicity tests. Nevertheless, recent genotoxicity studies have shown short-term exposures to 0.25-1.0 ppm ozone induce DNA strand breaks in cells recovered in bronchoalveolar lavage fluid (Haney et al., 1999; Bermudez et al., 1999; Bornholdt et al., 2002). However, Bornholdt et al. (2002) could not detect ozone-induced DNA strand breaks in whole lung, suggesting dilution beyond detection limits with whole lung homogenates or that ozone reacts chiefly with lung lining fluid and cells within the fluid. Continuous exposure of guinea pigs to 1 ppm ozone for 72 hours resulted in increased DNA strand breaks in epithelial cells subsequently isolated from the trachea and main bronchi (Ferng et al., 1997).

In one of the most rigorous Ames bacterial mutagenicity studies, Dillon et al. (1992) observed a weak mutagenic response in *Salmonella* strain TA102, but not strains TA100, TA104, or TA98 following 35 minute exposure to ozone

concentrations of 0.02 to 0.5 ppm, both with and without metabolic activation. Strain TA102 is uniquely sensitive to detecting mutations induced by oxygen radicals. However, a concentration-dependent mutagenic effect could not be demonstrated in this strain, possibly due to ozone's cytotoxic action. In earlier reports, no mutagenic effects could be found with the Ames *Salmonella* assay utilizing an ozone concentration of 0.5 ppm (Shepson et al., 1985), or with several concentrations in the range of 0.1 to 2.0 ppm with strains TA100, TA102, or TA104, either with or without metabolic activation (Victorin and Stahlberg, 1988). Cultures of rat tracheal epithelial cells exposed to 0.7 ppm ozone twice weekly for about five weeks exhibited roughly a two-fold increase in the frequency of preneoplastic variants compared with controls (Thomassen et al., 1991). However, single exposures of rat tracheal cells to 0.7 ppm ozone, or two- to four-week intermittent exposures *in vivo* did not induce increases in preneoplastic variants. Exposure of rat tracheal cells to 0.7-0.8 ppm ozone before exposure to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) reduced the transforming potency of MNNG. Exposure to MNNG prior to ozone exposure had no effect on the transforming potency of MNNG. In other *in vitro* studies, 5-minute exposure to 5 ppm, but not 1 ppm, ozone induced neoplastic transformation in mouse fibroblast cultures (Borek et al., 1986; Borek et al., 1989). Bornholdt et al. (2002) exposed Muta<sup>TM</sup>Mice to 2 ppm ozone, 90 min per day for 5 days. No treatment-related mutations could be detected in the *cII* transgene.

The only well-designed carcinogenicity study of ozone indicated that it is weakly carcinogenic in selected rodent species. Two-year and lifetime (30 months) exposure of female B6C3F<sub>1</sub> mice to 0.12 (2-year group only), 0.5 and 1.0 ppm ozone showed an increased induction of alveolar or bronchiolar adenomas and carcinomas at the 1.0 ppm level (Herbert et al., 1996). In male B6C3F<sub>1</sub> mice, there was a statistically significant increase in alveolar/bronchiolar neoplasms at the highest exposure level, but the increase was still within the range of historical controls. An increasing trend for neoplasms with increasing ozone concentration was present in both sexes in both the 24- and 30-month exposure groups. Unique mutations, together with a higher frequency of mutations, were found on the K-ras gene of ozone-induced neoplasms compared to lung neoplasms from controls, suggesting ozone may cause direct and/or indirect DNA damage on the K-ras proto-oncogene of the mice (Sills et al., 1995). In a concurrent study, exposure of F344/N rats to a similar ozone exposure regimen produced no increased incidence of neoplasms at any site, including lung (Boorman et al., 1994).

Other studies that investigated ozone's effect on lung tumor development employed less-than-lifetime exposures. No pulmonary tumors were observed in Syrian Golden hamsters exposed continuously to 0.8 ppm ozone for 6 months (Witschi et al., 1993). Ichinose et al. (1992) did not observe an increase in lung neoplasms in Wistar rats exposed to a mean ozone concentration of 0.05 ppm for 13 months. Hassett et al. (1985b) reported a slight but significant increase in pulmonary adenomas seen grossly in A/J mice following intermittent 6-month exposures to 0.31 and 0.5 ppm ozone. This strain of mice is very susceptible to

lung tumor formation following exposure to some carcinogens, but also has a high spontaneous incidence of tumors. Later analysis of the data indicated that only mice in the 0.5 ppm group had a significant increase in pulmonary adenomas (Mustafa et al., 1988). In another study on A/J mice, exposure to 0.4 or 0.8 ppm ozone for 4.5 months resulted in increased lung adenomas at the 0.8 ppm level (Last et al., 1987). Swiss Webster mice exposed under the same exposure protocol did not show an increase in lung neoplasms. The weakly positive results in A/J mice from both studies should be interpreted with caution due to the abnormally low tumor incidences in their accompanying control groups and the difficulty interpreting the carcinogenicity of ozone in mouse strains with high spontaneous tumor formation (Witschi, 1991; 1988). A reexamination of ozone carcinogenesis in A/J mice found no evidence for carcinogenesis with up to 9-month intermittent exposure (6 hr/day, 5 days/wk) to 0.12, 0.5, or 1.0 ppm ozone (Witschi et al., 1999). Although the average number of tumors per lung was somewhat higher in all mice exposed to ozone than in controls, there was no indication of a dose-response.

In studies investigating co-exposures of ozone with pulmonary carcinogens, F344/N rats were administered 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) during the first 20 weeks of a 2-year exposure to 0.5 ppm ozone (Boorman et al., 1994). Inhalation of ozone did not affect the incidence of pulmonary tumors in rats administered NNK. Male Syrian Golden hamsters administered *N*-nitrosodiethylamine during continuous 6-month exposure to 0.8 ppm ozone showed a marginal reduction in lung neoplasm incidence (Witschi et al., 1993). Exposure of A/J mice to 0.5 ppm ozone concurrently with urethane injections over 6 months resulted in increased lung tumors per animal (Hasset et al., 1985b). In studies investigating the tumor promotion potential of ozone, male Wistar rats administered a single dose of *N*-bis(2-hydroxypropyl)nitrosamine followed by exposure to a mean ozone concentration of 0.05 ppm for 13 months resulted in an increase in lung tumors, which was not statistically significant (Ichinose and Sagai, 1992). Exposure of A/J mice to 0.3 ppm ozone for 6 months following a single injection of urethane did not affect the lung tumorigenic response (Hasset et al., 1985b). In a similar experiment with Swiss Webster and A/J mice, a single injection of urethane was administered one day prior to the start of 0.4 or 0.8 ppm ozone exposure for 4.5 months (Last et al., 1987). Ozone decreased tumor multiplicity in urethane-treated mice in both strains in a dose-dependent fashion, but was significant only in A/J mice. The mouse findings suggest that the sequence of exposure is an important factor during co-exposure to pulmonary carcinogens (Witschi, 1991). When ozone is administered first, it may have a cytotoxic action on previously initiated cells destined to grow tumors, thus preventing tumor development. However, the cell proliferative activity of ozone might expand the cell population at risk to undergo transformation, thus increasing tumor formation when carcinogen administration follows ozone exposure.

In studies investigating the effect of ozone on cancer cell metastasis, infusion of melanoma cells following 12-week intermittent exposure of C57 BL/6 mice to 0.15 or 0.3 ppm ozone did not enhance lung cancer cell colonization (Richters,

1988). In another study, infusion of fibrosarcoma cells following continuous exposure to ozone concentrations as low as 0.1 ppm for up to 14 days showed a significant enhancement in the incidence of lung metastasis (Kobayashi et al., 1987). Maximal enhancement occurred in mice exposed to 0.8 ppm for 1 day.

In summary, ozone has been shown to be genotoxic and mutagenic in some, but not all, *in vitro* and *in vivo* bacterial and animal test systems. The extreme reactivity, gaseous nature, and toxicity of ozone likely present difficulties for these test systems. Lung tumor development studies that employed less-than-lifetime exposures in rats, hamsters and mice were either negative or ambiguous for carcinogenicity. These studies included carcinogenicity experiments with A/J mice, reported to be susceptible to lung tumor formation by some carcinogens. In two-year and lifetime carcinogenicity studies conducted by the National Toxicology Program, ozone was determined to be carcinogenic in female mice, uncertain in male mice, and not carcinogenic in rats. In mice, there was a trend toward increased incidences of lung neoplasms with increasing ozone exposure (0.12, 0.5, and 1.0 ppm), but only female mice exposed to 1.0 ppm ozone exhibited an increased incidence of lung neoplasms over control values. Unique mutations, together with a higher frequency of mutations, were found on the K-ras gene of ozone-induced neoplasms of mice, suggesting ozone exposure leads to DNA damage on this proto-oncogene. Co-carcinogenicity studies with pulmonary carcinogens are negative or ambiguous for ozone acting as a tumor promoter. The accumulated data thus far suggests that ozone is a weak carcinogen at high concentrations (1.0 ppm), at best, and may be related to the extensive pulmonary toxicity associated with these high levels of exposure. Hence, the potential for animal carcinogenicity, and by extrapolation, human carcinogenicity, at ambient air levels is presently uncertain.

### **A.3 Systemic Effects**

Studies presented in the first ozone review (ARB, 1987) showed that ozone can cause effects in organ systems and tissues outside of the respiratory tract. Relatively few of these studies have examined the extrapulmonary effects of ozone at concentrations of 0.5 ppm or less, and the mechanisms of many of these effects were unknown. Due to the reactive nature of ozone, it is unlikely that ozone can directly affect extrapulmonary organs. However, it is likely that ozone reaction products are transported from the lung to affect other organs and tissues.

#### **A.3.1 Liver**

Hepatocytes isolated from rats 48 hours after exposure to 0.5, 1.0 or 2.0 ppm ozone for 3 hours produced significantly more nitric oxide spontaneously and in response to inflammatory mediators (Laskin et al., 1994; 1998). Nitric oxide generation has been implicated in host defense and in tissue injury. Likewise, there was a dramatic increase in hepatocyte protein synthesis. These effects were dose dependent and statistically significant at the two highest ozone exposure levels compared to controls. It was suggested that the release of cytokines into the circulation due to the pulmonary inflammatory effects of ozone

resulted in an acute phase response of the liver to injury. Whether these findings indicate tissue injury to hepatocytes as a result of ozone-induced pulmonary injury is unknown.

Because pulmonary infections by *Listeria monocytogenes* can readily gain access to the circulation and infect other organs, van Loveren et al. (1988) examined livers of rats that were continuously exposed to 0.75 ppm ozone for seven days and then intratracheally infected with *Listeria monocytogenes*. The severity of bacteria-caused liver lesions associated with pulmonary infection of *Listeria monocytogenes* was increased as a result of the exposure to ozone.

### **A.3.2 Hematopoietic System Effects**

In general, the function of the immune system is to protect the body from damage by infectious microorganisms and neoplastic cells. Two types of immune mechanisms can be initiated by inhalation of antigens, including cell-mediated and antibody-mediated (humeral) immune responses. Cell-mediated mechanisms enhance the microbicidal capacity of alveolar macrophages (AM) in defense against intracellular bacteria and generate a class of lymphocytes that are cytotoxic for virus-infected cells. Humoral mechanisms neutralize viruses and microbial toxins, enhance the ingestion of bacteria by phagocytes, and play an important role in defense of the lung against fungal and parasitic infections. Recent immunological investigations have greatly expanded the database on ozone-induced immunological effects and suggest that ozone can impair and/or stimulate the immune system of experimental animals.

The effect of ozone exposure on lymphoid tissue weights and/or cellularity has been investigated. In particular, the thymus, spleen, and mediastinal lymph nodes have been of greatest interest. Continuous exposure to 1 ppm ozone in mice resulted in considerable loss of thymus weight by day 2, which remained depressed throughout the 28-day exposure (Goodman et al., 1989). Continuous exposure to 0.5 ppm ozone for 7 days caused a slight but insignificant reduction in thymus weight. Intermittent (4 hr/day) exposure to 0.5 ppm for 7 days had no effect on thymus weight. Exposure of mice to 0.4 or 0.8 ppm ozone continuously (Fujimaki et al., 1984;1987) or 0.7 ppm ozone for 20-24 hr/day (Dziedzic and White, 1986b; Bleavins and Dziedzic, 1990; Li and Richters, 1991b) resulted in reduced weight and cellular loss in the thymus. Fujimaki et al. (1987) noted that continuous exposure of mice to 0.8 ppm ozone for three days results in reduced lymphocytes in both thymus and blood, though the percentage of T and B lymphocytes remained the same in blood. Continuous exposure of mice to 0.7 ppm ozone resulted in suppressed thymocyte DNA synthesis and the presence of peroxidation products in blood plasma and thymus (Li and Richters, 1991b). Associated studies *in vitro* found that ozone-exposed plasma and serum decreased thymocyte survival and thymocyte DNA synthesis (Li and Richters, 1991b). The authors speculated that circulating lipid peroxidation products resulting from ozone exposure could have toxic manifestations in the thymus. Dziedzic et al. (1990) and Bleavins et al. (1990) observed thymus weights to be reversible by two weeks of exposure, suggesting adaptation to ozone exposure. In contrast, murine thymic weight remained depressed with continuous exposure

to 0.4 ppm ozone for 14 days (Fujimaki et al., 1984) or 0.8 ppm ozone for up to 56 days (Fujimaki, 1989). Lower continuous exposure of mice to 0.3 ppm ozone did not significantly affect thymus weight, though thymocyte numbers were reduced the last two weeks of a three week exposure (Li and Richters, 1991a). Shifts in specific thymocyte subpopulations over the three-week exposure were also noted.

In other lymphoid tissues, mediastinal lymph nodes in mice showed an initial decrease in weight in the first three days followed by a hyperplastic response and increased weight with prolonged ozone exposure (0.7 ppm, 20 hr/day for 4 to 28 days) (Dziedzic and White, 1986b; Bleavins and Dziedzic, 1990). In a companion study, the hyperplastic response was observed to be dose-dependent over a range of ozone levels (0.3, 0.5, or 0.7 ppm, 20 hr/day for 28 days) with apparent significance at the lowest ozone exposure tested (Dziedzic and White, 1986a). Using a similar exposure protocol, Gilmour et al. (1991) observed an initial reduction in the number of cells recovered from the mediastinal lymph nodes after 1 day of exposure to 0.8 ppm ozone (23 hr/day). This was followed by an increase and maintenance of cell number above baseline levels during the second week of exposure. In another study investigating the effects of ozone on the mediastinal lymph nodes, exposure of rats to 0.25 ppm ozone, but not 0.13 ppm ozone, for 1 week significantly increased T/B lymphocyte ratios suggesting a proliferation of T-cells (Van Loveren et al., 1988). The T/B cell ratio was still elevated 5 days post-exposure. Dziedzic et al. (1990) investigated the response of the bronchus-associated lymphoid tissue (BALT) and mediastinal lymph nodes in rats exposed to ozone. Similar to mice, rats exposed to ozone (0.5 ppm for 20 hr/day) for up to 14 days resulted in lymphocyte proliferation in BALT and mediastinal lymph nodes, which peaked on day 3 of exposure.

Altered spleen weight and spleen cellularity has also been observed following ozone exposure. Continuous exposure of mice to 0.3 ppm ozone resulted in lower spleen weights 1 week after exposure but these effects were not significantly different from controls after 2 and 3 weeks of exposure (Li and Richters, 1991a). Lower percentages of specific spleen T lymphocyte cells and decreased spleen T lymphocyte DNA synthesis were noted during the first two weeks of exposure, with subsequent recovery at the end of 3 weeks of exposure. Continuous exposure to 0.8 ppm ozone for 1 or 3 days reduced spleen weights in mice (Fujimaki et al., 1984). However, spleen weights were not significantly different from controls after 7 and 14 days of exposure. Similar results were observed in murine spleen weights during near-continuous (20 hr/day) exposure to 0.7 ppm ozone for 2 weeks (Bleavins and Dziedzic, 1990), and in murine spleen/body weight ratios during 14 day exposure to 0.8 ppm ozone (23 hr/day) (Gilmour and Jakab, 1991). Moreover, Gilmour et al. (1991) noted that the spleen/body weight ratios had increased above basal levels by day 14 in ozone exposed mice. In contrast to these findings, Fujimaki (1989) observed depressed spleen weights in mice exposed continuously to 0.8 ppm ozone for 56 days. In a long-term exposure study, mice continuously exposed to 0.31 ppm ozone (103 hr/week for 6 months) followed by a 5 month post-exposure period had increased spleen weights (Hassett et al., 1985a). However,

histopathological examination revealed no consistent alteration in spleen morphology.

A number of studies investigated the effect of ozone on immune function in the absence of antigenic stimulation. Two areas of study include ozone's effect on natural killer activity and ozone's effect on the blastogenic response of lymphocytes to nonspecific mitogens. Natural killer activity targets neoplastic and virus-infected cells and is considered an immediate defense mechanism or an innate immune response. Natural killer cells are primarily a specific subpopulation of lymphocytes found in lymphoid tissues but may also include other cells such as monocytes and neutrophils, depending on how organ tissue is processed. Whole lung homogenates from rats continuously exposed to 1.0 ppm ozone for up to 10 days exhibited decreased natural killer activity against YAC-1 tumor cell targets 1, 5, or 7 days after the beginning of ozone exposure, but had returned to control levels by the tenth day of exposure (Burlison et al., 1989). Pulmonary natural killer activity was also suppressed at 0.5 ppm ozone, but not 0.1 ppm ozone, following 23.5 hours of exposure. In another study, lung lymphoid cell suspensions obtained from rats continuously exposed to a range of ozone concentrations for 7 days were tested for natural killer activity toward YAC lymphoma cells (Van Loveren et al., 1990). Inhalation exposure to 0.2 and 0.4 ppm ozone resulted in stimulation of natural killer activity, while exposure to 0.8 ppm ozone resulted in suppression of natural killer activity. In mice exposed to 0.8 ppm ozone (23 hr/day), natural killer activity of splenic lymphocytes towards YAC-1 cells was reduced following 1 and 3 days of exposure but was restored by the second week of continued exposure (Gilmour and Jakab, 1991).

Acute exposure of rats to a high level of ozone (1 ppm for 3 hours) did not alter the response of spleen cells to the T-cell mitogens concanavalin A (ConA) and phytohemagglutinin (PHA) and B-cell mitogen *Salmonella typhimurium* glycoprotein (STM) (Selgrade et al., 1990). Exposure of mice to 0.7 ppm ozone (20 hr/day) showed little effect on mediastinal lymph node T-cell responsiveness to mitogenic stimulation with ConA during the first week of exposure (Dziedzic and White, 1986a). However, enhanced reactivity was observed by day 14 of exposure that continued to increase through end of exposure on day 28. Cells obtained from both mediastinal lymph nodes and spleen showed reduced responsiveness to PHA mitogen after 1 day of exposure to 0.8 ppm ozone (Gilmour and Jakab, 1991). However, this effect was abolished by day 3 of continued exposure (23 hr/day) through the end of exposure on day 14. Rat splenic cell responses to T-cell mitogens PHA and ConA and a B-cell mitogen (*Escherichia coli* LPS) were significantly enhanced by 7 days of intermittent exposure (8 hr/day) to 1 ppm ozone (Eskew et al., 1986).

In exposure studies of longer duration, mice exposed to 0.1 ppm ozone (5 hr/day, 5 days/wk for up to 103 days) had suppressed splenic cell responses to T-cell mitogens ConA and PHA, but not to the B-cell mitogen *Salmonella typhosa* LPS (Aranyi et al., 1983). In a long-term exposure study in rats, spleen cells were assessed for response to T-cell (ConA and PHA) and B-cell (STM) mitogens and natural killer cell activity towards YAC-1 cells following exposure to a simulated

urban profile of ozone (Selgrade et al., 1990). Daily exposure for 5 days/wk consisted of a background level of 0.06 ppm for a period of 13 hours, a broad exposure spike rising from 0.06 to 0.25 ppm and returning to 0.06 ppm over 9 hours, and a 2 hour downtime. Ozone exposure had no effect on response to the mitogens or natural killer cell activity at 78 weeks of exposure, or at 1, 3, 13, or 52 weeks of exposure. The authors speculated that the different outcomes between the long-term mouse study (Aranyi et al., 1983) and their rat study (Selgrade et al., 1990) might be due to species sensitivity differences to the immune parameters measured.

Recent studies have examined the effect of ozone exposure on the allergic response to antigenic stimulation. Ozone has been found to have an effect on protective antibody production, in that the oxidant gas appears to suppress non-allergic antibody production in response to an antigenic stimulation that is strongly dependent on TH1 lymphocytes. Suppression of this humoral antibody response by ozone could enhance infectious diseases in the respiratory tract.

Spleen cells collected from mice exposed continuously to 1 ppm ozone exhibited suppressed plaque-forming antibody production (IgM) when subsequently immunized with sheep erythrocytes (Goodman et al., 1989). This decreased T-lymphocyte-dependent immune response was noted for only the first two weeks of a three-week exposure. No consistent change in the secondary immune response (IgM + IgG) to sheep erythrocytes was seen. Continuous exposure of mice to 0.8 ppm ozone for up to 56 days suppressed plaque-forming antibody production (mostly IgM) in spleens when subsequently immunized with sheep erythrocytes (Fujimaki et al., 1984; Fujimaki, 1989). This T-lymphocyte-dependent antigen response occurred in mice exposed to ozone for as little as one day prior to immunization. These findings are similar to those of an *in vitro* human study by Becker et al. (1991), in which human lymphocytes exposed to ozone resulted in suppressed immune response to a T-cell-dependent stimulus but not to a T-cell-independent stimulus.

Immunization of previously ozone-exposed mice with a T-lymphocyte-independent antigen (dinitrophenol) had no effect on plaque-forming antibody production with one day or 56 days of exposure, but appeared to have an enhancing effect on T-lymphocyte-independent antigen stimulation of antibody production with 14 days of exposure (Fujimaki et al., 1984; Fujimaki, 1989). Gilmour et al. (1991) observed splenic suppression of ovalbumin-stimulated lymphoproliferation in mice on days 7-14 of continuous two-week exposure to 0.8 ppm ozone. However, mediastinal lymph node ovalbumin-stimulated lymphoproliferation was unaffected by ozone during the first week of exposure and enhanced by two-weeks of exposure to ozone. In addition, pulmonary ovalbumin-specific IgA and IgG in bronchoalveolar lavage fluid was frequently depressed during days 1-14 of the two-week exposure while the serum antibody titers to ovalbumin antigen were unaffected by any period of ozone exposure.

In a study examining the effect of ozone exposure on a delayed hypersensitivity reaction, mice continuously exposed to 0.8 ppm showed suppressed antibody response to sheep erythrocytes, as measured by footpad swelling (Fujimaki et

al., 1987). Maximal antibody response suppression occurred after 7 days of exposure, but had returned to control levels after 14 days of exposure. Inhibition of T-lymphocyte function by ozone was indicated as the underlying cause.

A synthesis of the hematopoietic system effects of ozone can be made as follows: Four general response patterns have been observed with ozone-induced effects on immunologic endpoints involving prolonged (up to 4 weeks) continuous or near-continuous exposures. First, an initial suppression followed by recovery has been observed with spleen weights, spleen/body weight ratios, thymus weight, pulmonary natural killer and splenic natural killer cell activity, and mediastinal lymph node proliferative response. It should also be mentioned that AM phagocytosis and AM-dependent intrapulmonary bacterial killing fit this pattern of response. Second, a response pattern of initial suppression followed by an increased response has been observed with mediastinal lymph node cell numbers. Third, initial absence of a response followed by increased activity has been observed with mediastinal lymph node and splenic proliferative responses. Finally, a sustained response of thymic atrophy has also been observed. Ozone exposures as low as 0.2-0.25 ppm have resulted in altered immunotoxic effects. However, continuous exposure for up to 1 week was necessary to elicit these effects. In addition, other experimental animal studies have had to employ multi-day continuous or near-continuous ozone exposures at levels in excess of current ambient and peak urban ozone concentrations to demonstrate an immunotoxic effect. In this regard, the long-term study by Selgrade et al. (1990) found no effect on multiple immune parameters when rats were chronically exposed to a simulated urban pattern of ozone.

### **A.3.3 Reproductive and Developmental Effects**

Few pertinent reproductive and developmental studies were available when the first California ozone review document (ARB, 1987) was released. In one previously reviewed study, Kavlock et al. (1979) noted intrauterine toxicity in rats only at high exposure concentrations (1.49-1.97 ppm). Exposure *in utero* to 1.0 or 1.5 ppm ozone continuously during mid- or late gestation (Days 9-12 or 17-20) resulted in reduced neonatal growth rates of the offspring (both gestational periods), delayed eye opening and delayed development of reflexes and responses (late gestation only) (Kavlock et al., 1980).

Female mice were exposed continuously during pregnancy (Days 7-17) to ozone concentrations of 0.4, 0.8 or 1.2 ppm (Bignami et al., 1994). To avoid confounding by postnatal maternal effects, all litters were assigned shortly after birth to foster dams neither treated nor handled during pregnancy. Ozone exposure had no effect on any measures of reproductive performance of dams or several measures of physical and neurobehavioral development in pups. However, postnatal body weight gain in pups at the highest exposure (1.2 ppm) was slightly but significantly depressed. Only a transient, dose-dependent depression in food and water intake and body weight gain was noted in dams early in exposure. Subsequent studies in mice used more prolonged, continuous ozone exposures up to 0.6 ppm from several days before start of pregnancy until either day 17 of pregnancy (Petruzzi et al., 1995b) or weaning of the offspring 3

weeks after birth (Dell'Omo et al., 1995a). In spite of transient depressed dam body weights, both exposure schedules found no deficits in reproductive performance or clear neurobehavioral effects due to ozone exposure. However, combined gestational and postnatal exposure to 0.6 ppm ozone produced long-lasting depressed body weights in pups and attenuation of sex differences in some activities that suggest persistent neural and endocrine changes similar to early stress effects (Bignami, 1996). Using an exposure protocol similar to that of Petruzzi et al. (1995b), exposed offspring were subjected to swimming navigation tests, which are a sensitive indicator for hippocampal damage (Dell'Omo et al., 1995b). With the exception of left-turning preference during swimming navigation, consistent developmental effects were not evident.

The turning preference findings generated interest for tests in handedness following exposure to ozone. Mice were exposed continuously to 0.3, 0.6, or 0.9 ppm ozone from six days before the start of pregnancy until weaning of the offspring 26 days after birth (Petruzzi et al. 1999). Forepaw preference for delivery of food pellets was not statistically significantly different from controls, though there was a tendency for exposed female offspring to show a left paw preference while exposed males exhibited a right paw preference. The offspring were also tested for morphine reactivity to the hot plate. The findings indicated that exposed offspring injected with morphine had a general tendency towards reduced drug sensitivity at the highest concentration (0.9 ppm), but this result was, at best, only suggestive of subtle CNS changes.

In studies using higher ozone concentrations, exposure of pregnant female rats to 1.0 ppm for 12 hr/day during gestation resulted in morphological anomalies of the cerebellum in offspring, including damaged Purkinje cells and a diminished folding pattern over the surface of the anterior lobe (Rivas-Manzano and Paz, 1999). Haro et al. (1993) used a similar ozone exposure protocol in pregnant rats and noted long-lasting sleep disturbances in offspring, including decreased paradoxical sleep duration and inversion of the light-dark cycle.

#### **A.3.4 Central Nervous System and Behavioral Effects**

In studies reported in the last Ozone Review (ARB, 1987), minimally detectable depression in operant behavior and motor activity in rodents was reported to appear at exposure levels as low as 0.12 ppm. These activities decreased further with increasing ozone concentration, but attenuation of the altered response has been observed with continuous exposure of sufficient length. Numerous studies investigating behavioral or central nervous system effects of ozone have been published since. These reports were largely generated as a result of possible evidence for CNS effects in humans (impaired mental performance, complaints of fatigue, lethargy, and headache) exposed to ozone. While many of the ozone effects reported in these studies are indicative of sensory irritation or odor aversion, other investigators suggest that some effects may be the result of ozone-derived products having a direct or indirect effect on the central nervous system.

A transient suppression of drinking behavior was observed in adult rats exposed continuously to 0.2 ppm ozone for seven days (Umezu et al., 1987). In mice exposed continuously to 0.4 ppm ozone, food and water intake also showed a transient decrease (Musi et al., 1994). In the mice exposed to higher levels of ozone (0.8 and 1.2 ppm) an immediate, but transient, increase in certain activities such as rearing and sniffing during the first hour of exposure occurred, suggesting a response to a strong unfamiliar stimulus followed by habituation. Continuous exposures up to 10 days at these three concentrations resulted in a dose-dependent decrease in certain activities (locomotion and wall climbing) but an increase in other activities (grooming), suggesting a process aimed at counteracting the consequences of stress. Overall activity changes due to ozone exposure were interpreted as a consequence of response competition rather than an overall depression (Musi et al., 1994). In a study investigating effects of ozone on isolation-induced aggressive behavior in male mice, Petruzzi et al. (1995a) observed an abatement of aggressive behavior and enhanced fear-associated displays as a result of continuous exposure to 1.2 ppm ozone for 20 days.

Exposure of rats to 0.1 - 0.2 ppm ozone for four hours resulted in long-term, but not short-term, memory deterioration as measured by a passive avoidance test (Rivas-Arancibia et al., 1998). The effect on long-term memory was not dose-dependent over a range of ozone concentrations (0.1-1.0 ppm) but seem to correlate with brain and lung Cu/Zn SOD levels, suggesting that deficits in oxidant defenses result in increased ozone-derived products reaching the brain and affecting learning and memory.

In rats implanted with electrodes to trace EEG and EMG recordings, exposure to 0.1 or 0.2 ppm ozone continuously for 5 days did not result in differences of wakefulness, slow-wave sleep, and paradoxical sleep compared to controls (Arito et al., 1990). However, exposure to 0.5 ppm for 6 hours suppressed wakefulness, and paradoxical sleep at the expense of an increase in slow-wave sleep (Arito et al., 1992). Administration of atropine blocked the ozone-induced decrease in wakefulness and increased slow-wave sleep but did not change the paradoxical sleep effects. Comparable disruptions in sleep patterns were observed in cats exposed to 0.8 ppm, but not 0.4 ppm, ozone for 24 hours (Paz and Bazan-Perkins, 1992). Under similar experimental protocols, 0.35 ppm ozone depressed slow-wave and paradoxical sleep in rats during a 24-hour exposure period (Paz and Huitron-Resendiz, 1996). A dose-dependent increase in serotonin was found in the pontine structures of the rat brain, which was significant at the highest ozone concentration (1.5 ppm). An increased level of serotonin in this area of the brain is known to reduce paradoxical sleep. While ozone would be unlikely to exert a direct effect on these sleep disturbances, it has been suggested that the increased circulation of prostaglandins resulting from pulmonary inflammation may also play a role in sleep-wake regulation in the brain (Paz, 1997). Reaction products of ozone that enter the circulation via the lung and thereby reach the brain have also been implicated in sleep disturbances (Paz, 1997).

Rahman et al. (1992) observed that exposure of rats to 0.25 ppm ozone for 5 days resulted in an increased concentration of thiobarbituric acid-reactive material, indicative of lipid peroxidation, in brain tissue. Levels of the peroxide scavengers catalase and GSH peroxidase were also elevated in brain tissue.

### **A.3.5 Hematology and Serum Chemistry**

In the previous Ozone Review (ARB, 1987), ozone was reported to have a variety of effects on red blood cells (RBC), such as increased osmotic fragility, decreased survival, Heinz body formation, morphological changes, and decreased levels of acetylcholinesterase and reduced GSH. Some of these effects appear to begin at ozone concentrations as low as 0.12 ppm. However, exposure of rabbits to 1 or 3 ppm ozone had no effect on the oxygen delivery capacity of RBC's, including oxyhemoglobin affinity, heme-oxygen binding site interaction, and red cell 2,3-diphosphoglycerate concentrations (Ross et al., 1979). Nor did continuous exposure to 0.8 ppm ozone for 7 days lead to altered levels of hemoglobin, methemoglobin or reticulocyte counts in rats (Chow and Kaneko, 1979). In addition, biochemical measures of RBC status, including levels of glucose-6-phosphate dehydrogenase, catalase, SOD, and thiobarbituric acid reactants were unaffected by ozone exposure. However, increased levels of GSH peroxidase, pyruvate kinase, and lactate dehydrogenase were observed in RBC's of ozone-exposed rats. These changes could be related to enzyme activation and/or leakage of enzymes from damaged lungs. The sequestering of old or damaged RBC's in the spleen may account for the mostly negative results. Increased spleen weights were observed in mice continuously exposed to 0.31 ppm ozone (103 hours every other week) for 6 months, followed by a 5 month post-exposure period (Hassett et al., 1985a). However, histopathological examination revealed no consistent alteration in spleen morphology. It was suggested spleen weight was indirectly affected by circulating ozone-damaged blood cells.

The early burst-forming erythroid progenitor (BFU-E) in bone marrow was found to be increased in mice for the first two weeks of a three-week continuous exposure to 1 ppm ozone (Goodman et al., 1989). Continuous, but not intermittent (4 hr/day), exposure to 0.5 ppm ozone for one week also resulted in an increase in BFU-E. The changes in BFU-E do not appear to be related to reduced food and water intake of exposed mice; consistent changes in levels of other measured blood cell progenitors did not occur.

In the serum, there is some evidence that exposure of rats and guinea pigs to high concentrations of ozone (1 ppm or greater) results in increased cholesterol (Mole et al., 1985; Vaughan et al., 1984). However, there were conflicting species-specific results with respect to ozone's effect on triglyceride levels (elevated in guinea pigs; depressed in rats). Thiobarbituric acid-reactive substances, an indicator of peroxidation products, were detected in the blood plasma and thymus of mice exposed to 0.7 ppm ozone for 3 days (Li and Richters, 1991b). This level of exposure also had adverse effects on thymus tissue. In associated studies, ozone-exposed plasma and serum were found to be toxic to thymocytes *in vitro* and suppressed DNA synthesis, suggesting that

ozone inhalation induces harmful intermediates that could reach the thymus via the circulation and exert a toxic effect (Li and Richters, 1991b). Thiobarbituric acid has also been found to be higher in human blood in persons who visited Mexico City, where air pollution is characterized by high levels of ozone (Hicks et al., 1996).

### **A.3.6 Cardiovascular Effects**

Cardiopulmonary measurements in rats (blood gases, pH, blood pressure) were not significantly affected by 1.0 ppm ozone exposure for 135 minutes except for a slight decrease in  $p\text{CO}_2$  (Tepper et al., 1990). In rats implanted with electrodes for ECG recordings, continuous exposure to 0.1 or 0.2 ppm ozone for 5 days resulted in decreased heart rate and increased prevalence of bradyarrhythmic episodes that was dose-dependent and statistically significant at the 0.1 ppm level (Arito et al., 1990). No effects on sleep-wakefulness or circadian rhythm were noted and habituation to the cardiac effects occurred by day 3 or 4 of exposure. The adaptive response of heart rate effects to intermittent ozone exposure (8 hr/day) was also observed in rats exposed to 0.1 ppm for 4 days (Iwasaki et al., 1998). Young rats (4 or 8 weeks old) exposed to 1 ppm ozone for 3 hours exhibited a smaller depressant effect on heart rate and mean arterial blood pressure and caused fewer bradyarrhythmic episodes compared to older rats (11 weeks old) (Uchiyama et al., 1986). Gender differences to the cardiac effects of ozone were not apparent. Exposure of elastase-treated emphysematous rats to 0.5 ppm ozone for 6 hours or continuously to 0.2 ppm ozone for 4 weeks did not increase susceptibility for cardiac responses (i.e., heart rate or mean arterial blood pressure) (Uchiyama and Yokoyama, 1989). In rats exposed to 0.5 ppm ozone, atropine prevented ozone-induced bradycardia (Arito et al., 1992). It was suggested that enhanced cardiac parasympathetic nerve activity resulting from ozone inhibition of cholinesterase activity in the vagal nerve terminals of the heart produced the bradycardia, which was blocked by atropine. Watkinson et al. (1993; 1995) observed significant decreases in heart rate in ozone-exposed rats that was dependent on the temperature at which exposure was conducted and on length of ozone exposure. In concentration-response experiments, 2-hour exposure to ozone concentrations as low as 0.37 ppm significantly decreased heart rate (Watkinson et al., 1993). Cool ambient temperatures (22°C) resulted in a greater magnitude and duration of decreased heart rate in rats exposed to 0.5 ppm ozone continuously or intermittently (6 hr/day) (Watkinson et al., 1995). However, adaptation to both exposure protocols occurred by day three of exposure.

Reaction products of ozone that enter the circulation via the lung and reach the heart have been implicated in cardiac injury. Rahman et al. (1992) observed an increased concentration of thiobarbituric acid-reactive material (an indicator of lipid peroxidation) in heart tissue of rats exposed continuously to 0.25 ppm ozone for 5 days. Elevated levels of the peroxide scavengers catalase and GSH peroxidase were also observed in the hearts of ozone-exposed rats. Examination of heart tissue revealed evidence of extracellular and intracellular edema in ozone-exposed rats.

### **A.3.7 Thermoregulatory Effects**

Rats exposed to 0.6 or 0.8 ppm, but not 0.2 or 0.4 ppm, ozone exhibited a decrease in rectal temperature during the third hour of a 3 hour exposure (Mautz and Bufalino, 1989). In rats exposed to 0.8 ppm ozone, the decline in rectal temperature progressed with the decline in minute ventilation and oxygen consumption, beginning at about 60 min into the exposure. Similar to the heart rate effects, Watkinson et al. (1993; 1995) observed significant decreases in body temperature in ozone-exposed rats that was dependent on the temperature at which exposure was conducted and the length of ozone exposure. Significant decreases in core body temperature occurred at acute ozone exposures (2 hours) as low as 0.37 ppm (Watkinson et al., 1993). Cool ambient temperatures (22°C) resulted in a greater magnitude and duration of decreased body temperature in rats exposed to 0.5 ppm ozone continuously or intermittently (6 hr/day) and adaptation to both exposure protocols occurred by day three of exposure (Watkinson et al., 1995). Body temperature was also diminished in rats exposed intermittently (8 hr/day) to ozone concentrations of 0.3 and 0.5 ppm, but exhibited adaptation by the end of the 4-day exposure (Iwasaki et al., 1998). Intermittent exposure to 0.1 ppm ozone had no effect on body temperature of rats. Interestingly, guinea pigs do not appear to demonstrate a hypothermic response during exposure to 1.0 ppm ozone for 2 hours (Campen et al., 2000).

Thermoregulatory control is generally more labile among rodents than other mammals such as dogs and humans. Rodent species commonly exhibit heterothermy as an adaptation to thermal, hydric, and nutritional environmental variation. Both the heart rate and thermoregulatory effects on rodent species resulting from ozone exposure may be more of a physiological response than a toxic effect (Watkinson et al., 1993; Watkinson et al., 2001). A physiological response implies a temporary change or resetting of functional parameters that may serve to attenuate overall toxicity while a toxic effect implies a harmful change. This premise is largely based on the finding that heart rate and thermoregulatory effects are not unique to ozone exposure, but occur in rodents following exposure to a variety of toxic compounds. In addition, it is unclear how relevant these ozone-related responses in rodents are compared to larger mammals and humans. Significant heart rate and thermoregulatory responses that occur in rodent species but have not been reported in humans suggest that these effects may not be reliable for predicting animal-to-human extrapolations resulting from ozone exposure.

In summary, the highly reactive nature of ozone likely precludes a direct action on extrapulmonary tissues. Potential ozone reaction products have been detected in blood plasma following ozone exposure and have been implicated in extrapulmonary tissue injury. Release of cytokines as a result of ozone-induced pulmonary injury has also been proposed as a potential source of extrapulmonary tissue injury. The immune system, which protects the body from damage by infectious microorganisms and neoplastic cells, can be affected by ozone exposure. Ozone exposures as low as 0.2-0.25 ppm have resulted in immunotoxic effects on T-cell lymphocyte function and immune system organs,

including the spleen and thymus, but generally require continuous or near-continuous multi-day exposures to achieve an effect. A long-term study mimicking urban ozone exposures (daily spikes of 0.25 ppm) was negative for immune effects. Recent developmental studies in rodents require continuous exposures of 0.6 ppm or greater to elicit an effect. Neurobehavioural developmental effects at equivalent or higher ozone concentrations have yielded ambiguous or negative results. Ozone has been shown to alter bone marrow erythroid progenitor formation. But similar to developmental effects, require multi-day continuous exposure at high ambient levels (0.5 ppm) to elicit an effect. Central nervous system (CNS) and behavioural effects have been recorded at ozone concentrations as low as 0.1-0.2 ppm but are probably indicative of sensory irritation or ozone-mediated products having a direct or indirect effect on the CNS. Cardiac effects, including slowed heart rate and bradyarrhythmic episodes were noted in rodents at ozone levels of 0.1 ppm. These effects were transient and likely related to the labile thermoregulatory control in the experimental rodent species. These ozone-induced thermoregulatory effects have not been reported in humans and may not be relevant for animal-to-human toxicity extrapolation.

#### **A.4 Interactions of Ozone with Other Pollutants**

This section summarizes the interactive effects of ozone exposure in combination with other air pollutants at near-ambient concentrations, relative to ozone exposure alone. Since most people are exposed to several air pollutants simultaneously or sequentially, experimental studies that reproduce these complex interactions can represent more realistic environmental conditions than studies with ozone alone. Pollutants can interact toxicologically in three basic modes: additive, more than additive (synergistic), or less than additive (antagonistic). Potentiation is a sub-classification of synergism and refers to a situation in which the response to a mixture is greater than the sum of the responses to individual components, only one of which produced a response different from control when administered alone. While antagonism implies lesser risk, some antagonistic interactions may increase the risk of disease through diminished protective or reparative abilities.

The major air pollutants that have been studied in combination with ozone include sulfur oxides (i.e., sulfuric acid, sulfur dioxide, sulfates), nitrogen-containing pollutants (i.e., nitrogen dioxide, nitric oxide), and particulate matter, including complex mixtures containing numerous pollutants.

##### **A.4.1 Ozone and Sulfur Oxides**

Inhalation studies with sulfur oxides, such as sulfuric acid, in the form of respirable aerosols have often exhibited a lack of toxicity to lungs at ambient levels. However, previous studies reviewed (ARB, 1987) have shown a synergistic interaction between sulfur oxide aerosols and ozone at environmentally relevant concentrations. In particular, Warren et al. (1987) noted increased total lavage protein following exposure of rats to 0.2 ppm ozone for 3 days in combination with 0.1, 0.5, or 1.0 mg/m<sup>3</sup> sulfuric acid aerosol when

compared to levels following exposure to 0.2 ppm ozone alone. In addition, combined ozone and acid sulfate aerosol exposures have shown synergistic increases in lung collagen synthesis in rats at similar ozone concentrations (Warren et al., 1986).

It has been postulated that a sulfur oxide-induced shift in pH of the alveolar milieu (intracellularly or extracellularly) increases the reactivities of free radicals generated by ozone interaction with the lung fluid lining and epithelium, resulting in a synergism of toxicologic effects.

Intermittent exposure (5 hr/day) of sheep to a combination of 0.3 ppm ozone and 3 ppm sulfur dioxide (SO<sub>2</sub>) for three days resulted in a 40% depression of tracheal mucus velocity immediately after exposure (Abraham et al., 1986). Tracheal mucus velocity was still depressed 24 hours after exposure (25% depression). Tracheal ciliary beat frequency was unaffected by coexposure to the pollutants, though this may have been a result of the *in vitro* measurement technique employed. While this study did not include exposures of the sheep to individual pollutants, in earlier work these researchers observed depressed airway mucociliary clearance in sheep exposed to 1.0 ppm ozone, but not 0.5 ppm ozone, for 2 hours (Allegra et al., 1983; Abraham et al., 1980). The authors also note that previous studies with SO<sub>2</sub> alone at levels of  $\leq 5$  ppm had shown no effect on mucociliary clearance.

Long-term exposure of rabbits to sulfuric acid (0.125 mg/m<sup>3</sup>), ozone (0.1 ppm) and their combination for 2 hr/day, 5 days/wk for up to one year accelerated mucociliary clearance in rabbits exposed to sulfuric acid or to the mixture (Schlesinger et al., 1992a). However, clearance rates became progressively slower in all treatment groups during a six-month post-exposure period. The slower post-exposure clearance suggests an attempt to reach a new level of homeostasis during prolonged irritant exposures. However, it is unclear if this represented a permanent alteration. A synergistic increase in bronchial secretory cell number occurred at four months in rabbits exposed to the mixture, but the response became attenuated with continued exposure. The characteristics of cells recovered in bronchoalveolar lavage (BAL) fluid showed no difference between treatment groups in total cell count or in the viability of recovered cells.

Chen et al. (1991) developed an exposure protocol that simulated some human exposure conditions, in that acid aerosol exposures precede ozone exposure. Exposure of guinea pigs to 0.084 mg/m<sup>3</sup> sulfuric acid layered on ultrafine zinc oxide (ZnO) particles for 1 hour, with subsequent 1-hour exposure to 0.15 ppm ozone, produced more than additive reductions in vital capacity and diffusing capacity than exposure to the pollutants alone. Sulfuric acid layered on a metal oxide, as produced in the smelting of metals and from combustion of coal with high sulfur content, are known to be more bioactive than pure sulfuric acid mist alone. In guinea pigs acutely exposed for 1 hour to 0.3 mg/m<sup>3</sup> pure sulfuric acid mist, subsequent exposure to 0.15 ppm ozone for 1 hour did not produce additional change in pulmonary function. In a second exposure regimen, guinea pigs were exposed to 0.024 mg/m<sup>3</sup> sulfuric acid layered on ZnO particles for 3 hr/day for 5 days followed by 1 hour exposure to 0.15 ppm ozone on day 9 (Chen

et al., 1991). This exposure regimen induced reductions in lung volumes and diffusing capacity that were not seen in animals receiving exposures to either ozone or sulfuric acid-layered ZnO alone. The results show that single or repeated exposures to ambient and near-ambient levels of surface-layered sulfuric acid aerosols can sensitize guinea pigs to subsequent exposure to ambient level ozone.

El-Fawal et al. (1995) examined the ability of 3-hour exposures to ozone and ozone-sulfuric acid mixtures to induce nonspecific airway hyperresponsiveness in rabbits. Using an acetylcholine challenge model, exposure to mixtures of 0.1-0.6 ppm ozone and 0.05-0.125 mg/m<sup>3</sup> sulfuric acid aerosols resulted in a general antagonism of bronchial responsiveness compared to ozone alone. Both ozone alone and sulfuric acid alone (based on a previous study by El-Fawal et al. (1994)) induced nonspecific airway hyperresponsiveness to acetylcholine, but their interaction appeared to reduce the effect of both pollutants. The authors had no explanation for this antagonistic effect, but noted that other studies have observed the toxicologic interactions of ozone and sulfuric acid to be highly endpoint specific.

In an acute exposure assessment of inflammatory responses to sulfuric acid-ozone interactions, rabbits were exposed for 3 hours to sulfuric acid aerosol (0.050, 0.075, or 0.125 mg/m<sup>3</sup>), ozone (0.1, 0.3, or 0.6 ppm), or their combination, following which BAL was performed (Schlesinger et al., 1992b). None of the exposures altered the total number or types of cells recovered from BAL fluid. Phagocytic activity of macrophages was depressed at the two highest acid levels and at all levels of ozone. However, the magnitude of the pollutant interaction generally appeared to be independent of the concentration of either pollutant in the mixture and was considered antagonistic due to a less than additive response. Zymosan-stimulated superoxide production in macrophages was not affected by ozone exposure and was depressed by the two highest levels of sulfuric acid. However, antagonistic interaction was observed to mixtures of 0.075 or 0.125 mg/m<sup>3</sup> acid with 0.1 or 0.3 ppm ozone. In the assessment of tumor necrosis factor secreted by stimulated macrophages, a synergistic interaction of increased activity was seen following mixtures of 0.125 mg/m<sup>3</sup> acid with 0.3 or 0.6 ppm ozone. The authors concluded that the type of interaction that occurs between sulfuric acid and ozone depends upon the endpoint and that the magnitude of the interaction was not always related to the exposure concentrations of the constituent pollutants.

Short exposure (4 hr/day for 2 days) of rats to the combination of 0.6 ppm ozone and 0.5 mg/m<sup>3</sup> ultrafine sulfuric acid aerosol (mass median diameter = 0.06 microns) resulted in a synergistic increase in volume percentage of markedly or severely injured parenchymal tissue when compared to ozone exposure alone (Kimmel et al., 1997). In contrast, combined exposure to ozone and 0.5 mg/m<sup>3</sup> fine sulfuric acid aerosol (mass median diameter = 0.3 microns) did not result in a synergistic effect. A synergistic interaction between ozone and fine sulfuric acid, but not ultrafine sulfuric acid, was observed for cellular proliferation in the periacinar region. No differences were noted for pulmonary function parameters

between the ozone and either acid groups. Effects from exposure to fine or ultrafine sulfuric acid alone for all endpoints were similar to controls. In contrast to ozone, patterns of aerosol deposition are strongly influenced by aerosol droplet size. The known differences in regional deposition patterns of fine and ultrafine sulfuric acid aerosols is thought to account for some of the differences in the interactive effects of the aerosols with ozone.

In rabbits exposed to sulfuric acid (0.050 mg/m<sup>3</sup>), ozone (0.6 ppm), or their combination for 3 hours, a synergistic effect of the combination was observed on intracellular pH regulatory mechanisms of alveolar macrophages (AM), while the same pollutant mixtures at higher concentrations (0.125 mg/m<sup>3</sup> sulfuric acid and 0.6 ppm ozone) produced an antagonistic effect on the resting intracellular pH of the AMs (Chen et al., 1995). Thus, it was suggested that the interaction between ozone and sulfuric acid on intracellular pH regulatory mechanisms of AMs is dependent on the concentration of the pollutant mixtures.

Lung biochemical and structural responses were examined in rats exposed to either 0.12 or 0.20 ppm ozone, 20, 100, or 150 ppm sulfuric acid aerosol (0.4-0.8 micron diameter), or their mixtures for up to 90 days (Last and Pinkerton, 1997). Both continuous and intermittent exposures (12 hr/day) were used. The ozone/sulfuric acid mixtures did not affect the extent or magnitude of the morphometric changes of the alveolar duct induced by ozone-alone exposures. A trend towards increased lung 4-hydroxyproline content in rats exposed to ozone was noted, with or without sulfuric acid aerosol, in the intermittent exposure groups, but not in the continuously exposed groups. Sulfuric acid alone exposures produced no changes on any biochemical or morphometric parameters measured. The ozone/sulfuric acid mixtures did not exhibit synergistic interactions after 90-day exposures in rats at concentrations that previously showed synergistic interactions with acute exposure (Warren and Last, 1987). It was suggested that the synergistic interactions in the acute experiments represented reversible responses of the lung to injury and that prolonged (90-day) exposure resulted in an adaptive response with no indication of a synergistic interaction.

#### **A.4.2 Ozone and Nitrogen-Containing Pollutants**

Ozone and nitrogen dioxide (NO<sub>2</sub>) are the two most common oxidant air pollutants in photochemical smog. Therefore, numerous animal studies have investigated the interactive effects of exposure to ozone and NO<sub>2</sub>. Due to ozone's greater oxidant potency relative to NO<sub>2</sub>, ozone is often the driver of pulmonary effects. Estimates of the relative effects of ozone and NO<sub>2</sub> have shown that ozone can cause 15- to 20-fold greater lung injury than NO<sub>2</sub> at the same concentration. Thus, the relative contribution of ozone and NO<sub>2</sub> and the resulting exposure ratio is significant for the ensuing pulmonary injury. However, previous animal exposure studies reported a synergistic interaction between the two oxidant gases. Ozone and NO<sub>2</sub> are known to react chemically to form higher oxides of nitrogen that may be more reactive in lung tissue, though these chemical products and their observed toxicologic responses have not been fully

determined. The formation of nitric acid vapor in the lung is also thought to play a role in ozone-NO<sub>2</sub> synergism in causing lung injury.

In a study of time-concentration (C x T) relationships, rats were exposed to mixtures of ozone (0.2-0.8 ppm) and NO<sub>2</sub> (3.6-14.4 ppm) for 6-24 hr/day for three days using four different protocols in which the C x T product was held constant (Gelzleichter et al., 1992a). Responses were quantified by changes in BAL cells and protein. The response to the combined exposure was additive at the low dose rate (0.2 ppm ozone, 3.6 ppm NO<sub>2</sub>) with an exposure duration of 24 hours. The response of rats to the combined exposures at higher dose rates with exposure durations of 12, 8 and 6 hours were considered synergistic, though the threshold for synergism was dependent on the biological endpoint measured. The interaction between ozone and NO<sub>2</sub> appeared to be concentration-dependent, so that the responses were disproportionately greater at the higher concentrations (higher dose rates) of these gases. At the highest dose rate (0.8 ppm ozone, 14.4 ppm NO<sub>2</sub>), sequential exposure, as opposed to concurrent exposure, resulted in additive rather than synergistic toxicological effects (Gelzleichter et al., 1992b). This finding suggested a substantial chemical reaction occurs between ozone and NO<sub>2</sub> and generates a highly reactive species that is at least partly responsible for the synergistic effects. In addition, when the concentration of NO<sub>2</sub> is held constant at 14.4 ppm, the threshold for synergism with ozone co-exposure can be as low as 0.2 ppm. A similar time-concentration relationship study using the same exposure protocol quantified effects in lung epithelium using a cumulative cell labeling technique of DNA-synthesizing cells (Rajini et al., 1993). There was a greater than additive (synergistic) airway response to the ozone/NO<sub>2</sub> mixture for the three higher dose rates in the large airways (0.4 ppm ozone + 7.2 ppm NO<sub>2</sub> for 12 hr/night; 0.6 ppm ozone + 10.8 ppm NO<sub>2</sub> for 8 hr/night; 0.8 ppm ozone + 14.4 ppm NO<sub>2</sub> for 6 hr/night), and for the highest dose rate in the peripheral airways. It was suggested that this synergistic response could be due to different cell populations being targeted by each of the gases.

Bhalla et al. (1987) investigated bronchoalveolar mucosal permeability after 2-hour exposures of resting and exercising rats to ozone (0.6 ppm), ozone (0.6 ppm) + NO<sub>2</sub> (2.5 ppm), or NO<sub>2</sub> (6 and 12 ppm). Exposure to ozone + NO<sub>2</sub> at rest increased bronchoalveolar permeability, but was not different from exposure to ozone alone. However, exposure to ozone + NO<sub>2</sub> during exercise led to significantly greater permeability than did exposure to ozone alone during exercise. Only exposure to 12 ppm NO<sub>2</sub> alone during exercise led to increased permeability. In another study examining the effect of exercising rats and exposure to the oxidant gases, mixtures of ozone (0.35 or 0.6 ppm) with NO<sub>2</sub> (respectively 0.6 or 2.5 ppm) doubled the level of focal lung injury produced by ozone alone in resting exposures to the higher concentrations and in exercising exposures to the lower concentrations (Mautz et al., 1988). Exposure durations were 3 or 4 hours. Exercising rats exposed to NO<sub>2</sub> alone (0.6 ppm level only) did not result in increased lung injury.

Exposure of rats and rabbits to 0.15 ppm ozone, 0.05 mg/m<sup>3</sup> nitric acid, or the mixture for 4 hr/day, 3 days/wk, for 12 or 40 weeks did not alter BAL fluid levels of total protein or elastase-like activity in any group (Mautz and Nadziejko, 2000). The negative results were attributed to the low level of ozone used and oxidant adaptation with repeated exposure.

Graham et al. (1987) used a bacterial infectivity model to determine the response of mice to NO<sub>2</sub> when combined with ozone. Animals were exposed to basal levels of the two gases for 15 days on which were superimposed 2 daily 1-hour spikes of the gases. The quantified response was mortality due to Streptococcus infection. A significant synergistic response was recorded at the intermediate exposure level (baseline of 0.5 ppm NO<sub>2</sub> with peaks of 1.0 ppm NO<sub>2</sub> and a baseline of 0.05 ozone with peaks of 0.1 ppm ozone), as well as the highest level. Exposure to the gas combination at the lowest level (baseline of 0.05 ppm NO<sub>2</sub> with peaks of 0.1 ppm NO<sub>2</sub> and a baseline of 0.05 ozone with peaks of 0.1 ppm ozone) did not increase mortality.

In a study by Last et al. (1993a), exposure of rats to a mixture of 0.8 ppm ozone and 14.4 ppm NO<sub>2</sub> for 6 hr/day resulted in severe progressive pulmonary fibrosis and 40% mortality by 90 days. Marked increases in collagen content and epithelial injury, including interstitial thickening with stainable collagen and inflammatory cell infiltrate, were observed in lung parenchyma. Inhalation of ozone and NO<sub>2</sub> alone at these same concentrations produced lesser degrees of histological change in the rats and no mortality. A follow-up study was conducted using the same exposure model to examine the pulmonary fibrotic process at the gene level (Farman et al., 1999). High levels of messenger RNA for procollagen types I and III were observed only in central acini of rats exposed to the oxidant mixture; the pulmonary injury extended twice as far into the acini with the combined exposure. In addition, the severity of lesions in rats exposed to the mixture increased over time, indicating that exposure to the combined gases results in progressive pulmonary fibrosis. Exposure to the individual gases demonstrated lessened severity of lesions over time.

In a related study, Ishii et al. (2000) continuously exposed rats to an ozone/NO<sub>2</sub> mixture that was half the concentration (0.4 ppm ozone and 7 ppm NO<sub>2</sub>) and twice the cumulative dose as that used by Last et al. (1993a) for a period of 90 days. Interstitial fibrosis and alveolar collapse in the lungs were apparent by day 90. However, no rats died during exposure and the degree of histologic changes was mild compared to the study by Last et al. (1993a) (see above). Similar to the findings of acute lung damage by Gelzleichter et al. (1992a), chronic pulmonary responses appear to be more dependent on the concentrations of oxidants than on the cumulative doses. In other findings by Ishii et al. (2000), the development of early pulmonary events (i.e., pulmonary inflammation, adaptation, and pulmonary fibrosis) is consistent with the events observed by Chang et al. (1992) in which rats were chronically exposed to an urban pattern of ozone. Lung collagen content was unchanged at day 45, but elevated to 1.7 and 2.0 times that of controls on days 60 and 90, respectively. Increased lung collagen content coincided with AM activation to produce tumor necrosis factor, a cytokine that

may play a role in regulation of the fibrotic process. The expression of antioxidant enzymes manganese-superoxide dismutase (Mn-SOD) and glutathione (GSH) from lung homogenates was not altered during exposure.

Immune function following ozone/NO<sub>2</sub> exposure was examined by Fujimaki (1989). Continuous exposure of mice to 0.8 ppm ozone, 4.0 ppm NO<sub>2</sub>, or the mixture for 56 days resulted in increased lung weights and decreased spleen weights in the mice exposed to the mixture, which appeared to be no different from those in mice exposed only to ozone. Mice exposed to the mixture had significantly lower thymus and spleen weights during the first two weeks of exposure but the response to ozone alone was not examined at these shorter exposure durations. Exposure to NO<sub>2</sub> alone had little or no effect on these organ weights. Continuous exposure of mice for two weeks to the ozone/NO<sub>2</sub> mixture suppressed plaque-forming antibody production (mostly IgM) in spleens when subsequently immunized with sheep erythrocytes (Fujimaki, 1989). Exposure to NO<sub>2</sub> alone did not produce this effect. Exposure to ozone for 56 days suppressed plaque-forming antibody production whereas exposure to the mixture had no effect. Finally, immunization of mice previously exposed to the ozone/NO<sub>2</sub> mixture with a T-lymphocyte-independent antigen (dinitrophenol) enhanced plaque-forming antibody production on day 14 of exposure, but was similar to controls by day 56 of exposure. In another study (Fujimaki et al., 1984) using similar exposure protocols for antibody responses to sheep red blood cells or dinitrophenol-ficoll in mice exposed only to ozone, exposure to ozone alone at shorter exposure durations (i.e., up to two weeks) gave similar results compared to ozone/NO<sub>2</sub> mixtures. These immune function studies suggest that NO<sub>2</sub> at the exposures specified did not have a synergistic effect on immune system responses when combined with ozone.

Lee et al. (1990) investigated the effects of 3-day exposures of rats to 1.20 ppm NO<sub>2</sub>, 0.30 ppm ozone, or their combination on a number of enzyme activities in whole lung homogenates. The combined exposures resulted in synergistic increases in GSH-reductase, SOD, and enzyme activities related to NADPH generation, and additive increases for GSH-peroxidase and disulfide reductase activities. Exposure to NO<sub>2</sub> alone did not alter any parameters measured while ozone alone increased activities of all parameters except for SOD. An earlier study by the same research group exposed rats to higher levels of the gases (1.8 ppm NO<sub>2</sub>, 0.45 ppm ozone, and their combination) under the same exposure conditions (Lee et al., 1989). Exposure to ozone alone increased all enzyme activities, including activities related to NADPH generation, sulfhydryl metabolism, and cellular detoxification. Exposure to NO<sub>2</sub> alone increased levels of some enzymes activities, including isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, disulfide reductase, and NADPH-cytochrome c reductase. Exposure to the mixture resulted in synergistic increases in glucose-6-phosphate dehydrogenase, GSH-peroxidase, and GSH-disulfide transhydrogenase activities while increases in the other enzyme activities, including SOD and GSH-reductase, were mostly additive.

Ichinose et al. (Ichinose and Sagai, 1989) examined lungs of rats and guinea pigs for biochemical changes following two-week continuous exposures to 0.4 ppm NO<sub>2</sub>, 0.4 ppm ozone, or their combination. Thiobarbituric acid values, used as an index of lipid peroxidation in the lungs, had synergistically increased in guinea pigs exposed to the mixture, whereas rats showed no change in thiobarbituric acid values in any group. In contrast, guinea pigs showed no change in lung antioxidant content in any group, whereas the mixture synergistically increased antioxidant levels in rat lung (primarily nonprotein sulfhydryl content, ascorbate, glucose-6-phosphate dehydrogenase, and GSH-peroxidase). The authors suggested that a reason guinea pigs are known to be sensitive to oxidant gas combinations is because they show low increases in antioxidant factors following exposure, resulting in high levels of lipid peroxidation in the lung.

In a lifetime exposure study by the same authors, rats were exposed to ozone, ozone + 0.04 ppm NO<sub>2</sub>, and ozone + 0.4 ppm NO<sub>2</sub> for up to 22 months and examined for pulmonary biochemical effects (Sagai and Ichinose, 1991). Ozone exposure duration was 10 hr/day, with a mean of 0.05 ppm and a daily peak level of 0.1 ppm. Nitrogen dioxide exposures were continuous. Thiobarbituric acid values had synergistically increased in the ozone/NO<sub>2</sub> mixtures at 9 months, but were similar to control values after 18 and 22 months of exposure. Ozone alone did not alter thiobarbituric acid values. In general, both ozone/NO<sub>2</sub> groups and the ozone-only group showed increased lung vitamin E and nonprotein sulfhydryl contents at 9 months, which decreased to control or below control levels at 18 and 22 months. Whole lung antioxidant protective enzyme activities (GSH enzymes and SOD) did not show any changes from control values in any groups during exposure.

Wong et al. (1996) examined lungs of rats for changes in stress-inducible heat shock protein 70 (HSP 70) following 40-week intermittent (4 hr/day, 3 days/wk) exposure to 0.15 ppm ozone alone, 0.050 mg/m<sup>3</sup> nitric acid alone, or their combination. Ozone or nitric acid alone elevated lung levels of HSP 70 by 277% and 221%, respectively. However, combined exposure to ozone and nitric acid increased HSP 70 levels only 177% above the control group. No explanation was given for the apparent antagonistic effect of combined ozone/nitric acid exposure.

Several studies have investigated the interaction of ozone and NO<sub>2</sub> on genotoxic, mutagenic, or carcinogenic endpoints.

Exposure of rats continuously for 3 days to 0.3 ppm ozone or a combination of ozone and NO<sub>2</sub> (0.3 ppm and 1.2 ppm, respectively) resulted in a significant increase in DNA single-strand breaks in AMs (Bermudez et al., 1999). This interaction between ozone and NO<sub>2</sub> was characterized as additive at best, though exposure to NO<sub>2</sub> alone (1.2 ppm) did not cause a significant increase in DNA single-strand breaks.

In a study investigating the effect of ozone and NO<sub>2</sub> on cancer cell metastasis, infusion of mouse B16 melanoma cells following 12-week intermittent combined

exposure (7 hr/day, 5 days/wk) of mice to ozone (0.15 ppm) and NO<sub>2</sub> (0.35 ppm) enhanced lung cancer cell colonization (Richters, 1988). However, NO<sub>2</sub> alone was not tested, while ozone alone (0.15 or 0.3 ppm) did not enhance lung cancer cell colonization. In another assay, melanoma cells that were treated *in vitro* with spleen cells from mice exposed to the combined gas mixture produced significantly more melanoma colonies in the lungs, suggesting that the cytotoxic/cytostatic effects of the spleen cells was suppressed by exposure.

In another study investigating the tumor promotion potential of a mixture of ozone and NO<sub>2</sub>, male rats were administered a single dose of N-bis(2-hydroxypropyl)nitrosamine (BHPN) followed by exposure to a mean ozone concentration of 0.05 ppm plus 0.4 ppm NO<sub>2</sub> for 13 months (Ichinose and Sagai, 1992). Exposure to ozone alone resulted in an increase in lung tumors, though not statistically significant. Exposure to the ozone/NO<sub>2</sub> mixture produced an additional increase in incidence of lung tumors that was significantly greater than the control group exposed to clean air and BHPN. The authors suggested that exposure to the mixture may have a synergistic action as a tumor promoter.

#### **A.4.3 Ozone and Particulate Matter including Complex Mixtures**

Recent epidemiological evidence has found an association between high levels of small airborne particulates and increased morbidity and mortality, particularly among individuals with preexisting lung and heart disease. Respirable particulate matter is generally referred to as PM<sub>10</sub> (particulate matter with a median aerodynamic diameter of 10 microns), which encompasses a coarse mode and a fine mode. The fine mode is referred to as PM<sub>2.5</sub> (median aerodynamic diameter of 2.5 microns) and is generally comprised of combustion emissions and photochemical pollution in California and elsewhere. It has been postulated that coexposure of particulate matter with oxidant pollutants, such as ozone, can result in increased exacerbation of lung injury and enhance centriacinar lesions. Animal studies have observed potentiation of the ozone response by co-exposure to particulate matter, in that low ambient levels of particulate matter by itself do not cause observable effects but can increase the pulmonary response to ozone when combined with the oxidant gas. In addition, a number of studies have investigated multi-chemical exposures in animal models to simulate urban air pollution. These complex mixtures may include other pollutant gases in addition to ozone, acid aerosols, and particulate matter. However, the current state of knowledge of interactions among pollutants in complex urban atmospheres is relatively primitive compared to that for interactions among gaseous pollutants.

Rats were intermittently exposed (4 hr/day, 5 days/wk) for up to 20 days to dilute diesel exhaust containing 0.250 or 0.500 mg/m<sup>3</sup> diesel soot particles and nitric oxide, and mixed with 0.4 or 0.6 ppm ozone, respectively (Kleinman et al., 1993). Due to secondary chemical reactions in the mixture, a separate group of rats were exposed to ozone and NO<sub>2</sub> at the same concentrations present in the diesel soot mixture. After one day of exposure, the diesel soot-containing mixture at high concentrations produced histopathological evidence of airway inflammation and increased bronchoalveolar permeability compared to clean air controls.

Following five days of exposure, the diesel soot-containing mixture at high concentration caused reduced phagocytosis and altered Fc receptor binding in macrophages, and permeability was still increased over clean air controls. However, there was no difference between groups exposed to the diesel soot mixture and the groups exposed to ozone + NO<sub>2</sub>. The findings from histopathology and macrophage phagocytosis after 20 days of exposure suggested that effects of the ozone + NO<sub>2</sub> mixture were worse than those of the diesel soot-containing mixture and that diesel soot particles in the oxidant gas mixture did not modify the attenuation of responses with repeated exposure. At the concentrations tested, it was concluded that diesel soot plus oxidant gas mixtures was not more toxic than the oxidant gases alone (Kleinman et al., 1993).

To examine whether ozone can directly react and affect particulate matter bioactivity, Madden et al. (2000) exposed diesel exhaust particles (DEP) to ozone (0.1 ppm or 1.0 for 48 hours) and then instilled the DEP intratracheally into rats. The DEP exposed to 0.1 ppm ozone was a more potent inducer of lung inflammation and injury compared to unexposed DEP. However, DEP exposed to 1.0 ppm ozone decreased the bioactivity of the particles. In contrast, carbon black particles, low in organic content relative to DEP, did not exhibit an increase in any of the bioactivities examined after exposure to 0.1 ppm ozone. These results indicate that there is an optimal ozonation of DEP that increases biological potency and that the organic component of the DEP is important for the increased bioactivity induced by ozone exposure.

In a study to simulate exposure of a sensitive population to ozone/PM10-containing atmospheres, geriatric rats (age 22-24 months) were exposed nose-only for 4 hr/day, 3 days/wk for 4 weeks to a low-level of carbon black (0.050 mg/m<sup>3</sup>) plus ammonium bisulfate (0.070 mg/m<sup>3</sup>) plus ozone (0.2 ppm), a high level of carbon (0.100 mg/m<sup>3</sup>) plus ammonium bisulfate (0.140 mg/m<sup>3</sup>) and ozone (0.2 ppm), or to ozone alone (0.2 ppm) (Bolarin et al., 1997). No young-animal controls were used for comparison, apparently because this experiment was considered a pilot study. However, ozone exposures were based on earlier studies in young adult rats. Markers of airway permeability and inflammation in BAL fluid (protein and albumin concentrations) and markers of collagen synthesis in blood plasma (immunoreactive prolyl 4-hydroxylase) did not show consistent, significant differences among the exposure groups. However, plasma fibronectin was increased in the group exposed to ozone alone, but not in rats exposed to the ozone/particle combinations. Plasma fibronectin is an indicator of pathological conditions associated with injury of the reticuloendothelial system, including pulmonary endothelial cells. No rationale for the seemingly antagonistic effects of combined exposure to carbon particles and ozone on plasma fibronectin levels was provided. However, the total rats/group used for this particular endpoint was low (5 rats/group).

Rats were exposed for 4 hours to ozone (0.8 ppm), the urban dust EHC-93 (5 mg/m<sup>3</sup> or 50 mg/m<sup>3</sup>), or the mixture and injected with tritiated thymidine to label proliferating airway cells (Vincent et al., 1997). The effects of ozone were

potentiated by co-exposure with either concentration of urban dust, exhibiting increased labeling in both the bronchiolar and parenchymal compartments. Exposure to the urban dust alone had no effect on cell labeling. Among individual lung cell types, exposure to the mixtures increased type 2 cell and macrophage (high dust group only) labeling over animals exposed only to ozone. In a follow-up study by Bouthillier et al. (1998), rats exposed to the mixture of ozone (0.8 ppm) and EHC-93 urban dust (40 mg/m<sup>3</sup>) for 4 hours exhibited markedly increased interstitial septal cellularity and neutrophilic infiltration of lung interstitium compared to animals exposed only to ozone. Morphometric measurements noted increased type 2 cell and centriacinar septal volume in rats exposed to the mixture. In contrast, exposure to the urban dust did not enhance the response to ozone with regard to measurements of cells and protein in BAL fluid. Phagocytosis and viability of macrophages from ozone-exposed rats were also unaffected by co-exposure with urban dust. In another study by the researchers, Adamson et al. (1999) exposed groups of rats to 0.8 ppm ozone, urban particulate matter (50 mg/m<sup>3</sup>), or their combination for 4 hours. While exposure to the urban dust alone had little effect on the lung, coexposure of rats to dust and ozone resulted in potentiation of ozone toxicity. Epithelial injury and regeneration, as determined by percent of tritiated thymidine-labeled cells, was greatest in the ozone plus dust group, and was three times higher in the periductal areas than in whole-lung counts. Morphological analysis revealed higher numbers of PMNs and AMs in air spaces in the coexposure group, but counts were significantly higher for these cells in the interstitial tissue compartment compared to the other exposure groups. Altogether, this series of studies show that exposure to particulate matter (urban dust) causes a potentiation of the lung injury induced by ozone. Adamson et al. (1999) also indicated that analysis of changes in BAL fluid of animals exposed to ozone/particulate atmospheres may not represent the most sensitive indicator of lung injury.

In a study examining the adaptive responses of rats exposed to ozone alone or in mixtures with acid-coated carbon particles, repeated exposure (4 hr/day for 5 days) to 0.2 and 0.4 ppm ozone alone resulted in persistent suppression of macrophage FcR binding activity while exposure to a high concentration ozone/acidic particle mixture (0.4 ppm, 0.500 mg/m<sup>3</sup> and 0.250 mg/m<sup>3</sup>, respectively) elicited much greater suppression than did a low mixture concentration (0.2 ppm, 0.100 mg/m<sup>3</sup> and 0.050 mg/m<sup>3</sup>, respectively) or either concentration of ozone (Kleinman et al., 1999). However, tidal volume changes over 5 days of exposure to ozone alone or the ozone + acid particle mixtures did not appear to differ. A typical pattern of diminished lung inflammatory response, measured as numbers of inflammatory cells in alveolar lumens and numbers of cells in the interstitium of alveolar septa, was observed with repeated exposure to 0.4 ppm ozone. However, repeated exposure to the high concentration of ozone/acid particle mixture did not show adaptation in lung inflammatory response with 5-day exposure. The results indicate that some cell defense systems (e.g., macrophage functions, inflammatory responses) do not become

attenuated to repeated exposure to ozone and that adaptive mechanisms can become altered if ozone is presented in combinations with airborne particles.

To investigate the pulmonary injury-repair response following exposure to PM<sub>2.5</sub>/ozone atmospheres on aged rats, animals were exposed 4 hr/day, 3 consecutive days/wk for 4 weeks to 0.05 mg/m<sup>3</sup> carbon particles alone, 0.07 mg/m<sup>3</sup> ammonium bisulfate (ABS) + carbon particles, 0.2 ppm ozone alone, and ABS + carbon particles + ozone (Kleinman et al., 2000). Elemental carbon and ABS are two important components of PM<sub>2.5</sub>. Cell number and cell viability of lavaged cells was not affected by any of the exposure atmospheres. However, atmospheres containing ozone and particles were the only ones to significantly increase the magnitudes of several other measured biological responses. Epithelial cell labeling with 5-bromo-2-deoxyuridine to identify the location of injury-repair-related cell replication was elevated among rats exposed to the ABS + carbon particles + ozone mixture. Lung tissue collagen content was also decreased in this exposure group. Macrophages lavaged from the rats in the ABS + carbon particles + ozone group showed increased respiratory burst activity and phagocytic activity over the control group. Finally, superoxide anion production by macrophages was increased in atmospheres containing ozone and carbon particles.

Creutzenberg et al. (1995) investigated AM function in rats intratracheally instilled with various amounts of carbon black (0.15, 0.5 or 1.5 mg/animal) followed either by 7-day or subchronic 2-month intermittent exposure (6 hr/day, 5 days/wk) to 0.5 ppm ozone. In general, ozone alone was found to have no effect or to marginally stimulate phagocytic activity and chemotactic migration of AMs, whereas carbon black alone impaired these functions. Combined treatment resulted in a slightly activating effect of ozone partially counterbalancing the impairment caused by carbon black.

In a study of the effects of combined ozone/particle exposure on airway responsiveness, both normal and ovalbumin-sensitized ("asthmatic") mice were intermittently exposed (5 hr/day) for 3 days to 0.100-0.500 mg/m<sup>3</sup> concentrated ambient particles (CAPs), or 0.3 ppm ozone, or both, immediately after daily challenge to ovalbumin or saline aerosols (Goldsmith et al., 2002). Exposure to both CAPs alone and CAPs + ozone produced a small, transient increase in airway responsiveness, approximately 0.9% per 0.100 mg/m<sup>3</sup> increase in CAPs. Combined exposure to the pollutants was considered additive, not synergistic. Allergic inflammation was not detected in any of the exposure groups. Due to the variable composition of CAPs, analysis of the effects of particle composition on airway responsiveness revealed an association between the AlSi (aluminum silica) particle fraction and increased airway responsiveness in "asthmatic" mice exposed to ozone and particles. This finding suggested that airway responsiveness may be correlated with specific elements in the particle mixture.

A few studies examined the impact of inhaled particles following or preceding induction of pulmonary inflammation resulting from ozone inhalation.

As discussed in Section A.3.2.1 (Clearance), preexposure to an urban pattern of ozone followed by inhalation of aerosolized asbestos fibers resulted in increased retention of fiber mass and fiber number in the lungs 1 month after exposure (Pinkerton et al., 1989). These findings indicated that ambient levels of ozone could impair clearance of inhaled fibrogenic and carcinogenic insoluble materials from the lungs. Rat tracheal explants exposed to ozone (0.01-1.0 ppm) were shown to enhance uptake of mineral fibers in a dose-response fashion (Churg et al., 1996).

Prior exposure of mice to aged and diluted sidestream cigarette smoke (ADSS) sensitized the lungs to greater ozone-induced injury (Yu et al., 2002). Mice were exposed to 30 mg/m<sup>3</sup> ADSS for 6 hr/day for three days followed by exposure to 0.5 ppm ozone for 24 hours. ADSS alone had little or no inflammatory effect. Exposure to ADSS/ozone potentiated cell proliferation in the centriacinar regions of the lung, increased the number of cells recovered in BAL fluid, and increased the proportion of neutrophils, lymphocytes and total protein level in BAL fluid compared to all other groups.

In rats preexposed to 0.8 ppm ozone for 8 hours to induce pulmonary injury, a single exposure (6 hr) to high levels of freshly generated diesel exhaust particles (not exceeding 10 mg/m<sup>3</sup>; particle size  $\leq$  2.5 microns) one day after the end of ozone exposure did not influence the pattern of mild inflammation present in the centriacinar region or in the nasal epithelium (Cassee et al., 2002). However, bromodeoxyuridine-labeling of cells in terminal bronchiolar epithelium, a measure of cell proliferation, was markedly enhanced by diesel particles in rats pre-exposed to ozone. Diesel particles exposure also increased GSH levels in BAL fluid for up to 4 days after exposure, suggesting increased oxidant stress in the lungs. Slight increases in lactate dehydrogenase, protein and albumin were found in BAL fluid of rats exposed to diesel particulate but was considered to be primarily due to the ozone pretreatment. These results indicate that increased bromodeoxyuridine-labeling and increased GSH levels in lung airways are sensitive indicators of diesel particle exposure in ozone-compromised rats.

Ulrich et al. (2002) pre-exposed rats to 0.8 ppm ozone for 8 hours to induce a mild inflammatory reaction prior to intratracheal instillation of 0.5, 1.5, or 5.0 mg/m<sup>3</sup> particulate matter from Ottawa Canada (EHC-93). Groups of rats were exposed to ozone alone or 5.0 mg/m<sup>3</sup> EHC-93 alone. Parameters in BAL fluid used to measure the inflammatory effect of ozone alone (total protein, alkaline phosphatase and lactate dehydrogenase activity, total cells) indicated no difference from control values 2 days after EHC-93 instillation. The high concentration of EHC-93 alone was sufficient to induce an inflammatory reaction at day 2 after EHC-93 instillation, but pre-exposure to ozone did not exacerbate the reaction. Transudation of plasma protein and elevation of fibrinogen in plasma were slightly elevated in pollutant combination animals at 4-7 days after EHC-93 instillation, but were not statistically significant different from controls. Plasma and mRNA expression levels of various cytokines thought to play a role in the progression of heart failure were also measured. Small, but statistically insignificant, changes were observed in inducible nitric oxide synthase and

endothelin-1 mRNA levels in pollutant combination animals. However, the ozone/EHC-93 mixtures did not affect levels of other cytokines such as lung tumor necrosis factor- $\alpha$ . It was speculated that some effects of ozone/EHC-93 mixtures on inflammatory measures and cytokine levels may have occurred within 2 days of EHC-93 instillation and were missed.

The following studies exposed experimental animals to complex pollutant atmospheres to simulate photochemical air pollution present in urban settings.

Rats were exposed to a complex pollutant atmosphere consisting of ozone (0.4 ppm), nitric acid (0.7 mg/m<sup>3</sup>), sulfuric acid (0.6 mg/m<sup>3</sup>), and hydroxymethanesulfonate (HMSA) (0.6 mg/m<sup>3</sup>) for 4 hours (Mautz et al., 1991). The pollutants in this mixture are key components found in acid fogs. Other exposure groups consisted of ozone alone (0.4 ppm) and ozone (0.4 ppm) plus HMSA (0.5 mg/m<sup>3</sup>). Ozone alone induced typical changes in inflammatory response (total protein in BAL and lung parenchymal lesions), breathing pattern, metabolic rate, and fatty acid composition of pulmonary surfactant, but exposure to the mixture or HMSA with ozone did not significantly modify the response to ozone alone. Nasal respiratory epithelium was unaffected by exposure to any of the pollutant groupings. The authors suggested that the exposures in this study might have been too short to show acid-induced enhancement of ozone injury or that the rats may have been insensitive to these acid-oxidant atmospheres. In a related study, Mautz et al. (Mautz and Nadziejko, 2000) exposed rats to a 26-week episodic exposure (4 hr/day, 3 days/wk) of 0.3 ppm ozone alone or a mixture of 0.3 ppm ozone, 0.2 ppm NO<sub>3</sub>, 0.05 mg/m<sup>3</sup> nitric acid, 0.1 mg/m<sup>3</sup> NH<sub>4</sub>NSO<sub>4</sub>, and 0.06 mg/m<sup>3</sup> carbon particles. Analysis of BAL fluid in rats exposed to ozone alone showed a slight but significant increase in total protein and a possible increase in nonspecific esterase activity. However, exposure to the mixture did not result in changes of these inflammatory parameters. Protease inhibitor levels (elastase inhibitory capacity (EIC) and cetyl trimethyl ammonium bromide-resistant EIC) were unchanged in BAL fluid of all exposure groups. It was presumed that adaptation to the low level of pollutants had occurred over the 26-week exposure.

Exposure of rats for 3-4 hours to ozone (0.6 ppm) combined with sulfuric acid (1 mg/m<sup>3</sup>) in the presence of 5 ppm SO<sub>2</sub> and iron and manganese ions did not increase lung parenchymal injury compared to exposure to ozone alone if rats were at rest during exposure (Kleinman et al., 1989). However, when rats were exposed during exercise, the acid-ozone mixture increased lung injury 2.5 times that observed in rats exposed to ozone alone, at the same exercise level. Other than noting that exercise appears to be an important factor in this process, no conclusions were drawn.

Bhalla et al. (1987) exposed rats for 2 hours to a 7-component particle and gas mixture to represent urban air pollution in a photochemical environment. The mixture consisted of ozone (0.6 ppm), NO<sub>2</sub> (2.5 ppm), SO<sub>2</sub> (5 ppm), ferric oxide (0.241 mg/m<sup>3</sup>), ammonium sulfate (0.308-0.364 mg/m<sup>3</sup>), ferric sulfate (0.411-0.571 mg/m<sup>3</sup>), and manganese sulfate (0.007-0.009 mg/m<sup>3</sup>). The response to this mixture was compared to that following exposure to ozone alone (0.6 or 0.8

ppm), ozone (0.6 ppm) plus NO<sub>2</sub> (2.5 ppm), or NO<sub>2</sub> alone (6 or 12 ppm). Exposure to ozone, ozone + NO<sub>2</sub>, and the mixture all increased bronchoalveolar permeability to tracers. The complex mixture produced effects that were similar to ozone alone, though there appeared to be a prolongation of bronchoalveolar permeability compared to ozone alone.

To examine the effects of a similar urban pollutant atmosphere on macrophage function, Prasad et al. (1988) exposed rats for 4 hr/day for 7 or 21 days to a 7-component pollutant atmosphere. This pollutant atmosphere is comparable to that found in the South Coast Air Basin. The effect of the pollutant mixture (0.30 ppm ozone, 1.2 ppm NO<sub>2</sub>, 2.5 ppm SO<sub>2</sub>, 0.27 mg/m<sup>3</sup> ammonium sulfate, 0.22 mg/m<sup>3</sup> iron sulfate, 0.004 mg/m<sup>3</sup> manganese sulfate and an insoluble aerosol of 0.15 mg/m<sup>3</sup> iron oxide) was compared to effects resulting from ozone exposure alone (0.8 ppm for 4 hours). Both the 7-day exposure to the pollutant mixture and acute exposure to ozone alone caused a similar reduction in macrophage Fc receptor activity, a surface receptor crucial for macrophages to become cytotoxic against target cells. However, 21-day exposure to the pollutant mixture caused an even greater reduction in Fc activity compared to ozone alone. The pollutant mixture following 7 days, but not 21 days, of exposure reduced macrophage phagocytic activity. However, an ozone-only exposure for comparison was not performed.

Mautz et al. (2001) examined cumulative and adaptive responses of 3 concentrations of a simulated Southern California air pollutant mixture in rats intermittently exposed (4 hr/day, 3 days/wk) for 4 weeks. Direct comparisons with ozone exposure alone were not performed. Exposure to the high dose (0.6 ppm ozone, 0.4 ppm NO<sub>2</sub>, 0.2 mg/m<sup>3</sup> ammonium bisulfite, 0.12 mg/m<sup>3</sup> carbon particles, 0.1 mg/m<sup>3</sup> nitric acid) exacerbated irritant-induced rapid shallow breathing responses while exposure to the medium concentration (0.3 ppm ozone, 0.2 ppm NO<sub>2</sub>, 0.1 mg/m<sup>3</sup> ammonium bisulfite, 0.06 mg/m<sup>3</sup> carbon particles, 0.05 mg/m<sup>3</sup> nitric acid) showed diminished responses over the 4-week exposure period. Lavaged AMs showed dose-dependent depressions of Fc-receptor binding and phagocytosis that was significantly decreased at the medium (Fc-binding) or high (phagocytosis) concentrations. The pollutant atmospheres did not alter respiratory tract clearance of tracer particles but bronchoalveolar permeability, measured as total protein in BAL fluid, and histological evidence of parenchymal inflammation was increased at the high concentration. Epithelial cell proliferation labeling, a marker of cell injury, showed a dose-dependent increase at all respiratory tract levels but was markedly elevated in the nose and terminal bronchioles of the high concentration group. It was indicated that exposure to the lower levels of pollutants induced a response that then attenuates on repeated exposure, but higher doses delivered in repetition result in an exacerbated response.

A few animal studies investigated effects from actual urban pollutant exposures.

Saldiva et al. (1992) exposed rats to the urban pollutant atmosphere in Sao Paulo for six months and compared them to rats kept in a clean-air area. Mean levels of recorded pollutants included 1.25 ppm carbon monoxide, 0.011 ppm

ozone, 0.035 mg/m<sup>3</sup> particulates, and 0.029 mg/m<sup>3</sup> SO<sub>2</sub>. The urban atmosphere-exposed rats developed airway secretory cell hyperplasia, ultrastructural ciliary alterations, and more rigid mucus, and mucociliary clearance impairment. These rats also experienced greater mortality than clean air controls, likely due to *Mycoplasma pulmonis* infection. It was unclear, however, whether decreased host defense against infection due to exposure to the pollutant atmosphere was a factor in the increased mortality.

Calderón-Garciduenas et al. (2001a) performed a histopathological study on stray mongrel dogs exposed to a complex mixture of pollutants, predominantly particulate matter and ozone, in a severely polluted urban environment (Mexico City and Cuernavaca) and compared them to dogs living in less polluted regions. Dogs were chosen for the study due to their long life span and their similarities to humans in regard to pulmonary development, structure, and function relative to rodents. The crucial lesion in the lungs resulting from life-long exposure was epithelial and endothelial injury, leading to persistent chronic parenchymal lung inflammation and focal fibrosis. The high load of particulate material in lung cells and tissue suggested that simultaneous exposure to pollutants such as ozone and NO<sub>2</sub> likely contribute to the particle uptake and translocation into the interstitium by increasing epithelial permeability. In an associated study, Calderón-Garciduenas et al. (2001b) also observed cardiac abnormalities in the dogs exposed to severely polluted urban environments, including apoptotic myocytes, degranulated mast cells, microthrombi in capillaries, particulate matter deposition, and other pathological findings. The close association between the myocardial findings and lung changes noted in these dogs appear to support the epidemiological findings of increased cardiovascular morbidity and mortality in people exposed to particulate matter and other pollutants.

Taken together, the studies suggest that the types of interactions produced with ozone and co-occurring pollutants are dependent on many factors. Investigations of sulfuric acid and ozone co-exposures indicate that the type of interaction is dependent on the health endpoint, composition of the aerosol, and size of the aerosol. A further complication is that the magnitude of the sulfuric acid/ozone interaction is not always related to the exposure concentrations of the constituent pollutants. Striking synergistic interactions have been observed with acute exposures to ozone/ultrafine sulfuric acid aerosol combinations and ozone/sulfuric acid layered on metal mixtures. Interactions of ozone and NO<sub>2</sub> have also produced antagonistic or synergistic effects, depending on factors such as exposure concentrations used, animal species tested, and health endpoint examined. One of the more sensitive measures of ozone/NO<sub>2</sub> interactions utilized a bacterial infectivity model in which 15-day exposure to a simulated urban pollutant atmosphere (baseline of 0.5 ppm NO<sub>2</sub> with peaks of 1.0 ppm, and a baseline of 0.05 ppm ozone with peaks of 0.1 ppm) produced a synergistic interaction on mortality in mice. With particulate matter/ozone co-exposures, the type of interaction produced has been shown to depend on the ozone concentration used, the organic content of the particulate, and the endpoint measured. Of note, potentiation of ozone injury has been observed with co-exposure to urban-type dusts, and preexposure to ozone followed by

toxic particle instillation resulted in a marked retention of the toxic particles in small airways.

## A.5 References

Abraham WM, Januszkiewicz AJ, Mingle M, Welker M, Wanner A, Sackner MA (1980 May). Sensitivity of bronchoprovocation and tracheal mucous velocity in detecting airway responses to O<sub>3</sub>. *J Appl Physiol* 48(5):789-93.

Abraham WM, Sielczak MW, Delehunt JC, Marchette B, Wanner A (1986 Feb). Impairment of tracheal mucociliary clearance but not ciliary beat frequency by a combination of low level ozone and sulfur dioxide in sheep. *Eur J Respir Dis* 68(2):114-20.

Adamson IY, Vincent R, Bjarnason SG (1999 May). Cell injury and interstitial inflammation in rat lung after inhalation of ozone and urban particulates. *Am J Respir Cell Mol Biol* 20(5):1067-72.

Allegra L, Abraham WMCGA, Wanner A (1983). Targets of allergic airway challenge and tracheobronchial irritation with ozone in an animal model (sheep). *European Journal of Respiratory Diseases* 64 (suppl 126):45-52.

Aranyi C, Vana SC, Thomas PT, Bradof JN, Fenters JD, Graham JA, et al. (1983 Jul). Effects of subchronic exposure to a mixture of O<sub>3</sub>, SO<sub>2</sub>, and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> on host defenses of mice. *J Toxicol Environ Health* 12(1):55-71.

ARB (1987). Effects of Ozone on Health. Technical Support Document. Prepared by the California Air Resources Board. September.

Arito H, Uchiyama I, Arakawa H, Yokoyama E (1990 Jul). Ozone-induced bradycardia and arrhythmia and their relation to sleep- wakefulness in rats. *Toxicol Lett* 52(2):169-78.

Arito H, Uchiyama I, Yokoyama E (1992). Acute effects of ozone on EEG activity, sleep-wakefulness and heart rate in rats. *Ind Health* 30(1):23-34.

Armstrong LC, Watkins K, Pinkerton KE, Last JA (1994 Jul). Collagen mRNA content and distribution in the lungs of rats exposed to ozone. *Am J Respir Cell Mol Biol* 11(1):25-34.

Bassett DJ, Bowen-Kelly E, Elbon CL, Reichenbaugh SS (1988). Rat lung recovery from 3 days of continuous exposure to 0.75 ppm ozone. *J Toxicol Environ Health* 25(3):329-47.

Becker S, Quay J, Koren HS (1991 Nov). Effect of ozone on immunoglobulin production by human B cells in vitro. *J Toxicol Environ Health* 34(3):353-66.

Ben-Jebria A, Hu SC, Kitzmiller EL, Ultman JS (1991 Dec). Ozone absorption into excised porcine and sheep tracheae by a bolus- response method. *Environ Res* 56(2):144-57.

Bermudez E, Ferng SF, Castro CE, Mustafa MG (1999 Jul). DNA strand breaks caused by exposure to ozone and nitrogen dioxide. *Environ Res* 81(1):72-80.

Bhalla DK, Mannix RC, Kleinman MT, Crocker TT (1986). Relative permeability of nasal, tracheal, and bronchoalveolar mucosa to macromolecules in rats exposed to ozone. *J Toxicol Environ Health* 17(2-3):269-83.

Bhalla DK, Crocker TT (1986 Sep). Tracheal permeability in rats exposed to ozone. An electron microscopic and autoradiographic analysis of the transport pathway. *Am Rev Respir Dis* 134(3):572-9.

Bhalla DK, Crocker TT (1987). Pulmonary epithelial permeability in rats exposed to O<sub>3</sub>. *J Toxicol Environ Health* 21(1-2):73-87.

Bhalla DK, Mannix RC, Lavan SM, Phalen RF, Kleinman MT, Crocker TT (1987). Tracheal and bronchoalveolar permeability changes in rats inhaling oxidant atmospheres during rest or exercise. *J Toxicol Environ Health* 22(4):417-37.

Bhalla DK, Rasmussen RE, Daniels DS (1993 Dec). Adhesion and motility of polymorphonuclear leukocytes isolated from the blood of rats exposed to ozone: potential biomarkers of toxicity. *Toxicol Appl Pharmacol* 123(2):177-86.

Bhalla DK (1996 Dec). Alteration of alveolar macrophage chemotaxis, cell adhesion, and cell adhesion molecules following ozone exposure of rats. *J Cell Physiol* 169(3):429-38.

Bhalla DK, Hoffman L (1997). Time course of airway epithelial and inflammatory changes in rats exposed to moderate levels of ozone. *Inhalation Toxicology* 9:829-42.

Bhalla DK (1999 Jan-1999 Mar). Ozone-induced lung inflammation and mucosal barrier disruption: toxicology, mechanisms, and implications. *J Toxicol Environ Health B Crit Rev* 2(1):31-86.

Bignami G, Musi B, Dell'Omo G, Laviola G, Alleva E (1994 Dec). Limited effects of ozone exposure during pregnancy on physical and neurobehavioral development of CD-1 mice. *Toxicol Appl Pharmacol* 129(2):264-71.

Bignami G (1996 Apr). Economical test methods for developmental neurobehavioral toxicity. *Environ Health Perspect* 104 Suppl 2:285-98.

Bleavins MR, Dziedzic D (1990 Aug). An immunofluorescence study of T and B lymphocytes in ozone-induced pulmonary lesions in the mouse. *Toxicol Appl Pharmacol* 105(1):93-102.

Boehme DS, Hotchkiss JA, Henderson RF (1992 Feb). Glutathione and GSH-dependent enzymes in bronchoalveolar lavage fluid cells in response to ozone. *Exp Mol Pathol* 56(1):37-48.

Bolarin DM, Bhalla DK, Kleinman MT (1997). Effects of repeated exposures of geriatric rats to ozone and particle-containing atmospheres: an analysis of bronchoalveolar lavage and plasma proteins. *Inhalation Toxicology* 9:423-34.

Boorman GA, Hailey R, Grumbein S, Chou BJ, Herbert RA, Goehl T, et al. (1994 Sep-1994 Oct). Toxicology and carcinogenesis studies of ozone and ozone 4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone in Fischer-344/N rats. *Toxicol Pathol* 22(5):545-54.

Boorman GA, Sills RC, Grumbein S, Hailey R, Miller RA, Herbert RA (1995 Dec). Long-term toxicity studies of ozone in F344/N rats and B6C3F1 mice. *Toxicol Lett* 82-83:301-6.

Borek C, Zaider M, Ong A, Mason H, Witz G (1986 Sep). Ozone acts alone and synergistically with ionizing radiation to induce in vitro neoplastic transformation. *Carcinogenesis* 7(9):1611-3.

Borek C, Ong A, Zaider M (1989 Aug). Ozone activates transforming genes in vitro and acts as a synergistic co-carcinogen with gamma-rays only if delivered after radiation. *Carcinogenesis* 10(8):1549-51.

Bornholdt J, Dybdahl M, Vogel U, Hansen M, Loft S, Wallin H (2002 Sep). Inhalation of ozone induces DNA strand breaks and inflammation in mice. *Mutat Res* 520(1-2):63-71.

Bouthillier L, Vincent R, Goegan P, Adamson IY, Bjarnason S, Stewart M, et al. (1998 Dec). Acute effects of inhaled urban particles and ozone: lung morphology, macrophage activity, and plasma endothelin-1. *Am J Pathol* 153(6):1873-84.

Burleson GR, Keyes LL, Stutzman JD (1989). Immunosuppression of pulmonary natural killer activity by exposure to ozone. *Immunopharmacol Immunotoxicol* 11(4):715-35.

Calderon-Garciduenas L, Mora-Tiscareno A, Fordham LA, Chung CJ, Garcia R, Osnaya N, et al. (2001a Jun). Canines as sentinel species for assessing chronic exposures to air pollutants: part 1. Respiratory pathology. *Toxicol Sci* 61(2):342-55.

Calderon-Garciduenas L, Gambling TM, Acuna H, Garcia R, Osnaya N, Monroy S, et al. (2001b Jun). Canines as sentinel species for assessing chronic exposures to air pollutants: part 2. Cardiac pathology. *Toxicol Sci* 61(2):356-67.

Campen MJ, Norwood J, McKee JL, Mebane R, Hatch GE, Watkinson WP (2000). Ozone-induced hypothermia and bradycardia in rats and guinea pigs in nose-only or whole-body inhalation systems. *Journal of Thermal Biology* 25:81-9.

Canning BJ, Hmieleski RR, Spannhake EW, Jakab GJ (1991 Oct). Ozone reduces murine alveolar and peritoneal macrophage phagocytosis: the role of prostanoids. *Am J Physiol* 261(4 Pt 1):L277-82.

Cassee FR, Boere AJ, Bos J, Fokkens PH, Dormans JA, van Loveren H (2002 Jul). Effects of diesel exhaust enriched concentrated PM<sub>2.5</sub> in ozone preexposed or monocrotaline-treated rats. *Inhal Toxicol* 14(7):721-43.

Chang LY, Huang Y, Stockstill BL, Graham JA, Grose EC, Menache MG, et al. (1992 Aug). 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(2):241-52.

Chang MM, Wu R, Plopper CG, Hyde DM (1998 Sep). IL-8 is one of the major chemokines produced by monkey airway epithelium after ozone-induced injury. *Am J Physiol* 275(3 Pt 1):L524-32.

Cheek JM, Buckpitt AR, Li C, Tarkington BK, Plopper CG (1994 Mar). Ozone injury to alveolar epithelium in vitro does not reflect loss of antioxidant defenses. *Toxicol Appl Pharmacol* 125(1):59-69.

Chen LC, Miller PD, Lam HF, Guty J, Amdur MO (1991 Nov). Sulfuric acid-layered ultrafine particles potentiate ozone-induced airway injury. *J Toxicol Environ Health* 34(3):337-52.

Chen LC, Qu Q, Amdur MO, Schlesinger RB (1995 Jan-1995 Feb). Alteration of pulmonary macrophage intracellular pH following inhalation exposure to sulfuric acid/ozone mixtures. *Exp Lung Res* 21(1):113-28.

Cheng PW, Boat TF, Shaikh S, Wang OL, Hu PC, Costa DL (1995 May-1995 Jun). Differential effects of ozone on lung epithelial lining fluid volume and protein content. *Exp Lung Res* 21(3):351-65.

Chow CK, Kaneko JJ (1979 Jun). Influence of dietary vitamin E on the red cells of ozone-exposed rats. *Environ Res* 19(1):49-55.

Christman CA, Schwartz LW (1982 Aug). Enhanced phagocytosis by alveolar macrophages induced by short-term ozone insult. *Environ Res* 28(2):241-50.

Churg A, Brauer M, Keeling B (1996 Apr). Ozone enhances the uptake of mineral particles by tracheobronchial epithelial cells in organ culture. *Am J Respir Crit Care Med* 153(4 Pt 1):1230-3.

Cohen Hubal EA, Kimbell JS, Fedkiw PS (1996). Incorporation of nasal-lining mass-transfer resistance into a CFD model for prediction of ozone dosimetry in the upper respiratory tract. *Inhalation Toxicology* 8:831-57.

Cohen MD, Zelikoff JT, Chen L-C, Schlesinger RB (1997). Pulmonary retention and distribution of inhaled chromium: effects of particle solubility and coexposure to ozone. *Inhalation Toxicology* 9:843-65.

Cohen MD, Zelikoff JT, Chen LC, Schlesinger RB (1998 Sep). Immunotoxicologic effects of inhaled chromium: role of particle solubility and co-exposure to ozone. *Toxicol Appl Pharmacol* 152(1):30-40.

Cohen MD, Sisco M, Li Y, Zelikoff JT, Schlesinger RB (2001 Mar). Ozone-induced modulation of cell-mediated immune responses in the lungs. *Toxicol Appl Pharmacol* 171(2):71-84.

Cohen MD, Sisco M, Baker K, Li Y, Lawrence D, van Loveren H, et al. (2002 Jun). Effects of inhaled ozone on pulmonary immune cells critical to antibacterial responses in situ. *Inhal Toxicol* 14(6):599-619.

Cohen MD, Sisco M, Baker K, Bowser D, Chen LC, Schlesinger RB (2003 Jan). Impact of coexposure to ozone on the carcinogenic potential of inhaled chromium. 1. effects on retention and on extra- and intracellular distribution. *J Toxicol Environ Health A* 66(1):39-55.

Creutzenberg O, Bellmann B, Klingebiel R, Heinrich U, Muhle H (1995 May). Phagocytosis and chemotaxis of rat alveolar macrophages after a combined or separate exposure to ozone and ARB on black. *Exp Toxicol Pathol* 47(2-3):202-6.

Dell'Omo G, Fiore M, Petrucci S, Alleva E, Bignami G (1995a). Neurobehavioral development of CD-1 mice after combined gestational and postnatal exposure to ozone. *Arch Toxicol* 69(9):608-16.

Dell'Omo G, Wolfer D, Alleva E, Lipp HP (1995b Nov). Developmental exposure to ozone induces subtle changes in swimming navigation of adult mice. *Toxicol Lett* 81(2-3):91-9.

DeLucia AJ, Adams WC (1977 Jul). Effects of O<sub>3</sub> inhalation during exercise on pulmonary function and blood biochemistry. *J Appl Physiol* 43(1):75-81.

Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schreinemachers D, et al. (1991 Jan). Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am J Respir Cell Mol Biol* 4(1):72-81.

Devlin RB, Folinsbee LJ, Biscardi F, Hatch G, Becker S, Madden MC, et al. (1997). Inflammation and cell damage induced by repeated exposure of humans to ozone. *Inhalation Toxicology* 9:211-35.

Dillon D, Combes R, McConville M, Zeiger E (1992). Ozone is mutagenic in *Salmonella*. *Environ Mol Mutagen* 19(4):331-7.

Donaldson K, Brown GM, Brown DM, Slight J, Maclaren WM, Davis JM (1991 Oct). Leukocyte-mediated epithelial injury in ozone-exposed rat lung. *Res Rep Health Eff Inst* (44):1-27.

Donaldson K, Brown GM, Brown DM, Slight J, Maclaren W, Davis JMG (1993). Characteristics of bronchoalveolar leucocytes from the lungs of rats inhaling 0.2-0.8 ppm of ozone. *Inhalation Toxicology* 5:149-64.

Dormans JA, Rombout PJ, van Loveren H (1990 Sep). Surface morphology and morphometry of rat alveolar macrophages after ozone exposure. *J Toxicol Environ Health* 31(1):53-70.

Dormans JA, van Bree L, Boere AJ, Marra M, Rombout PJ (1999 Apr). Interspecies differences in time course of pulmonary toxicity following repeated exposure to ozone. *Inhal Toxicol* 11(4):309-29.

Dormans JAMA, Boere AJF, van Loveren H, Rombout PJA, Marra M, van Bree L (1996). Age-related toxicity in rat lungs following acute and repeated ozone exposure. *Inhalation Toxicology* 8:903-25.

Driscoll KE, Vollmuth TA, Schlesinger RB (1987). Acute and subchronic ozone inhalation in the rabbit: response of alveolar macrophages. *J Toxicol Environ Health* 21(1-2):27-43.

Duan X, Buckpitt AR, Pinkerton KE, Ji C, Plopper CG (1996 Jan). Ozone-induced alterations in glutathione in lung subcompartments of rats and monkeys. *Am J Respir Cell Mol Biol* 14(1):70-5.

Dubick MA, Keen CL (1983 Jul). Tissue trace elements and lung superoxide dismutase activity in mice exposed to ozone. *Toxicol Lett* 17(3-4):355-60.

Dziedzic D, White HJ (1986a Dec). T-cell activation in pulmonary lymph nodes of mice exposed to ozone. *Environ Res* 41(2):610-22.

Dziedzic D, White HJ (1986b Dec). Thymus and pulmonary lymph node response to acute and subchronic ozone inhalation in the mouse. *Environ Res* 41(2):598-609.

Dziedzic D, Wright ES, Sargent NE (1990 Apr). Pulmonary response to ozone: reaction of bronchus-associated lymphoid tissue and lymph node lymphocytes in the rat. *Environ Res* 51(2):194-208.

el-Fawal HA, Schlesinger RB (1994 Mar). Nonspecific airway hyperresponsiveness induced by inhalation exposure to sulfuric acid aerosol: an in vitro assessment. *Toxicol Appl Pharmacol* 125(1):70-6.

el-Fawal HA, McGovern T, Schlesinger RB (1995 Jan-1995 Feb). Nonspecific bronchial responsiveness assessed in vitro following acute inhalation exposure to ozone and ozone/sulfuric acid mixtures. *Exp Lung Res* 21(1):129-39.

Elsayed NM, Kass R, Mustafa MG, Hacker AD, Ospital JJ, Chow CK, et al. (1988). Effect of dietary vitamin E level on the biochemical response of rat lung to ozone inhalation. *Drug Nutr Interact* 5(4):373-86.

Eskew ML, Scheuchenzuber WJ, Scholz RW, Reddy CC, Zarkower A (1986 Aug). The effects of ozone inhalation on the immunological response of selenium- and vitamin E-deprived rats. *Environ Res* 40(2):274-84.

Farman CA, Watkins K, van Hoozen B, Last JA, Witschi H, Pinkerton KE (1999 Feb). Centriacinar remodeling and sustained procollagen gene expression after exposure to ozone and nitrogen dioxide. *Am J Respir Cell Mol Biol* 20(2):303-11.

Ferng SF, Castro CE, Afifi AA, Bermudez E, Mustafa MG (1997 Jul). Ozone-induced DNA strand breaks in guinea pig tracheobronchial epithelial cells. *J Toxicol Environ Health* 51(4):353-67.

Foster WM, Freed AN (1999 Feb). Regional clearance of solute from peripheral airway epithelia: recovery after sublobar exposure to ozone. *J Appl Physiol* 86(2):641-6.

Freed AN, Cueto R, Pryor WA (1999 Nov). Antioxidant transport modulates peripheral airway reactivity and inflammation during ozone exposure. *J Appl Physiol* 87(5):1595-603.

Fujimaki H, Ozawa M, Imai T, Shimizu F (1984 Dec). Effect of short-term exposure to O<sub>3</sub> on antibody response in mice. *Environ Res* 35(2):490-6.

Fujimaki H, Shiraishi F, Ashikawa T, Murakami M (1987 Jun). Changes in delayed hypersensitivity reaction in mice exposed to O<sub>3</sub>. *Environ Res* 43(1):186-90.

Fujimaki H (1989 Apr). Impairment of humoral immune responses in mice exposed to nitrogen dioxide and ozone mixtures. *Environ Res* 48(2):211-7.

Gelzleichter TR, Witschi H, Last JA (1992a Jan). Concentration-response relationships of rat lungs to exposure to oxidant air pollutants: a critical test of Haber's Law for ozone and nitrogen dioxide. *Toxicol Appl Pharmacol* 112(1):73-80.

Gelzleichter TR, Witschi H, Last JA (1992b Sep). Synergistic interaction of nitrogen dioxide and ozone on rat lungs: acute responses. *Toxicol Appl Pharmacol* 116(1):1-9.

Gilmour MI, Jakab GJ (1991). Modulation of immune function in mice exposed to 0.8 ppm ozone. *Inhalation Toxicology* 3:293-308.

Gilmour MI, Hmieleski RR, Stafford EA, Jakab GJ (1991 May-1991 Jun). Suppression and recovery of the alveolar macrophage phagocytic system during continuous exposure to 0.5 ppm ozone. *Exp Lung Res* 17(3):547-58.

Gilmour MI, Park P, Selgrade MK (1993a Mar). Ozone-enhanced pulmonary infection with *Streptococcus zooepidemicus* in mice. The role of alveolar macrophage function and capsular virulence factors. *Am Rev Respir Dis* 147(3):753-60.

Gilmour MI, Park P, Doerfler D, Selgrade MK (1993b May-1993b Jun). Factors that influence the suppression of pulmonary antibacterial defenses in mice exposed to ozone. *Exp Lung Res* 19(3):299-314.

Gilmour MI, Selgrade MK (1993 Dec). A comparison of the pulmonary defenses against streptococcal infection in rats and mice following O<sub>3</sub> exposure: differences in disease susceptibility and neutrophil recruitment. *Toxicol Appl Pharmacol* 123(2):211-8.

Goldsmith CA, Ning Y, Qin G, Imrich A, Lawrence J, Murthy GG, et al. (2002 Apr). Combined air pollution particle and ozone exposure increases airway responsiveness in mice. *Inhal Toxicol* 14(4):325-47.

Goodman JW, Peter-Fizaine FE, Shipcock SG, Hall EA, Fahmie DJ (1989 May-1989 Jun). Immunologic and hematologic consequences in mice of exposure to ozone. *J Environ Pathol Toxicol Oncol* 9(3):243-52.

Graham JA, Gardner DE, Blommer EJ, House DE, Menache MG, Miller FJ (1987). Influence of exposure patterns of nitrogen dioxide and modifications by ozone on susceptibility to bacterial infectious disease in mice. *J Toxicol Environ Health* 21(1-2):113-25.

Grose EC, Stevens MA, Hatch GE, Jaskot RH, Selgrade MJK, Stead AG, et al. (1989). The impact of a 12-month exposure to a diurnal pattern of ozone on pulmonary function, antioxidant biochemistry and immunology. 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, Elsevier Science Publishers B. V., Amsterdam, The Netherlands.* pp. 535-543 (*Studies in environmental science* 35).

Gross KB, White HJ (1987). Functional and pathologic consequences of a 52-week exposure to 0.5 PPM ozone followed by a clean air recovery period. *Lung* 165(5):283-95.

Grotberg JB, Sheth BV, Mockros LF (1990 May). An analysis of pollutant gas transport and absorption in pulmonary airways. *J Biomech Eng* 112(2):168-76.

Guth DJ, Warren DL, Last JA (1986 Aug). Comparative sensitivity of measurements of lung damage made by bronchoalveolar lavage after short-term exposure of rats to ozone. *Toxicology* 40(2):131-43.

Hackney JD, Linn WS, Buckley RD, Pedersen EE, Karuza SK, Law DC, et al. (1975 Aug). Experimental studies on human health effects of air pollutants: I. Design considerations. *Arch Environ Health* 30(8):373-8.

Haney JT Jr, Connor TH, Li L (1999 Apr). Detection of ozone-induced DNA single strand breaks in murine bronchoalveolar lavage cells acutely exposed in vivo. *Inhal Toxicol* 11(4):331-41.

Hanna LM, Frank R, Scherer PW (1989). Absorption of soluble gases and vapors in the respiratory system. In: Chang, H. K.; Paiva, M., eds. *Respiratory physiology: an analytical approach*. New York: Marcel Dekker, Inc.; pp. 277-316.

Haro R, Paz C (1993 Dec). Effects of ozone exposure during pregnancy on ontogeny of sleep in rats. *Neurosci Lett* 164(1-2):67-70.

Hassett C, Mustafa MG, Coulson WF, Elashoff RM (1985a Aug). Splenomegaly in mice following exposure to ambient levels of ozone. *Toxicol Lett* 26(2-3):139-44.

Hassett C, Mustafa MG, Coulson WF, Elashoff RM (1985b Oct). Murine lung carcinogenesis following exposure to ambient ozone concentrations. *J Natl Cancer Inst* 75(4):771-7.

Hatch GE, Slade R, Stead AG, Graham JA (1986). Species comparison of acute inhalation toxicity of ozone and phosgene. *J Toxicol Environ Health* 19(1):43-53.

Hatch GE, Wiester MJ, Overton JH 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 international symposium; May 1989; Nijmegen, The Netherlands, Elsevier Science Publishers B. V., Amsterdam, The Netherlands*. pp. 553-560 (*Studies in environmental science* 35).

Hatch GE, Slade R, Harris LP, McDonnell WF, Devlin RB, Koren HS, et al. (1994 Sep). Ozone dose and effect in humans and rats. A comparison using oxygen-18 labeling and bronchoalveolar lavage. *Am J Respir Crit Care Med* 150(3):676-83.

Herbert RA, Hailey JR, Grumbein S, Chou BJ, Sills RC, Haseman JK, et al. (1996 Sep-1996 Oct). Two-year and lifetime toxicity and carcinogenicity studies of ozone in B6C3F1 mice. *Toxicol Pathol* 24(5):539-48.

Hicks JJ, Medina-Navarro R, Guzman-Grenfell A, Wachter N, Lifshitz A (1996 Summer). Possible effect of air pollutants (Mexico City) on superoxide dismutase activity and serum lipoperoxides in the human adult. *Arch Med Res* 27(2):145-9.

Highfill JW, Hatch GE, Slade R, Crissman KM, Norwood J, Devlin RB, et al. (1992). Concentration-time models for the effects of ozone on bronchoalveolar lavage fluid protein from rats and guinea pigs. *Inhalation Toxicology* 4:1-16.

Hoffer E, Baum Y, Tabak A, Frevert C (1999 Sep). Adhesion molecules of blood polymorphonuclear leukocytes and alveolar macrophages in rats: modulation by exposure to ozone. *Hum Exp Toxicol* 18(9):547-51.

Hornof WJ, Schelegle E, Kammerman M, Gunther RA, Fisher PE, Cross CE (1989 Sep). Ozone-induced accelerated lung clearance of <sup>99m</sup>Tc-DTPA aerosol in conscious sheep. *Respir Physiol* 77(3):277-90.

Horstman DH, Folinsbee LJ, Ives PJ, Abdul-Salaam S, McDonnell WF (1990 Nov). 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(5):1158-63.

Hotchkiss JA, Harkema JR, Kirkpatrick DT, Henderson RF (1989a). Response of rat alveolar macrophages to ozone: quantitative assessment of population size, morphology, and proliferation following acute exposure. *Exp Lung Res* 15(1):1-16.

Hotchkiss JA, Harkema JR, Sun JD, Henderson RF (1989b Apr). Comparison of acute ozone-induced nasal and pulmonary inflammatory responses in rats. *Toxicol Appl Pharmacol* 98(2):289-302.

Hu SC, Ben-Jebria A, Ultman JS (1992 Oct). Longitudinal distribution of ozone absorption in the lung: quiet respiration in healthy subjects. *J Appl Physiol* 73(4):1655-61.

Hyde DM, Hubbard WC, Wong V, Wu R, Pinkerton K, Plopper CG (1992 May). Ozone-induced acute tracheobronchial epithelial injury: relationship to granulocyte emigration in the lung. *Am J Respir Cell Mol Biol* 6(5):481-97.

Ichinose T, Sagai M (1989 Dec). Biochemical effects of combined gases of nitrogen dioxide and ozone. III. Synergistic effects on lipid peroxidation and antioxidative protective systems in the lungs of rats and guinea pigs. *Toxicology* 59(3):259-70.

Ichinose T, Sagai M (1992 Sep). Combined exposure to NO<sub>2</sub>, O<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>-aerosol and lung tumor formation in rats. *Toxicology* 74(2-3):173-84.

Ishii Y, Hirano K, Morishima Y, Masuyama K, Goto Y, Nomura A, et al. (2000 Sep). Early molecular and cellular events of oxidant-induced pulmonary fibrosis in rats. *Toxicol Appl Pharmacol* 167(3):173-81.

Iwasaki T, Takahashi M, Saito H, Arito H (1998 Jan). Adaptation of extrapulmonary responses to ozone exposure in conscious rats. *Ind Health* 36(1):57-60.

Jackson RM, Frank L (1984 Mar). Ozone-induced tolerance to hyperoxia in rats. *Am Rev Respir Dis* 129(3):425-9.

Jakab GJ, Hmieleski RR (1988). Reduction of influenza virus pathogenesis by exposure to 0.5 ppm ozone. *J Toxicol Environ Health* 23(4):455-72.

Jakab GJ, Bassett DJ (1990 May). Influenza virus infection, ozone exposure, and fibrogenesis. *Am Rev Respir Dis* 141(5 Pt 1):1307-15.

Joad JP, Bric JM, Pino MV, Hyde DM, McDonald RJ (1993 Jun). Effects of ozone and neutrophils on function and morphology of the isolated rat lung. *Am Rev Respir Dis* 147(6 Pt 1):1578-84.

Kavlock R, Daston G, Grabowski CT (1979 Mar). Studies on the developmental toxicity of ozone. I. Prenatal effects. *Toxicol Appl Pharmacol* 48(1 Pt 1):19-28.

Kavlock RJ, Meyer E, Grabowski CT (1980 Jan). Studies on the developmental toxicity of ozone: postnatal effects. *Toxicol Lett* 5(1):3-9.

Kimmel TA, Chen LC, Bosland MC, Nadziejko C (1997 Jun). Influence of acid aerosol droplet size on structural changes in the rat lung caused by acute exposure to sulfuric acid and ozone. *Toxicol Appl Pharmacol* 144(2):348-55.

Kirschvink N, Fievez L, Bureau F, Degand G, Maghuin-Rogister G, Smith N, et al. (2002 Jan). Adaptation to multiday ozone exposure is associated with a sustained increase of bronchoalveolar uric acid. *Free Radic Res* 36(1):23-32.

Kleeberger SR, Levitt RC, Zhang LY (1993 Jan). Susceptibility to ozone-induced inflammation. I. Genetic control of the response to subacute exposure. *Am J Physiol* 264(1 Pt 1):L15-20.

Kleeberger SR, Levitt RC, Zhang LY, Longphre M, Harkema J, Jedlicka A, et al. (1997 Dec). Linkage analysis of susceptibility to ozone-induced lung inflammation in inbred mice. *Nat Genet* 17(4):475-8.

Kleeberger SR, Longphre M, Tankersley CG (1999 Apr). Mechanisms of response to ozone exposure: the role of mast cells in mice. *Res Rep Health Eff Inst* (85):1-30; discussion 31-6.

Kleinman MT, Phalen RF, Mautz WJ, Mannix RC, McClure TR, Crocker TT (1989 Feb). Health effects of acid aerosols formed by atmospheric mixtures. *Environ Health Perspect* 79:137-45.

Kleinman MT, Bhalla DK, Ziegler B, Bucher-Evans S, McClure T (1993). Effects of inhaled fine particles and ozone on pulmonary macrophages and epithelia. *Inhalation Toxicology* 5:371-88.

Kleinman MT, Mautz WJ, Bjarnason S (1999 Mar). Adaptive and non-adaptive responses in rats exposed to ozone, alone and in mixtures, with acidic aerosols. *Inhal Toxicol* 11(3):249-64.

Kleinman MT, Bufalino C, Rasmussen R, Hyde D, Bhalla DK, Mautz WJ (2000 Sep-2000 Oct). Toxicity of chemical components of ambient fine particulate matter (PM 2.5) inhaled by aged rats. *J Appl Toxicol* 20(5):357-64.

Kobayashi T, Todoroki T, Sato H (1987). Enhancement of pulmonary metastasis of murine fibrosarcoma NR-FS by ozone exposure. *J Toxicol Environ Health* 20(1-2):135-45.

Kodavanti UP, Hatch GE, Starcher B, Giri SN, Winsett D, Costa DL (1995a Feb). Ozone-induced pulmonary functional, pathological, and biochemical changes in normal and vitamin C-deficient guinea pigs. *Fundam Appl Toxicol* 24(2):154-64.

Kodavanti UP, Costa DL, Dreher KL, Crissman K, Hatch GE (1995b Jul). Ozone-induced tissue injury and changes in antioxidant homeostasis in normal and ascorbate-deficient guinea pigs. *Biochem Pharmacol* 50(2):243-51.

Kodavanti UP, Costa DL, Richards J, Crissman KM, Slade R, Hatch GE (1996 Jul-1996 Aug). Antioxidants in bronchoalveolar lavage fluid cells isolated from ozone-- exposed normal and ascorbate-deficient guinea pigs. *Exp Lung Res* 22(4):435-48.

Koike E, Kobayashi T, Nelson DJ, McWilliam AS, Holt PG (1998 Feb). Effect of ozone exposure on alveolar macrophage-mediated immunosuppressive activity in rats. *Toxicol Sci* 41(2):217-23.

Koren HS, Devlin RB, Graham DE, Mann R, McDonnell WF (1989a). 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, Elsevier Science Publishers B. V., Amsterdam, The Netherlands. pp. 745-753 (Studies in environmental science 35).*

Koren HS, Devlin RB, Graham DE, Mann R, McGee MP, Horstman DH, et al. (1989b Feb). Ozone-induced inflammation in the lower airways of human subjects. *Am Rev Respir Dis* 139(2):407-15.

Koren HS, Devlin RB, Becker S, Perez R, McDonnell WF (1991). Time-dependent changes of markers associated with inflammation in the lungs of humans exposed to ambient levels of ozone. *Toxicol Pathol* 19(4 Pt 1):406-11.

Laskin DL, Pendino KJ, Punjabi CJ, Rodriguez del Valle M, Laskin JD (1994 Dec). Pulmonary and hepatic effects of inhaled ozone in rats. *Environ Health Perspect* 102 Suppl 10:61-4.

Laskin DL, Heck DE, Laskin JD (1998 Dec). Role of inflammatory cytokines and nitric oxide in hepatic and pulmonary toxicity. *Toxicol Lett* 102-103:289-93.

Last JA, Warren DL, Pecquet-Goad E, Witschi H (1987 Jan). Modification by ozone of lung tumor development in mice. *J Natl Cancer Inst* 78(1):149-54.

Last JA, Gelzleichter TR, Pinkerton KE, Walker RM, Witschi H (1993a Aug). A new model of progressive pulmonary fibrosis in rats. *Am Rev Respir Dis* 148(2):487-94.

Last JA, Gelzleichter T, Harkema J, Parks WC, Mellick P (1993b Nov). Effects of 20 months of ozone exposure on lung collagen in Fischer 344 rats. *Toxicology* 84(1-3):83-102.

Last JA, Pinkerton KE (1997 Jan). Chronic exposure of rats to ozone and sulfuric acid aerosol: biochemical and structural responses. *Toxicology* 116(1-3):133-46.

Lee JS, Mustafa MG, Afifi AA (1990). Effects of short-term, single and combined exposure to low-level NO<sub>2</sub> and O<sub>3</sub> on lung tissue enzyme activities in rats. *J Toxicol Environ Health* 29(3):293-305.

Lee S-L, Afifi AA, Mustafa MG (1989). Effects of short-term, single and combined exposure of rats to NO<sub>2</sub> and O<sub>3</sub> on lung tissue enzyme activities. *Inhalation Toxicology* 1:21-35.

Li AF, Richters A (1991a Jan-1991a Feb). Ambient level ozone effects on subpopulations of thymocytes and spleen T lymphocytes. *Arch Environ Health* 46(1):57-63.

Li AFY, Richters A (1991b). Effects of 0.7 ppm ozone exposure on thymocytes: in vivo and in vitro studies. *Inhalation Toxicology* 3:61-71.

Long NC, Suh J, Morrow JD, Schiestl RH, Murthy GG, Brain JD, et al. (2001 Oct). Ozone causes lipid peroxidation but little antioxidant depletion in exercising and nonexercising hamsters. *J Appl Physiol* 91(4):1694-700.

Madden MC, Richards JH, Dailey LA, Hatch GE, Ghio AJ (2000 Oct). Effect of ozone on diesel exhaust particle toxicity in rat lung. *Toxicol Appl Pharmacol* 168(2):140-8.

Mariassy AT, Abraham WM, Phipps RJ, Sielczak MW, Wanner A (1990 Jun). Effect of ozone on the postnatal development of lamb mucociliary apparatus. *J Appl Physiol* 68(6):2504-10.

Mautz WB, Nadziejko C (2000). California Air Resources Board. Effects of ozone on proteases and protease inhibitors of the human and rat lung. Sacramento, CA: Research Division; 2000. Contract No. A033-175.

Mautz WJ, Kleinman MT, Phalen RF, Crocker TT (1988). Effects of exercise exposure on toxic interactions between inhaled oxidant and aldehyde air pollutants. *J Toxicol Environ Health* 25(2):165-77.

Mautz WJ, Bufalino C (1989 Apr). Breathing pattern and metabolic rate responses of rats exposed to ozone. *Respir Physiol* 76(1):69-77.

Mautz WJ, Finlayson-Pitts BJ, Messer K, Kleinman MT, Norgren MB, Quirion J (1991). Effects of ozone combined with components of acid fogs on breathing pattern, metabolic rate, pulmonary surfactant composition, and lung injury in rats. *Inhalation Toxicology* 3:1-25.

Mautz WJ, Kleinman MT, Bhalla DK, Phalen RF (2001 Jun). Respiratory tract responses to repeated inhalation of an oxidant and acid gas-particle air pollutant mixture. *Toxicol Sci* 61(2):331-41.

McBride RK, Oberdoerster G, Marin MG (1991 Jun). Effects of ozone on the cholinergic secretory responsiveness of ferret tracheal glands. *Environ Res* 55(1):79-90.

Mercer RR, Anjilvel S, Miller FJ, Crapo JD (1991 May). Inhomogeneity of ventilatory unit volume and its effects on reactive gas uptake. *J Appl Physiol* 70(5):2193-205.

Miller FJ, Illing JW, Gardner DE (1978a). Effect of urban ozone levels on laboratory-induced respiratory infections. *Toxicology Letters* 2:163-9.

Miller FJ, Menzel DB, Coffin DL (1978b Aug). Similarity between man and laboratory animals in regional pulmonary deposition of ozone. *Environ Res* 17(1):84-101.

Miller FJ, Overton JH Jr, Jaskot RH, Menzel DB (1985 Jun). A model of the regional uptake of gaseous pollutants in the lung. I. The sensitivity of the uptake of ozone in the human lung to lower respiratory tract secretions and exercise. *Toxicol Appl Pharmacol* 79(1):11-27.

Miller FJ, Overton JH, Gerrity TR, Graham RC (1988). Interspecies dosimetry of reactive gases. In: Mohr U, Dungworth D, Kimmerle G, Lewkowski J, McClellan R, Stober W, editors. Inhalation toxicology: the design and interpretation of inhalation studies and their use in risk assessment. New York: Springer-Verlag; pp. 139-155.

Miller FJ, Conolly RB (1995). Uncertainties in health risk assessments: commentary on selected issues and research needs. In: Lee, S. D. //Schneider, T. eds. Comparative risk analysis and priority setting for air pollution issue: proceedings of the 4th US-Dutch international symposium; June 1993; Keystone, CO, Air and Waste Management Association, Pittsburg, PA. pp. 76-91.

Miller PD, Gordon T, Warnick M, Amdur MO (1986). Effect of ozone and histamine on airway permeability to horseradish peroxidase in guinea pigs. *J Toxicol Environ Health* 18(1):121-32.

Mills PC, Roberts CA, Smith NC (1996 Sep). Effects of ozone and airway inflammation on glutathione status and iron homeostasis in the lungs of horses. *Am J Vet Res* 57(9):1359-63.

Mochitate K, Ishida K, Ohsumi T, Miura T (1992 Apr). Long-term effects of ozone and nitrogen dioxide on the metabolism and population of alveolar macrophages. *J Toxicol Environ Health* 35(4):247-60.

Mole ML Jr, Stead AG, Gardner DE, Miller FJ, Graham JA (1985 Sep). Effect of ozone on serum lipids and lipoproteins in the rat. *Toxicol Appl Pharmacol* 80(3):367-76.

Musi B, Dell'Omo G, Ricceri L, Santucci D, Laviola G, Bignami G, et al. (1994 Winter). Effects of acute and continuous ozone (O<sub>3</sub>) exposure on activity/exploration and social behavior of CD-1 mice. *Neurotoxicology* 15(4):827-35.

Mustafa MG, Hassett CM, Newell GW, Schrauzer GN (1988). Pulmonary carcinogenic effects of ozone. *Ann N Y Acad Sci* 534:714-23.

Nikula KJ, Wilson DW, Giri SN, Plopper CG, Dungworth DL (1988 May). The response of the rat tracheal epithelium to ozone exposure. Injury, adaptation, and repair. *Am J Pathol* 131(2):373-84.

Oosting RS, van Golde LM, Verhoef J, Van Bree L (1991 Aug). Species differences in impairment and recovery of alveolar macrophage functions following single and repeated ozone exposures. *Toxicol Appl Pharmacol* 110(1):170-8.

Overton JH, Graham RC, Miller FJ (1987 May). A model of the regional uptake of gaseous pollutants in the lung. II. The sensitivity of ozone uptake in laboratory animal lungs to anatomical and ventilatory parameters. *Toxicol Appl Pharmacol* 88(3):418-32.

Overton JH, Graham RC (1989). Predictions of ozone absorption in human lungs from newborn to adult. *Health Phys* 57 Suppl 1:29-36.

Overton JH, Barnett AE, Graham RC (1989). Significances of the variability of tracheobronchial airway paths and their air flow rates to dosimetry model predictions of the absorption of gases. In: Crapo JD; Smolko ED; Miller FJ; Graham JA; Hayes AW; eds. *Extrapolation of dosimetric relationships for inhaled particles and gases*. San Diego: Academic Press, Inc.; pp. 273-291.

Paz C, Bazan-Perkins B (1992 Jun). Sleep-wake disorganization in cats exposed to ozone. *Neurosci Lett* 140(2):270-2.

Paz C, Huitron-Resendiz S (1996 Feb). The effects of ozone exposure on the sleep-wake cycle and serotonin contents in the pons of the rat. *Neurosci Lett* 204(1-2):49-52.

Paz C (1997 Summer). Some consequences of ozone exposure on health. *Arch Med Res* 28(2):163-70.

Pearson AC, Bhalla DK (1997 Feb). Effects of ozone on macrophage adhesion in vitro and epithelial and inflammatory responses in vivo: the role of cytokines. *J Toxicol Environ Health* 50(2):143-57.

Petruzzi S, Fiore M, Dell'Omo G, Alleva E (1995a). Exposure to ozone inhibits isolation-induced aggressive behavior of adult CD-1 male mice. *Aggressive Behavior* 21:387-96.

Petruzzi S, Fiore M, Dell'Omo G, Bignami G, Alleva E (1995b Jul-1995b Aug). Medium and long-term behavioral effects in mice of extended gestational exposure to ozone. *Neurotoxicol Teratol* 17(4):463-70.

Petruzzi S, De Acetis L, Chiarotti F, Sorace A, Alleva E (1999). Limited changes in handedness and morphine reactivity in CD-1 mice after pre- and postnatal ozone exposure. *Acta Neurobiol Exp (Warsz)* 59(2):115-22.

Phipps RJ, Denas SM, Sielczak MW, Wanner A (1986 Mar). Effects of 0.5 ppm ozone on glycoprotein secretion, ion and water fluxes in sheep trachea. *J Appl Physiol* 60(3):918-27.

Pickrell JA, Hahn FF, Rebar AH, Horoda RA, Henderson RF (1987a Apr). Changes in collagen metabolism and proteinolysis after repeated inhalation exposure to ozone. *Exp Mol Pathol* 46(2):159-67.

Pickrell JA, Gregory RE, Cole DJ, Hahn FF, Henderson RF (1987b Apr). Effect of acute ozone exposure on the proteinase-antiproteinase balance in the rat lung. *Exp Mol Pathol* 46(2):168-79.

Pinkerton KE, Brody AR, Miller FJ, Crapo JD (1989 Oct). Exposure to low levels of ozone results in enhanced pulmonary retention of inhaled asbestos fibers. *Am Rev Respir Dis* 140(4):1075-81.

Pino MV, Levin JR, Stovall MY, Hyde DM (1992a Jan). Pulmonary inflammation and epithelial injury in response to acute ozone exposure in the rat. *Toxicol Appl Pharmacol* 112(1):64-72.

Pino MV, Stovall MY, Levin JR, Devlin RB, Koren HS, Hyde DM (1992b Jun). Acute ozone-induced lung injury in neutrophil-depleted rats. *Toxicol Appl Pharmacol* 114(2):268-76.

Plopper CG, Duan X, Buckpitt AR, Pinkerton KE (1994 Jul). Dose-dependent tolerance to ozone. IV. Site-specific elevation in antioxidant enzymes in the lungs of rats exposed for 90 days or 20 months. *Toxicol Appl Pharmacol* 127(1):124-31.

Plopper CG, Hatch GE, Wong V, Duan X, Weir AJ, Tarkington BK, et al. (1998 Sep). Relationship of inhaled ozone concentration to acute tracheobronchial epithelial injury, site-specific ozone dose, and glutathione depletion in rhesus monkeys. *Am J Respir Cell Mol Biol* 19(3):387-99.

Postlethwait EM, Langford SD, Bidani A (1994 Mar). Determinants of inhaled ozone absorption in isolated rat lungs. *Toxicol Appl Pharmacol* 125(1):77-89.

Prasad SB, Rao VS, Mannix RC, Phalen RF (1988). Effects of pollutant atmospheres on surface receptors of pulmonary macrophages. *J Toxicol Environ Health* 24(3):385-402.

Pryor WA, Das B, Church DF (1991 May-1991 Jun). The ozonation of unsaturated fatty acids: aldehydes and hydrogen peroxide as products and possible mediators of ozone toxicity. *Chem Res Toxicol* 4(3):341-8.

Pryor WA (1992). How far does ozone penetrate into the pulmonary air/tissue boundary before it reacts? *Free Radic Biol Med* 12(1):83-8.

Rahman I, Clerch LB, Massaro D (1991 Jun). Rat lung antioxidant enzyme induction by ozone. *Am J Physiol* 260(6 Pt 1):L412-8.

Rahman I, Massaro GD, Massaro D (1992). Exposure of rats to ozone: evidence of damage to heart and brain. *Free Radic Biol Med* 12(4):323-6.

Rajini P, Gelzleichter TR, Last JA, Witschi H (1993 Aug). Alveolar and airway cell kinetics in the lungs of rats exposed to nitrogen dioxide, ozone, and a combination of the two gases. *Toxicol Appl Pharmacol* 121(2):186-92.

Reinhart PG, Bassett DJ, Bhalla DK (1998 May). The influence of polymorphonuclear leukocytes on altered pulmonary epithelial permeability during ozone exposure. *Toxicology* 127(1-3):17-28.

Reiser KM, Tyler WS, Hennessy SM, Dominguez JJ, Last JA (1987 Jul). Long-term consequences of exposure to ozone. II. Structural alterations in lung collagen of monkeys. *Toxicol Appl Pharmacol* 89(3):314-22.

Richters A (1988). Effects of nitrogen dioxide and ozone on blood-borne cancer cell colonization of the lungs. *J Toxicol Environ Health* 25(3):383-90.

Rietjens IM, Van Bree L, Marra M, Poelen MC, Rombout PJ, Alink GM (1985 Dec). Glutathione pathway enzyme activities and the ozone sensitivity of lung cell populations derived from ozone exposed rats. *Toxicology* 37(3-4):205-14.

Rivas-Arancibia S, Vazquez-Sandoval R, Gonzalez-Kladiano D, Schneider-Rivas S, Lechuga-Guerrero A (1998 Jan). Effects of ozone exposure in rats on memory and levels of brain and pulmonary superoxide dismutase. *Environ Res* 76(1):33-9.

Rivas-Manzano P, Paz C (1999 Nov). Cerebellar morphological alterations in rats induced by prenatal ozone exposure. *Neurosci Lett* 276(1):37-40.

Rombout PJA, van Bree L, Heisterkamp SH, Marra M (1989). The need for an eight hour ozone standard. 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, Elsevier Science Publishers B. V., Amsterdam, The Netherlands.* pp. 701-710 (Studies in environmental science 35).

Ross BK, Hlastala MP, Frank R (1979 May-1979 Jun). Lack of ozone effects on oxygen hemoglobin affinity. *Arch Environ Health* 34(3):161-3.

Ryer-Powder JE, Amoruso MA, Czerniecki B, Witz G, Goldstein BD (1988 Nov). Inhalation of ozone produces a decrease in superoxide anion radical production in mouse alveolar macrophages. *Am Rev Respir Dis* 138(5):1129-33.

Sagai M, Ichinose T (1991 Feb). Biochemical effects of combined gases of nitrogen dioxide and ozone. IV. Changes of lipid peroxidation and antioxidative protective systems in rat lungs upon life span exposure. *Toxicology* 66(2):121-32.

Saldiva PH, King M, Delmonte VL, Macchione M, Parada MA, Daliberto ML, et al. (1992 Feb). Respiratory alterations due to urban air pollution: an experimental study in rats. *Environ Res* 57(1):19-33.

Schelegle ES, Walby WF, Alfaro MF, Wong VJ, Putney L, Stovall MY, et al. (2003 Feb). Repeated episodes of ozone inhalation attenuates airway injury/repair and release of substance P, but not adaptation. *Toxicol Appl Pharmacol* 186(3):127-42.

Schlesinger RB, Gorczynski JE, Dennison J, Richards L, Kinney PL, Bosland MC (1992a Jul-1992a Aug). Long-term intermittent exposure to sulfuric acid aerosol, ozone, and their combination: alterations in tracheobronchial mucociliary clearance and epithelial secretory cells. *Exp Lung Res* 18(4):505-34.

Schlesinger RB, Zelikoff JT, Chen LC, Kinney PL (1992b Aug). Assessment of toxicologic interactions resulting from acute inhalation exposure to sulfuric acid and ozone mixtures. *Toxicol Appl Pharmacol* 115(2):183-90.

Selgrade MK, Illing JW, Starnes DM, Stead AG, Menache MG, Stevens MA (1988 Jul). Evaluation of effects of ozone exposure on influenza infection in mice using several indicators of susceptibility. *Fundam Appl Toxicol* 11(1):169-80.

Selgrade MK, Daniels MJ, Grose EC (1990). Acute, subchronic, and chronic exposure to a simulated urban profile of ozone: effects on extrapulmonary natural killer cell activity and lymphocyte mitogenic responses. *Inhalation Toxicology* 2:375-89.

Selgrade MK, Cooper KD, Devlin RB, van Loveren H, Biagini RE, Luster MI (1995 Jan). Immunotoxicity--bridging the gap between animal research and human health effects. *Fundam Appl Toxicol* 24(1):13-21.

Shepson PB, Kleindienst TE, Edney EO, Namie GR, Pittman JH, Cupitt LT, et al. (1985). The mutagenic activity of irradiated toluene/NO<sub>x</sub>/H<sub>2</sub>O/air mixtures. *Environ Sci Technol* 19:249-55.

Sherwood RL, Lippert WE, Goldstein E (1986 Dec). Effect of 0.64 ppm ozone on alveolar macrophage lysozyme levels in rats with chronic pulmonary bacterial infection. *Environ Res* 41(2):378-87.

Sielczak MW, Denas SM, Abraham WM (1983 Apr-1983 Jun). Airway cell changes in tracheal lavage of sheep after ozone exposure. *J Toxicol Environ Health* 11(4-6):545-53.

Sills RC, Hong HL, Greenwell A, Herbert RA, Boorman GA, Devereux TR (1995 Jul). Increased frequency of K-ras mutations in lung neoplasms from female B6C3F1 mice exposed to ozone for 24 or 30 months. *Carcinogenesis* 16(7):1623-8.

Slade R, Highfill JW, Hatch GE (1989). Effects of depletion of ascorbic acid or nonprotein sulfhydryls on the acute inhalation toxicity of nitrogen dioxide, ozone, and phosgene. *Inhalation Toxicology* 1:261-71.

Slade R, Crissman K, Norwood J, Hatch G (1993 Jul-1993 Aug). Comparison of antioxidant substances in bronchoalveolar lavage cells and fluid from humans, guinea pigs, and rats. *Exp Lung Res* 19(4):469-84.

Slade R, Watkinson WP, Hatch GE (1997 Jan). Mouse strain differences in ozone dosimetry and body temperature changes. *Am J Physiol* 272(1 Pt 1):L73-7.

Sterner-Kock A, Kock M, Braun R, Hyde DM (2000 Sep). Ozone-induced epithelial injury in the ferret is similar to nonhuman primates. *Am J Respir Crit Care Med* 162(3 Pt 1):1152-6.

Tepper JS, Costa DL, Lehmann JR, Weber MF, Hatch GE (1989 Aug). Unattenuated structural and biochemical alterations in the rat lung during functional adaptation to ozone. *Am Rev Respir Dis* 140(2):493-501.

Tepper JS, Wiester MJ, Weber MF, Menache MG (1990 Feb). Measurements of cardiopulmonary response in awake rats during acute exposure to near-ambient concentrations of ozone. *J Appl Toxicol* 10(1):7-15.

Thomassen DG, Harkema JR, Stephens ND, Griffith WC (1991 Jun). Preneoplastic transformation of rat tracheal epithelial cells by ozone. *Toxicol Appl Pharmacol* 109(1):137-48.

Tyler WS, Tyler NK, Last JA, Gillespie MJ, Barstow TJ (1988 Jul). Comparison of daily and seasonal exposures of young monkeys to ozone. *Toxicology* 50(2):131-44.

U.S. Environmental Protection Agency (1996). Air Quality Criteria for Ozone and Related Photochemical Oxidants. Volume III. Chapter 8. Extrapolation of Animal Toxicological Data to Humans. EPA/600/P-93/004a-cF p. 8-1 to 8-101. Can be obtained online at: <http://cfpub.epa.gov/ncea/cfm/ozone.cfm?ActType=default>.

Uchiyama I, Simomura Y, Yokoyama E (1986 Dec). Effects of acute exposure to ozone on heart rate and blood pressure of the conscious rat. *Environ Res* 41(2):529-37.

Uchiyama I, Yokoyama E (1989 Feb). Effects of short- and long-term exposure to ozone on heart rate and blood pressure of emphysematous rats. *Environ Res* 48(1):76-86.

Ulrich MM, Alink GM, Kumarathasan P, Vincent R, Boere AJ, Cassee FR (2002 Oct). Health effects and time course of particulate matter on the cardiopulmonary system in rats with lung inflammation. *J Toxicol Environ Health A* 65(20):1571-95.

Umezu T, Shimojo N, Tsubone H, Suzuki AK, Kubota K, Shimizu A (1987 Jan-1987 Feb). Effect of ozone toxicity in the drinking behavior of rats. *Arch Environ Health* 42(1):58-62.

van Bree L, Dormans JA, Boere AJ, Rombout PJ (2001 Aug). Time study on development and repair of lung injury following ozone exposure in rats. *Inhal Toxicol* 13(8):703-18.

van Bree L, Dormans JA, Koren HS, Devlin RB, Rombout PJ (2002 Aug). Attenuation and recovery of pulmonary injury in rats following short-term, repeated daily exposure to ozone. *Inhal Toxicol* 14(8):883-900.

Van Loveren H, Rombout PJ, Wagenaar SS, Walvoort HC, Vos JG (1988 Jul). Effects of ozone on the defense 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(3):374-93.

Van Loveren H, Krajnc EI, Rombout PJ, Blommaert FA, Vos JG (1990 Jan). Effects of ozone, hexachlorobenzene, and bis(tri-n-butyltin)oxide on natural killer activity in the rat lung. *Toxicol Appl Pharmacol* 102(1):21-33.

Vaughan WJ, Adamson GL, Lindgren FT, Schooley JC (1984 Jul). Serum lipid and lipoprotein concentrations following exposure to ozone. *J Environ Pathol Toxicol Oncol* 5(4-5):165-73.

Victorin K, Stahlberg M (1988). A method for studying the mutagenicity of some gaseous compounds in *Salmonella typhimurium*. *Environ Mol Mutagen* 11(1):65-77.

Victorin K (1996). Genotoxicity and carcinogenicity of ozone. *Scand J Work Environ Health* 22 Suppl 3:42-51.

Vincent R, Bjarnason SG, Adamson IY, Hedgecock C, Kumarathasan P, Guenette J, et al. (1997 Dec). Acute pulmonary toxicity of urban particulate matter and ozone. *Am J Pathol* 151(6):1563-70.

Warren DL, Guth DJ, Last JA (1986 Jul). Synergistic interaction of ozone and respirable aerosols on rat lungs. II. Synergy between ammonium sulfate aerosol and various concentrations of ozone. *Toxicol Appl Pharmacol* 84(3):470-9.

Warren DL, Last JA (1987 Apr). Synergistic interaction of ozone and respirable aerosols on rat lungs. III. Ozone and sulfuric acid aerosol. *Toxicol Appl Pharmacol* 88(2):203-16.

Watkinson WP, Aileru AA, Dowd SM, Doerfler DL, Tepper JS, Costa DL (1993). Acute effects of ozone on heart rate and body temperature in the unanesthetized, unrestrained rat maintained at different ambient temperatures. *Inhalation Toxicology* 5:129-47.

Watkinson WP, Wiester MJ, Highfill JW (1995 Mar). Ozone toxicity in the rat. I. Effect of changes in ambient temperature on extrapulmonary physiological parameters. *J Appl Physiol* 78(3):1108-20.

Watkinson WP, Campen MJ, Nolan JP, Costa DL (2001 Aug). Cardiovascular and systemic responses to inhaled pollutants in rodents: effects of ozone and particulate matter. *Environ Health Perspect* 109 Suppl 4:539-46.

Weller BL, Crapo JD, Slot J, Posthuma G, Plopper CG, Pinkerton KE (1997 Nov). Site- and cell-specific alteration of lung copper/zinc and manganese superoxide dismutases by chronic ozone exposure. *Am J Respir Cell Mol Biol* 17(5):552-60.

Wiester MJ, Williams TB, King ME, Menache MG, Miller FJ (1987 Jul). Ozone uptake in awake Sprague-Dawley rats. *Toxicol Appl Pharmacol* 89(3):429-37.

Wiester MJ, Tepper JS, King ME, Menache MG, Costa DL (1988 Oct). Comparative study of ozone (O<sub>3</sub>) uptake in three strains of rats and in the guinea pig. *Toxicol Appl Pharmacol* 96(1):140-6.

Wiester MJ, Tepper JS, Winsett DW, Crissman KM, Richards JH, Costa DL (1996a May). Adaptation to ozone in rats and its association with ascorbic acid in the lung. *Fundam Appl Toxicol* 31(1):56-64.

Wiester MJ, Watkinson WP, Costa DL, Crissman KM, Richards JH, Winsett DW, et al. (1996b Oct). Ozone toxicity in the rat III. Effect of changes in ambient temperature on pulmonary parameters. *J Appl Physiol* 81(4):1691-700.

Wiester MJ, Winsett DW, Richards JH, Jackson MC, Crissman KM, Costa DL (2000 Jul). Ozone adaptation in mice and its association with ascorbic acid in the lung. *Inhal Toxicol* 12(7):577-90.

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

Witschi H (1991 Mar-1991 Apr). Effects of oxygen and ozone on mouse lung tumorigenesis. *Exp Lung Res* 17(2):473-83.

Witschi H, Wilson DW, Plopper CG (1993 Jan). Modulation of N-nitrosodiethylamine-induced hamster lung tumors by ozone. *Toxicology* 77(1-2):193-202.

Witschi H, Espiritu I, Pinkerton KE, Murphy K, Maronpot RR (1999 Dec). Ozone carcinogenesis revisited. *Toxicol Sci* 52(2):162-7.

Wong CG, Bonakdar M, Mautz WJ, Kleinman MT (1996 Feb). Chronic inhalation exposure to ozone and nitric acid elevates stress- inducible heat shock protein 70 in the rat lung. *Toxicology* 107(2):111-9.

Wright ES, Kehrer JP, White DM, Smiler KL (1988 Mar). Effects of chronic exposure to ozone on collagen in rat lung. *Toxicol Appl Pharmacol* 92(3):445-52.

Yokoyama E, Frank R (1972 Aug). Respiratory uptake of ozone in dogs. *Arch Environ Health* 25(2):132-8.

Young C, Bhalla DK (1992 Feb). Time course of permeability changes and PMN flux in rat trachea following O<sub>3</sub> exposure. *Fundam Appl Toxicol* 18(2):175-80.

Yu M, Pinkerton KE, Witschi H (2002 Jan). Short-term exposure to aged and diluted sidestream cigarette smoke enhances ozone-induced lung injury in B6C3F1 mice. *Toxicol Sci* 65(1):99-106.

Zelikoff JT, Kraemer GL, Vogel MC, Schlesinger RB (1991 Dec). Immunomodulating effects of ozone on macrophage functions important for tumor surveillance and host defense. *J Toxicol Environ Health* 34(4):449-67.

## **Glossary**

|                 |   |
|-----------------|---|
| Antagonism      | less than additive effects with co-exposure of two pollutants   |
| Attenuation     | in reference to ozone exposure, a lessening of the effects of ozone as exposure progresses. Also has been referred to as tolerance or adaptation. However, the term attenuation also accounts for some responses, such as lung function, airway reactivity, airway inflammation, and permeability of airway epithelium becoming lessened with continued exposure to ozone, while other responses, such as morphological and biochemical effects, appear to progress with ongoing exposure |
| B cell          | any of the lymphocytes (bone marrow-derived) that have antibody molecules on the surface and comprise the antibody-secreting plasma cells when mature   |
| Carcinogenicity | the origin or production of cancer, including carcinomas and other malignant neoplasms  |
| Central acinus  | or central acinar region. The region of the airway between the distal portion of the terminal bronchiole to the proximal portion of the alveolar duct. Primary site of ozone-induced epithelial injury  |
| Chemotactic     | inducing orientation or movement of an organism or cell in relation to chemical agents  |
| Cytokine        | any class of immunoregulatory substances that are secreted by cells of the immune system. In relation to ozone-induced inflammation, cytokines released are involved in immunoregulation of the inflammatory response   |
| Fibrosis        | a condition in the lung marked by an increase of interstitial fibrous tissue  |
| Genotoxic       | substance or agent capable of damaging the genetic material of a cell. Generally considered the event prior to potential mutagenicity   |
| Hepatocyte      | an epithelial parenchymatous cell of the liver  |
| Histochemistry  | a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues   |
| Hyperplastic    | an abnormal or unusual increase in cells composing a part of a tissue   |
| In situ         | in the natural or original position or place  |

|                        |   |
|------------------------|---|
| In vitro               | outside the living body and in an artificial environment  |
| In vivo                | in the living body of a plant or animal   |
| Lymphocyte             | cells originating from stem cells and differentiating in lymphoid tissue (thymus or bone marrow) that are the typical cellular elements of lymph, include cellular mediators of immunity, and constitute 20 to 30 percent of the leukocytes of normal human blood |
| Mitogen                | substance that induces cell division, or mitosis  |
| Morphometry            | the measurement of the form of organisms or their parts. In relation to the lung, measurement of pulmonary subcompartments or cells types, such as interstitial thickness or volume of epithelial cells types   |
| Mutagen                | a substance or agent capable of damaging DNA such that subsequent divisions of the cell lead to a change in the sequence of base pairs in the chromosomal molecule  |
| Phagocytosis           | the engulfing of particulate matter or debris by a cell, such as the alveolar macrophage  |
| Potentiation           | greater effect with co-exposure of two pollutants, in which one of the pollutants alone would have no measurable effect   |
| Respiratory bronchiole | in higher mammals, the airway generation(s) between the terminal bronchiole and the alveolar duct consisting of conducting airway epithelium with outpockets of alveolar epithelium   |
| Synergism              | greater than additive effects with co-exposure of two pollutants  |
| Tachypneic             | rapid, shallow breathing; a characteristic response of mammalian exposure to high acute levels of ozone   |
| T cell                 | any of several lymphocytes that differentiate in the thymus, possess highly specific cell-surface antigen receptors, and include some that control the initiation or suppression of cell-mediated and humoral immunity and others that lyse antigen-bearing cells |
| Terminal bronchiole    | last conducting airway generation prior to the beginning of the alveolar duct, or in higher mammals, the respiratory bronchiole   |
| Thymocyte              | a cell of the thymus  |

## **Appendix B**

# **Office of Environmental Health Hazard Assessment Recommendations to the ARB for an Ambient Air Quality Standard for Ozone**

## Office of Environmental Health Hazard Assessment

---



Terry Tamminen  
Agency Secretary

Joan E. Denton, Ph.D., Director  
Headquarters • 1001 I Street • Sacramento, California 95814  
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010  
Oakland Office • Mailing Address: 1515 Clay Street, 16<sup>th</sup> Floor • Oakland, California 94612



Arnold Schwarzenegger  
Governor

### Recommendation for an Ambient Air Quality Standard for Ozone

June 2004

---

California Environmental Protection Agency

*The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption.*



Printed on Recycled Paper

# Office of Environmental Health Hazard Assessment



Terry Tamminen  
Agency Secretary

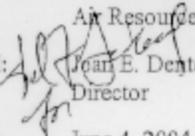
Joan E. Denton, Ph.D., Director  
Headquarters • 1001 I Street • Sacramento, California 95814  
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010  
Oakland Office • Mailing Address: 1515 Clay Street, 16<sup>th</sup> Floor • Oakland, California 94612



Arnold Schwarzenegger  
Governor

## MEMORANDUM

**TO:** Catherine Witherspoon  
Executive Officer  
Air Resources Board

**FROM:**  Joan E. Denton, Ph.D.  
Director

**DATE:** June 4, 2004

**SUBJECT:** RECOMMENDATION FOR AN AMBIENT AIR QUALITY STANDARD  
FOR OZONE

Attached is the Office of Environmental Health Hazard Assessment's (OEHHA) Recommendation for an Ambient Air Quality Standard for Ozone. We are recommending that the current 1-hour average standard of 0.09 parts per million (ppm) be retained and add an 8-hour standard of 0.070 ppm, both not to be exceeded. If the Board does not adopt an 8-hour standard, then the 1-hour standard should be lowered to 0.08 ppm, not to be exceeded.

These recommendations were developed pursuant to Health and Safety Code Section 39606(b), which requires OEHHA to develop health-based recommendations for Ambient Air Quality Standards for the criteria air pollutants. These recommendations will be included in the public review draft of the joint ARB/OEHHA staff report on ozone and has been sent electronically to staff for incorporation into the staff report.

We would like to thank both Research Division and Planning and Technical Support Division staff for working with us to provide information during the development of our recommendations.

Should you have any questions or concerns, please call me at (916) 322-6325.

### Attachment

cc: ✓ Bart Croes, Chief  
Research Division  
Air Resources Board

Robert Fletcher, Chief  
Planning and Technical Support Division  
Air Resources Board

California Environmental Protection Agency

*The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption.*



Printed on Recycled Paper

## **B Office of Environmental Health Hazard Assessment Recommendations to the ARB for an Ambient Air Quality Standard for Ozone**

### **B.1 OEHHA Recommendation for Standard**

This chapter presents the OEHHA recommendations for ozone ambient air quality standards (AAQSs) for California for the Board's consideration. The section begins with findings on the overall adequacy of the current standards for ozone with respect to protecting the health of the public, including infants and children. It continues with recommendations for the pollution indicators, averaging times, forms, and concentrations adequate to protect public health.

The recommended concentrations for the ozone standard should be based on scientific information about the health risks associated with ozone, recognizing the uncertainties in these data. With this in mind, the numerous human chamber and epidemiologic studies of ozone-associated morbidity and mortality indicate that, within the concentration ranges reported, there is no identified "bright line" or threshold ozone concentration below which health effects would not occur in at least some individuals. However, the Children's Environmental Health Protection Act [Senate Bill 25, Escutia; Stats. 1999, Ch. 731, specifically California Health & Safety Code Section 39606(d)(2)] requires a standard that "adequately protects the health of the public, including infants and children, with an adequate margin of safety." In the development of standards, SB25 called for, to the extent that information is available, that the following information be assessed:

1. Exposure patterns among infants and children that are likely to result in disproportionately high exposures relative to the general population
2. Special susceptibility of infants and children to ambient air pollution relative to the general population
3. The effects on infants and children of exposure to ambient air pollution and other substances that have common mechanisms of toxicity
4. The interaction of multiple air pollutants on infants and children, including between criteria air pollutants and toxic air contaminants.

The governing statutory language indicates that California's ambient air quality standards should also protect other vulnerable populations, in addition to infants and children, and the general public [(H&SC sections 39606(d)(2) and 39606(d)(3)]. This legislative directive is consistent with historical practice in California, where ambient air quality standards have been formulated to protect identifiable susceptible subgroups, as well as the general population. For instance, the one-hour sulfur dioxide standard was developed in order to protect the most sensitive recognized subgroup, exercising asthmatics. Nonetheless, even with standards tailored to shield vulnerable populations, there may be exquisitely sensitive individuals remaining outside the ambit of protection.

Although both the California Health & Safety Code (section 39606) and the federal Clean Air Act (section 109) refer to an adequate margin of safety, no specific legislative definition of “adequate” is provided. This judgment is left to the responsible regulatory agencies. As described in the preceding chapters, data from controlled exposure studies demonstrate that some individuals experience ozone-associated toxicity at relatively low concentrations, while several epidemiologic studies suggest a fairly linear relationships between adverse health outcomes and ambient ozone concentrations, with no clear demarcation of a “threshold” level of ozone exposure below which no adverse health effects would ever be expected to occur. The incorporation of a safety margin has been recognized by the California Supreme Court as integral to the process of promulgating ambient air quality standards [Western Oil and Gas Association v. Air Resources Board, 22 ERC 1178, 1184 (1984)]. To the extent that health effects associated with ambient ozone occur at low levels of exposure, and that there is substantial inter-individual variability in response to environmental insults, it is unlikely that any ozone standard will provide universal protection for every individual against all possible ozone-related effects. Thus, in this instance, applying the notion of an “adequate margin of safety” for ozone standards becomes somewhat challenging. Nevertheless, taking into account the limitations of the scientific data, we have operationalized the concept of an adequate margin of safety by recommending standards that, when attained, should protect nearly all of the California population, including infants and children, against ozone-associated effects throughout the year.

The Children’s Environmental Health Protection Act required the ARB and OEHHA to review all health-based ambient air quality standards to determine whether the standards were protective of the health of the public, including infants and children, with an adequate margin of safety. The Act also required that, depending on the outcome of these reviews, the various ambient air quality standards be prioritized for full review and possible revision. Five factors were considered in assessing the health protectiveness of each ambient air quality standard during the prioritization process:

- 1) The extent of the evidence of effects reported to occur at or near the existing ambient air quality standard.
- 2) The nature and severity of those effects.
- 3) The magnitude of risk of effects anticipated when ambient (outdoor) levels are at or near the level of the existing standard.
- 4) Any evidence indicating that children may be more susceptible to effects than adults.
- 5) The degree of outdoor exposure in California relative to the level of the standard.

Following these reviews, the various ambient air quality standards were prioritized for full review (California Air Resources Board and Office of Environmental Health Hazard Assessment 2000). The standard for ozone was

prioritized to undergo full review after the standards for particulate matter and sulfates. The SB25 review found that several clinical and epidemiological studies suggested effects of ozone exposure on lung function, asthma exacerbation, and other indices of acute respiratory morbidity in children and adults at concentrations at or below that of the current State standard of 0.09 ppm, averaged over one hour. Such evidence could indicate the need for a more stringent standard, an averaging time different from the current one-hour average, or both.

## **B.2 Defining an Adverse Effect**

A key issue in evaluating the public health consequences of ozone exposure is consideration of the definition of an “adverse health effect”. The term “adverse health effect” is incorporated in the legislative background of the Federal Clean Air Act, as well as the California Health and Safety Code, although neither provides a definition for the term. Because it is helpful to the standard review process to consider the available scientific literature in the context of guidelines as to what is meant by the term, we have used guidelines published by the Scientific Assembly for Environmental and Occupational Health of the American Thoracic Society, which developed the most commonly used guidelines in the US (American Thoracic Society 1985; American Thoracic Society 2000)). Both USEPA and ARB have referred to these guidelines over the intervening years in assessing the significance of pollutant-associated physiological, biological or pathological changes.

It is important to keep in mind the differences between statistical significance and medical or biological significance when considering what constitutes an adverse health effect. The 1985 ATS statement defined “adverse respiratory health effects” as medically significant physiologic or pathologic changes generally evidenced by one or more of the following: (1) interference with the normal activity of the affected person or persons, (2) episodic respiratory illness, (3) incapacitating illness, (4) permanent respiratory injury, and/or, (5) progressive respiratory dysfunction. The 2000 ATS statement expanded on the 1985 statement to include consideration of biomarkers, quality of life, physiological impact, symptoms, clinical outcomes, mortality, and population health versus individual risk when evaluating whether or not a change should be designated as an adverse health effect. The 2000 ATS review committee’s recommendations are summarized here:

1. *Biomarkers*: These should be considered, however it must be kept in mind that few biomarkers have been validated sufficiently to establish their use for defining a point at which a response becomes adverse, consequently, not all changes in biomarkers should necessarily be considered adverse.
2. *Quality of life*: In recent years, decreased health-related quality of life has become widely accepted as an adverse health effect. The review committee concluded that reduction in quality of life, whether in healthy persons or persons with chronic respiratory disease, should be considered as an adverse effect.

3. *Physiological impact:* The committee recommended that small, transient reductions in pulmonary function should not necessarily be regarded as adverse, although permanent loss of lung function should be considered adverse. The committee also recommended that reversible loss of lung function in conjunction with symptoms should be considered adverse.
4. *Symptoms:* Air pollution-related symptoms associated with reduced quality of life or with a change in clinical status (i.e., requiring medical care or a change in medications) should be considered adverse at the individual level. At the population level, the committee suggested that any detectable increase in symptom frequency should be considered adverse.
5. *Clinical outcomes:* Detectable effects of air pollution on clinical measures should be considered adverse. More specifically, the ATS committee cited as examples increases in emergency department visits for asthma or hospitalizations for pneumonia, at the population level, or an increased need to use bronchodilator medication, at the individual level. The committee recommended that: “no level of effect of air pollution on population-level clinical indicators can be considered acceptable.”
6. *Mortality:* Increased mortality should clearly be judged as adverse.
7. *Population health versus individual risk:* The committee concluded that a shift in risk factor distribution, and hence the risk profile of an exposed population, should be considered adverse when the relationship between the risk factor and the disease is causal, even if there is no immediate occurrence of obvious illness.

Based on these recommendations, many health outcomes found to be associated with ozone could be considered adverse including pulmonary function changes accompanied by symptoms, pulmonary function changes and respiratory symptoms that reduce quality of life, large changes in pulmonary function, clinical outcomes such as emergency department visits for asthma, hospitalization for respiratory and cardiovascular disease, and mortality. In additions, outcomes such as increase in airway reactivity and inflammation may be considered adverse if they signify increases in the potential risk profile of the population.

## **B.3 Summary of the Scientific Evidence**

### **B.3.1 Summary of Findings from Chamber Studies**

#### *B.3.1.1 Exposure protocol and effective dose*

Acute respiratory responses to inhaled ozone are roughly proportional to the “effective dose” (ED) of inhaled ozone. ED is defined as the simple product of ozone concentration, ventilation rate and duration of exposure. The concept has been refined to indicate that ozone concentration is the most significant of the three factors, explaining the largest share of the variance in responses. Ventilation rate explained the second largest portion, followed by exposure duration. Subsequent investigations revealed that increased ventilation rate

accentuated the observed pulmonary response at any given ozone concentration, and lowered the minimum ozone concentration at which significant pulmonary responses were evident. Further, there is a positive correlation between ozone concentration and the rate at which adverse responses develop: the higher the ozone concentration, the more rapidly adverse effects become apparent. Consequently, a large number of exposure scenarios, based on varied ozone concentrations, ventilation rates, and durations, could be developed that are likely to induce adverse health effects. An exposure scenario that has been used repeatedly in chamber studies over the last decade, involves multi-hour exposure durations of 6.6 to 8 hours to a constant level of ozone.

Ozone concentrations are highest outdoors, since there are few indoor sources of ozone, with varying penetration (20 to 80%) to indoor environments. Greater penetration occurs with open windows and doors and in the absence of air conditioning. Consequently, individuals at greatest risk of experiencing adverse health consequences from ozone exposure are those who spend prolonged periods of time outdoors while participating in activities that increase the breathing rate. This group is comprised primarily of children, outdoor workers and recreational and professional athletes. Thus, in order to emulate these likely exposure patterns, participants in most chamber studies have included healthy, exercising young adults (ages approximately 18 to 35) as part of the experimental protocol.

At this time, the susceptibility of certain subgroups, such as asthmatics, although not clearly demonstrated in experimental settings, can be inferred from results of both chamber studies and epidemiological studies. The range of responses to ozone exposure in people with compromised health status is largely unknown, although there is a growing body of literature addressing the responses of mild to moderate asthmatics. At near-ambient ozone concentrations, the asthmatics studied have typically had changes in symptoms and lung function in the same ranges as nonasthmatics. However, some studies have shown that asthmatics have experienced larger increases in airway reactivity and inflammation than healthy, nonasthmatic people. These ozone-associated changes are superimposed on pre-existing chronic airway inflammation and elevated airway responsiveness that are hallmarks of asthma. Furthermore, significant decrements in FEV1 in an asthmatic would lead to increased medication use including inhaled steroids (National Asthma Education and Prevention Program 2002). This would qualify as an adverse effect based on ATS and suggests that asthmatics may represent a sensitive subpopulation for ozone.

Because of ethical and major logistical considerations, there are few studies of individuals with cardiovascular disease or COPD. However, since seriously impaired individuals are unlikely to spend significant periods of time outdoors working or exercising, their response to ozone is unlikely to be well characterized in the multi-hour chamber studies. (However, some epidemiological studies are likely to include these potentially sensitive individuals.) Therefore, the findings derived from the clinical literature are likely representative of people who are physically able to perform moderate exertion for several hours, and by extension,

likely to experience the greatest ozone exposures from active outdoor work or play for multi-hour periods.

### *B.3.1.2 Changes in Pulmonary Function*

Collectively, the available literature exploring the responses of primarily healthy, young human subjects exposed to controlled concentrations of ozone indicates that one- to three-hr exposures to ozone concentrations as low as 0.12 ppm with moderate to heavy exercise can induce decrements in pulmonary function and increases in respiratory symptoms for some subjects. Statistically significant group mean decrements in lung function have been reported at ozone concentrations of 0.12 ppm, but there are no studies that show group mean differences below this level. For example, Horstman et al. (1990) and McDonnell et al. (1991) reported no statistically significant change in FEV<sub>1</sub> after a 1-hour exposure to 0.10 ppm (as part of a multi-hour exposure). The group mean responses with short exposures to 0.12 ppm ozone have been relatively small – about a 3 to 5 percent decrement in FEV<sub>1</sub>. However, the studies at 0.12 indicate that some individuals responded with large reductions in lung function. For example, as reported by McDonnell et al. (1983), McDonnell et al. (1985b) and Gong et al. (1986), the maximum individual decrements were 16, 21, and 29%, respectively.

These results illustrate that, in the controlled exposure studies, a modest to moderate percentage of volunteer subjects experience decrements in lung function (often accompanied by increases in symptoms) that are markedly greater than the rest of the study populations (McDonnell et al. 1983; McDonnell et al. 1991). While the notion of ozone “responders” and “nonresponders” has existed for many years, the constitutional factors that determine such responsiveness are largely unknown, except that increasing age among adults is associated with decreasing functional and symptomatic responses to ozone. Repeated exposures of the same individuals at intervals of up to a year or more indicate that ozone responsiveness is an intrinsic individual characteristic, which is likely related to genetic polymorphisms, possibly those involved in anti-oxidant defenses. In the 1987 review of the ozone standard, the Department of Health Services stated that such responders, although they could not yet be identified a priori, represented a subpopulation warranting protection by the ozone AAQS (California Department of Health Services 1987)

Concern about the impacts of longer averaging times led to studies in healthy adults who performed a protocol simulating a day of active outdoor work or play. These studies demonstrate that statistically significant group mean decrements in FEV<sub>1</sub> occur at 6.6 to 8-hour ozone concentrations as low as 0.08 ppm. The importance of multi-hour exposures was discussed in the review of the chamber studies, which clearly indicate an increasing response after the third hour of exposure. Except for one unpublished study, ozone concentrations between 0.04 and 0.08 ppm have not been investigated with multi-hour exposure protocols. Although the group mean effect on FEV<sub>1</sub> is relatively small in these studies of multi-hour exposures at 0.08 ppm (from approximately 2 to 8% with a median decrement of 3.5%), the evidence indicates that some individuals

experience large changes. For example, as indicated by Folinsbee et al. (1991), 26% of the subjects had FEV<sub>1</sub> decrements greater than 10% while about 10% had decrements greater than 30%. These data demonstrate that significant lung function decrements coupled with increased reporting of symptoms such as cough or pain upon deep inspiration can occur in certain individuals when they undergo multi-hour exposures to 0.08 ppm ozone. Thus, based on the recommendations of ATS, these outcomes should be labeled as adverse. In addition, further decrements in those with already compromised lung function, such as asthmatics, should be considered adverse. Finally, Adams (1998) tested 30 subjects at 6.6 hours exposure to 0.06 ppm. At this concentration, the changes in FEV<sub>1</sub> or symptoms were not statistically different relative to clean air, though some differences in group response were observed. However, five of the 30 subjects had FEV<sub>1</sub> decrements greater than 10%. The paper did not report whether these same individuals experienced symptoms or not so it is not clear whether these outcomes should be labeled as adverse, based on ATS recommendations.

#### *B.3.1.3 Symptoms*

Significantly increased symptoms of respiratory irritation have been reported with 1 to 3 hr exposures with moderate exercise at ozone concentrations as low as 0.12 ppm in healthy adults. Specifically, McDonnell et al. (1983) reported associations with cough at 0.12 ppm, and with shortness of breath and pain upon deep inspiration at 0.24 ppm, while Seal et al. (1993) reported increased cough at 0.18 ppm, but not lower. At 6.6 hours of exposure to 0.08 ppm ozone with moderate exercise, increases in cough, shortness of breath and pain on deep breath (McDonnell et al. 1991) and increases in total symptom score (but not pain on deep breath) (Adams 2002) were reported.

#### *B.3.1.4 Nonspecific Airway Responsiveness*

Increased nonspecific airway responsiveness, referring to the tendency of the airways to constrict in reaction to exposure to irritant chemicals, pharmaceutical spasmogens, or physical stimuli such as cold air, has been reported with one- to three- hr exposures to 0.40, but not 0.20 ppm ozone at rest. The lowest short-term ozone concentration at which an increase in nonspecific airway responsiveness has been reported in exercising subjects is 0.18 ppm, but there was no change at 0.12 ppm compared to FA exposure. Exposures to ozone concentrations as low as 0.08 ppm for 6.6 hr can increase nonspecific airway hyperresponsiveness.

#### *B.3.1.5 Airway Inflammation*

Increased levels of cellular (i.e., neutrophils) and various biochemical (i.e., lactate dehydrogenase and other proteins) indicators of airway inflammation have been observed following 1 to 3 hr exposures of healthy adults to 0.20, 0.30 and 0.40 ppm ozone with heavy exercise. There are no studies that have investigated airway inflammation after 1 to 3 hr exposures at ozone concentrations lower than 0.20 ppm. Analysis of BALF after 6.6-hr exposures with moderate exercise to 0.08 and 0.10 ppm ozone has demonstrated both cellular and biochemical

evidence for airway inflammation. Possible inflammatory effects of ozone concentrations lower than 0.08 ppm for 6.6 hr or longer have not been investigated.

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

#### *B.3.1.6 Pollutant Mixtures*

Although there are isolated findings to the contrary, the published data do not support the likelihood of clinically meaningful interactions in human subjects between ozone and gaseous nitrogen-based air pollutants, SO<sub>2</sub> or H<sub>2</sub>SO<sub>4</sub> aerosols at concentrations in the ambient range. Observed responses at the pollutant concentrations studied to date appear to be attributable to the O<sub>3</sub> in the mixture. Research also suggests that pre-exposure to fog (water or nitric acid) may mitigate the effects of subsequent ozone exposure, although inhalation of nitric acid gas had no effect on responses to ozone. There is evidence that concurrent exposures to high concentrations of PAN and ozone result in pulmonary function and symptom responses somewhat larger than those observed following exposure to the same concentration of O<sub>3</sub> alone. However, typical ambient PAN concentrations are considerably lower than those utilized in these studies. Consequently, even if ozone and PAN do interact in their effects on pulmonary function at high concentrations, it is unlikely that PAN contributes significantly to adverse health effects in healthy young and older adults at concentrations in the ambient range. There have been few human exposure studies on mixtures of ozone with particulate matter, with the exception of H<sub>2</sub>SO<sub>4</sub> aerosol.

An early report demonstrated that ozone exposure at 0.12 ppm at rest for one hour resulted in an increase in allergic asthmatics' sensitivity to the effects of subsequent exposure to allergen. Although two separate studies failed to replicate these results, other studies suggest that higher exposure concentrations (i.e., above 0.20 ppm) can result in allergic asthmatics' requiring a lower dose of allergen to produce a given degree of airway hyperresponsiveness.

#### *B.3.1.7 Effect Modifiers*

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

Data addressing the issue of age-related responsiveness to ozone are limited to studies that investigated pulmonary function and symptoms. The few data available do not identify children or adolescents as being either more or less responsive than young adults who have undergone similar exposure protocols, although children tend to report fewer symptoms (McDonnell et al. 1985a). The lack of symptoms reported by children suggests a lower level of somatic awareness of pain/discomfort among children, which might result in their failure to curtail exposure in real-life situations. In contrast, after about age 30 pulmonary function changes due to ozone exposure become progressively smaller (Drechsler-Parks et al. 1987; Drechsler-Parks et al. 1989; Seal et al. 1993). Middle-aged and older adults also tend to report few symptoms, even with exposure to ozone concentrations in excess of 0.4 ppm, while young adults are symptomatic following exposures at that level. Although children and adolescents do not appear to experience greater adverse responses than adults who complete similar exposures, they are among those most likely to spend significant periods of time outdoors while engaged in exercise, putting them at increased risk of adverse responses. There is no information available on other endpoints, such as airway inflammation or airway hyperreactivity, other than for young adults.

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

Though a variety of factors have been examined to explain differences in responsiveness to acute ozone exposure, only current smoking and increasing age have been linked with airway responsiveness, both in an inverse direction. This reduced responsiveness in smokers may wane after smoking cessation (Emmons and Foster 1991).

#### *B.3.1.8 Relationship between Short-Term Effects and Long-Term Outcomes*

The results of controlled human exposure studies utilizing ozone exposures up to about eight hr have clearly established that ozone induces acute responses that qualify as adverse and raise concern that residual effects from repeated acute exposures could accumulate over time and lead to chronic effects or disease. However, practical and logistic considerations are such that controlled human exposure studies are unable to shed light on the impact of long-term exposures to ozone. What is known about long-term exposures comes from results of both epidemiological and animal studies. There are limitations to both of these bodies of literature that cannot be fully overcome, but they do provide some guidance into evaluating the likelihood for chronic effects from ozone exposure. Only a few epidemiological studies have followed a cohort over a long period of time (i.e., several years). In addition, it is difficult to characterize long-term exposure to ozone because of the lack of high penetration rates into the indoor environment. Therefore, results from these studies of long-term exposure could be reviewed as suggestive. Animal toxicology studies are limited by incomplete knowledge of species sensitivity and dosimetry patterns compared to humans, although they

can offer controlled experimental conditions for chronic exposures, provide evidence of causal relationships, and also allow investigation of endpoints not possible to study in humans.

#### *B.3.1.9 Concentrations where adverse effects have been observed*

Taken together and using the ATS criteria for adverse health effects, many health outcomes found to be associated with ozone in chamber studies could be considered adverse including pulmonary function changes accompanied by symptoms, pulmonary function decrements and respiratory symptoms that reduce quality of life, and large changes in pulmonary function. In addition, outcomes such as increase in airway reactivity and inflammation may be considered adverse if they signify increases in the potential risk profile of the population exposure to ozone. These adverse outcomes are demonstrated among exercising individuals exposed to a 1-hour ozone concentration of 0.12 ppm and an 8-hour concentration of 0.08 ppm. At multi-hour exposures to 0.08 ppm, some individuals experienced both large changes in lung function and increases in respiratory symptoms. For asthmatics, a repeated decrease in FEV<sub>1</sub> of 20 to 30% could necessitate medical intervention through low-dose inhaled corticosteroids (National Asthma Education and Prevention Program 2002), which clearly qualifies as an adverse effect.

#### **B.3.2 Summary of Findings from Toxicological Studies**

Animal toxicological studies have shown that chronic ozone exposure can induce morphological changes throughout the respiratory tract, particularly at the junction of the conducting airways and the gas exchange zone in the deep lung. The morphological changes found in animals following chronic ozone exposures are similar to those characteristic of respiratory bronchiolitis, which may progress to fibrotic lung disease (Last et al. 1994; Reiser et al. 1987). The exposure concentrations that have caused morphological changes in these animal studies are typically considerably higher than ambient levels; however, uncertainties about low-dose extrapolation and animal-to-human extrapolation of the results make it unclear whether similar tissue changes also occur in humans with chronic exposures to ambient concentrations of ozone. Interestingly, morphological damage has been reported in rats exposed to 0.50 and 1.0 ppm ozone for 20 months, but not 0.12 ppm, while there were no alterations in pulmonary function with any exposure (Catalano et al. 1995; Pinkerton et al. 1998; Pinkerton et al. 1995; Szarek et al. 1995). Studies on monkeys exposed to ozone at 0.15 ppm for 8 hr/d for 6 to 90 days showed significant distal airway remodeling, with the morphological changes consistent with incipient peribronchiolar fibrogenesis (Harkema et al. 1993). There is some evidence from primate studies that intermittent challenge with a pattern of ozone exposure designed to simulate seasonal episodes, with extended periods of clean air in between extended periods of ozone exposure led to greater injury than regular exposures to similar conditions (Tyler et al. 1988).

A series of studies in monkeys has demonstrated that cyclic multi-day exposures to relatively high ozone concentrations (0.5 ppm) can impact development of the

lung. Cyclic exposure to ozone and to ozone plus house dust mite allergen (HDMA) alters the development of the tracheal basement membrane zone (BMZ) (Evans et al. 2003). The BMZ is important to the tracheal epithelial functioning as it serves as the attachment point for the epithelial cells, functions as a barrier to foreign substances, and is intimately involved in cell-to-cell communication. The BMZ is important to normal growth and development of the airway including storage and release of growth factors. (Schelegle et al. 2003) also noted that ozone in combination with airborne allergen (dust mite allergen, HDMA) can amplify the immune response to allergens in sensitized infants, resulting in an allergic phenotype airway. This phenotype was characterized by increased HDMA-induced histamine release as measured by serum histamine, elevated BAL eosinophils, and increased airway resistance and reactivity. The increased levels of serum HDMA-specific IgE is consistent with the concept that ozone may prime the developing immune system towards a Th2-type response.

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

The animal data provide a biologically plausible basis for considering that repeated inflammation associated with exposure to ozone over a lifetime may result in sufficient damage to the respiratory tissue such that individuals may experience some degree of chronic lung injury. However, uncertainties in interspecies extrapolation, and the use of high ozone concentrations in the animal studies compared to current ambient concentrations, pose difficulties in developing a quantitative relationship for chronic effects.

### **B.3.3 Summary of Findings from Epidemiologic Studies**

The experimental studies such as the chamber studies reported in this document provide valuable information about the acute effects of ozone exposure in humans under controlled conditions. Epidemiologic studies have added to that evidence by evaluating short-term effects of ozone on lung function and respiratory symptoms in free-living populations. As such, epidemiologic studies are able to examine a wide range of individuals, behaviors, subgroups, and exposure conditions. The studies have been able to provide information on the effects of short-term ozone exposure on acute mortality, emergency department visits and hospitalizations. In addition, epidemiologic studies supply evidence of associations between adverse health outcomes and longer-term (i.e., a year or more) exposures to ozone.

There are some limitations to epidemiologic studies. Firstly, it is not possible to characterize exposure in a precise manner similar to that of a chamber study.

Most of the epidemiologic studies rely on regional air pollution monitors, which may not reflect the true exposures at the residences of the study subjects. For ozone and other gases this may be an issue of significant exposure mismeasurement since some limited evidence suggests a low correlation between personal exposure and ambient concentrations of ozone (Sarnat et al. 2001). This finding is contradicted, however, by evidence from Linn et al. (1996) which reported a relatively high correlation ( $r = 0.61$ ) between ozone measured from a personal badge and from a fixed site monitor in a study in Southern California. In addition, study subjects move around from place to place during the day, so one measurement will not adequately reflect overall exposure. Secondly, epidemiologic studies may be subject to bias from uncontrolled or poorly controlled confounders such as seasonality, weather and co-pollutants. However, time series studies which examine the association between health and air pollution at a given site over a designated period of time (from several months to years) have employed sophisticated modeling techniques including non-parametric and parametric smoothing in an attempt to control for these potential confounders. However, ozone presents a particular challenge because of its seasonal nature and high correlation with temperature. More recent studies appear to be successful in addressing some of these limitations. Thirdly, the epidemiologic studies in this review used different averaging times of ozone for their exposure measurements. Many used a 1-hour maximum while others reported results for 8-hour or 24-hour average levels. Since these metrics tend to be highly correlated, if there is a positive association between ozone and a given health effect, it is difficult to attribute the effect to a precise averaging time.

Despite these limitations, a large number of studies published in the last several years have shown positive associations between ozone levels and several health effects including overall mortality, cardiopulmonary mortality, decreased lung function, respiratory symptoms, and emergency visits for asthma. The overall findings from these studies are supported by the consistency of effects, many of which are seen in the summer season only, the biological plausibility obtained from animal studies, and the finding of a concentration-response relationship in many of the studies. Thus, it is difficult to use these studies to determine a low or no effects level useful for standard setting. However, these studies often provide useful information on the type and magnitude of health effects associated with exposure to ozone and provide information on concentration-response relationships. As such, they contribute to important considerations of margin of safety and to the calculations of the potential benefits of controlling ozone. While any given epidemiologic study may have some limitations, taken together these studies provide a strong case for a causal relationship between ambient ozone and a suite of adverse health outcomes. A summary of the most important findings is presented here.

#### *B.3.3.1 Field Studies Addressing Acute Respiratory Effects of Ozone*

Nine of 11 newer studies presented in this document that tested for effects of ozone on lung function reported significant associations, although there were several inconsistent findings. In another particularly relevant study, investigators

measured lung function before and after outdoor summer work shifts on a group of 58 berry pickers, ages 10 to 69, in Fraser Valley, British Columbia (Brauer et al. 1996). These workers had an extended exposure period outdoors and elevated levels of exertion throughout exposure. Statistically significant changes in several measures of lung function were reported. Thus, this study suggests that, as demonstrated in the chamber studies, multi-hour exposures to ozone combined with exercise can generate enhanced response to ozone. There is some possibility of greater responsiveness in this cohort due to a generally less advantaged health and social status.

Among the 12 studies reporting results for daily symptoms, seven reported associations with ozone that appear fairly robust; two of those seven were conducted in the United States. One of the largest and best conducted studies was that of Gent and colleagues (Gent et al. 2003), where 271 asthmatic children under age 12 living in southern New England were each followed over six months (April through September) for daily symptoms. Significant effects of lag 1 daily maximum 1-hour and 8-hour ozone were observed for a variety of respiratory symptoms, including chest tightness and shortness of breath, in the group who used maintenance asthma medications (n=130). The effects of ozone, but not PM<sub>2.5</sub>, remained significant and even increased in two-pollutant models. Significant associations, such as with chest tightness were observed at 52 ppb or higher for both the 1- and 8-hour averages of ozone. However, there was no measurement of sulfate, which may have high temporal correlation with ozone in this region.

Absence from school was associated with ozone concentrations in a study of 1,933 fourth grade students from 12 southern California communities participating in the Children's Health Study (Gilliland et al. 2001). They found an 83% increase for absences due to respiratory disease and a 37% increase for non-respiratory causes per 20 ppb rise in 10am-6pm ozone concentrations. A wide range of exposures were captured while staying below the highest levels observed in the summer season.

#### *B.3.3.2 Effects of Ozone on Daily Hospital Admissions and Emergency Department visits*

Large, multi-city studies of hospital admissions have reported significant ozone associations with total respiratory hospitalizations (Burnett et al. 1997) and chronic obstructive pulmonary disease (Anderson et al. 1997). The largest such study to date was carried out using all-age respiratory hospital admissions from 16 Canadian cities from 1981-1991 (Burnett et al. 1997). Pooling the 16 cities, a significant positive association was observed between respiratory hospital admissions and lag 1 daily 1-hour maximum ozone concentration in spring and summer. There was no evidence of an ozone effect in the winter season. Other ozone metrics were also evaluated. However, the 1-hour maximum had the strongest associations with admissions. Other studies, such as the analysis of six European cities (Anderson et al. 1997) have found stronger effects in the summer or warm seasons. Many of the individual city studies have reported associations with total respiratory admissions and a few with asthma. In the

case of emergency department (ED) studies, asthma has been studied most often, with variable results. An important consideration in determining whether a safe level of ozone can be identified is whether the concentration-response (C-R) relationship is linear across the full concentration range or instead shows evidence of a threshold. Several studies on ED visits for asthma that have examined the impacts of increasing intervals of exposure report a non-linear response consistent with a potential threshold. The lowest effect level appears to be somewhere between 75 and 110 ppb 1-hour ozone. This range corresponds roughly with an 8-hour concentration of 90 to 130 ppb. The one study of emergency room visits that used 8-hour ozone (Tolbert et al. 2000) reported elevated (but not statistically significant) risks for concentrations starting in the interval of 70 to 80 ppb, with a more consistent response in the interval from 90 to 100 ppb, and statistical significance attained for the interval between 100 and 113 ppb 8-hour ozone. As noted above, due to the high correlation among ozone concentrations at varying averaging times, it is difficult to ascribe an effect solely to a one-hour or 8 hour ozone exposure.

#### *B.3.3.3 Ozone and Acute Mortality*

Though limited in some ways, a large and growing body of data now exists examining the association between daily mortality and ozone concentrations. These data support a preliminary conclusion that warm season ozone concentrations represent an independent risk factor for premature mortality, controlling for weather effects and other air pollutants. The most robust data on ozone effects on mortality come from the National Mortality and Morbidity Air Pollution Study (NMMAPS), a study of mortality in the largest 90 cities in the U.S. which was reanalyzed in 2003 using non-GAM methods (Dominici, 2003). Several other studies conducted both within the U.S. (Moolgavkar et al. 1997) and outside of the U.S. (Hoek et al. 2000; Simpson et al. 1997; Goldberg et al. 2003; Goldberg MS 2003; Vedal et al. 2003) reported larger excess mortality risks in the warm (or summer) season than in the cool (or winter) season. While there is a real potential for the occurrence of these outcomes, based on the inflammatory response generated from ozone exposure, additional studies need to be conducted to ensure that: (1) ozone is not confounded by other pollutants including particulate matter (PM10 and PM2.5); (2) ozone is not confounded by temperature and season using parametric (versus non-parametric) generalized linear models; and (3) personal exposure to ozone is sufficiently related to ambient concentrations of ozone. Finally, the ozone-specific models need to undergo the thorough sensitivity analysis of their results similar to that undertaken for studies on particulate matter.

#### *B.3.3.4 Effects of Long-Term Ozone Exposures*

Epidemiology has a key role to play in addressing the health impacts of long-term ozone exposures in humans, since it is impractical to study these effects using controlled human exposure studies. In recent years the following outcomes have been evaluated with respect to long-term ozone exposure: respiratory inflammation, lung function and respiratory symptoms, long-term mortality risks, growth or decline of lung function over many years, and asthma prevalence.

For example, Kinney et al. (1996) found greater cell damage, measured in bronchoalveolar lavage (BAL) fluids collected in summer compared with those collected in winter among adult joggers. Kinney and Lippmann (2000) found a larger decline in FEV<sub>1</sub> among subjects who had trained in high versus moderate ozone regions.

The results of studies of lung function and long-term ozone exposure have been variable. For example, Peters et al. (1999) found evidence for lung function declines in females but not males living in high ozone cities. In a longitudinal analysis of lung function growth in the fourth grade, decrements in lung function growth were associated with particulate matter and NO<sub>2</sub>, but not with ozone (Gauderman et al. 2000). Finally, studies of college students have shown decrements in lung function among students who had lived in areas with higher ozone (Galizia and Kinney 1999; Tager et al. 1998; Kunzli et al. 1997).

Two recent reports from longitudinal cohort studies have reported associations between the onset of asthma and long-term ozone exposures (Abbey et al. 1999; McConnell et al. 2002).

Finally, there is inconsistent and inconclusive evidence for a relationship between long-term ozone exposure and increased mortality risk (Abbey et al. 1999; Pope et al. 2002). However the Pope study of 500,000 members of the American Cancer Society cohort did find that the association between cardiopulmonary mortality and July-September daily 1-hour maximum ozone was positive and nearly significant.

#### **B.4 Consideration of Infants and Children**

As noted earlier, SB25 specifically asks that OEHHA assess the proposed standard in light of four factors related to infants and children, to the extent that information is available.

1. Exposure patterns among infants and children that are likely to result in disproportionately high exposures relative to the general population

As indicated above, children who are outdoors for extended periods of time, particularly while engaged in physical activity that increases their breathing rate, should be considered as a potentially susceptible subpopulation. Under these circumstances, their effective dose of ozone would be disproportionately high relative to the general population. Infants and children inhale more air per unit body weight than adults, even at rest. Thus, young children and infants experience a greater exposure per lung surface area than adults

2. Special susceptibility of infants and children to ambient air pollution relative to the general population

A number of animal studies have indicated that the developing lung is altered by multi-day exposure to ozone at relatively high concentrations (0.5 ppm) and also to ozone plus airborne allergen. Studies in primates have shown altered structural development of the tracheal epithelium, including areas where the

tracheal epithelial basement membrane is incompletely developed (Schelegle et al. 2003; Evans et al. 2003). In addition, ozone alters neuronal distribution in the midlevel airways, resulting in decreased neuronal density in the midlevel airways and abnormal clumping of neurons in larger airways (Larson et al. 2004). Ozone exposure enhances the allergic response of the developing primate infant lung to airborne allergens, promoting the development of an allergic airway (Schelegle et al. 2003). In addition, there is epidemiological evidence of lower lung function in 18 to 21 year-old males raised in areas with high ozone in the U.S (Kunzli et al. 1997; Galizia and Kinney 1999). Finally, one longitudinal epidemiological study found a consistent association between elevated long-term ozone concentrations and new-onset asthma in children playing outdoor team sports (McConnell et al. 2002). Thus, children may be more susceptible to the effects of ozone than the general population due to effects on the developing lung. The standard setting takes this into consideration.

3. The effects on infants and children of exposure to ambient air pollution and other substances that have common mechanisms of toxicity.

There are no data that can be used to assess the combined effects of oxidant chemicals in the ambient air on children's health. However, in considering the epidemiological studies (including field studies), it should be noted that exposures to highly correlated oxidant chemicals in the ambient air are inherently included in the evaluation. In addition, notwithstanding isolated findings to the contrary, the majority of controlled exposure studies with ozone in combination with nitrogen oxides or sulfur oxides indicated that there was little to no difference in symptoms and lung function changes for the combined exposures relative to ozone alone.

4. The interaction of multiple air pollutants on infants and children, including between criteria air pollutants and toxic air contaminants.

There are some studies that shed light on interactions of ozone and other criteria air pollutants. Current evidence from both chamber studies and the epidemiological literature for the most part indicates that other criteria air pollutants have little or no modification of the effects attributed to ozone such as decreased lung function and respiratory symptoms. There are no studies evaluating the interaction of ozone and toxic air contaminants.

## **B.5 Recommended Pollutant Indicator**

OEHHA recommends that ozone continue to be the indicator for oxidant air pollutants. It is generally recognized that control of ambient ozone levels provides the most effective means of controlling harmful photochemical oxidants. Furthermore, available health-related data suggest that, at current ambient levels of photochemical oxidants, only ozone is likely to play an important role in the genesis of adverse health effects. Thus, OEHHA recommends that ozone remain the sole pollutant indicator for protection of public health from exposure to all photochemical oxidants found in ambient air.

## **B.6 Recommended Averaging Times and Forms**

The current California ambient air quality standard for ozone has a 1-hr averaging time. Selection of this averaging period was based on the desire to protect the public against health effects associated with 1-hr exposures to ozone, based on typical ozone monitoring patterns in the South Coast Air Basin, and for historical reasons, in that the State standard has had a 1-hr averaging time since its inception. It was recognized in 1987 that multi-hour ozone exposures were likely associated with adverse health effects as well, but there were virtually no published data available at that time to support a longer averaging time. It was also recognized that a stringent 1-hr ozone standard would serve to drive multi-hour term average ozone concentrations down, and thereby also provide protection against health effects associated with exposures longer than one hr. The studies on which the 1-hr standard was based indicated that exposures to ozone as low as 0.12 ppm for 1-2 hr induced decrements in lung function and increased symptoms in exercising subjects. Other chamber studies have show increased airway resistance at 0.18 ppm. Airway inflammation has been noted at 0.20 ppm, but has not been studied at lower concentrations in 1 to 3 hr protocols.

Dozens of epidemiological studies also demonstrate an association between 1-hour daily maximum concentrations of ozone and a wide range of adverse health effects, including premature mortality, hospitalizations, emergency rooms visits, asthma exacerbation, and respiratory symptoms. Some of these studies have the potential to be confounded by season, weather and co-pollutants. In addition, some of the effects may be likely due to multi-hour exposures to ozone, which are highly correlated with one-hour averages. In other words, it is not possible to ascribe the measured health effects solely to one-hour ambient peak concentrations rather longer term exposures. However, short-term exposures still may be of concern given the nature of some of the health effects (i.e., cardiovascular mortality among the elderly and emergency room visits for infants), It is possible that at least some of the important exposures may be relatively short-term in nature (i.e., less than 2 hours), since these subgroups are unlikely to be engaged in multi-hour periods of moderate or heavy exercise. Therefore, OEHHA recommends that a short-term 1-hour standard be retained to protect against these possible effects. Further, OEHHA recommends that a substantial margin of safety be used in setting the 1-hour standard to account for the possibility of significant adverse health effects, as suggested by the epidemiologic studies.

Since the 1987 review of the California AAQS for ozone, a series of controlled human exposure studies have appeared that used a 6.6 to 8 hr protocol, in simulation of a full day of outdoor work, recreation or play. These studies indicated that multi-hour exposures to ozone concentrations as low as 0.08 ppm could induce statistically significant decrements in group mean lung function and respiratory symptoms, and increases in airway hyperreactivity and markers of airway inflammation. These studies, in concert with observations of a broad, low peak level pattern to ozone concentration profiles in much of the US led the US EPA to select an averaging time of 8 hr in its ozone standard recommendation in

1996. In California, different regions exhibit varying relationships between the 1- and 8-hour averages. Some exhibit narrow, high peaks (and relatively high ratios of 1 to 8-hour averages) while other exhibit a wide afternoon peak concentration and a relatively low ratio of 1- to 8-hour averages.

We analyzed the relationship between high 1-hour and high 8-hour ozone averages for the years 2000 - 2002 using the California Design Value approach, also known as the Expected Peak Day Concentration (EPDC). As discussed earlier in this Staff Paper, this procedure is used to determine "design values," which are concentrations that play the primary role in determining the attainment status of regions with respect to California's state standards. California Design Values are calculated values for which one exceedance per year is expected. The "one expected exceedance" criterion and the calculation procedure are applied to all pollutants, including ozone, with short-term standards of 24 hours or less. The procedure is based on a statistical model of the highest 20% of the daily maximum 1-hour or 8-hour values from the previous three years. The resulting design values are relatively robust to fluctuations in daily meteorological conditions and are not unduly influenced by any single day. State regulations permit exceptional events, such as forest or urban fires, to be excluded from the calculation. Based on 2000 - 2002 data, we compared the California Design Values for 1-hour and 8-hour ozone levels at each ozone monitoring site in the state.

We first calculated the projected 1-hr design value for alternative 8-hr targets using a simple rollback model. In this model, the ratio between the 8-hour target concentration (e.g., 0.08 ppm) and the current 8-hour maximum at each design monitor is calculated. This ratio is then applied to the current 1-hour maximum value at that monitor location to determine the projected 1-hour design value. These results indicate that, using the design monitors, an 8-hr average of 0.080 ppm is associated with 1-hour values greater than 0.09 at several sites (see Table 7.1). For example, a rollback to the 8-hour average of 0.08 ppm is associated with a 1-hour maximum of 0.111 ppm at the Calexico site, 0.102 ppm at the Livermore (Old First St.) site, 0.097 ppm at the Gilroy site, and 0.095 ppm at the Santa Clarita site. A rollback to a 0.75 8-hour average is associated with 1-hour averages of 0.102, 0.094, 0.090 and 0.088 ppm, respectively, at these four sites. Thus, these multi-hour exposures could co-exist with 1-hour exposures greater than 0.09 ppm; this suggests the need for standards with both averaging times.

We also examined the 8-hour EPDC assuming that each site is rolled back to meet the current state 1-hour standard of 0.09 ppm. As indicated in Table 7.2, 6 of the 84 design monitor sites would have 8-hour averages above 0.08, 27 sites (~32%) would still have an EPDC 8-hour average above 0.075 ppm, and 78 (~93%) would have an EPDC 8-hour average above 0.070 ppm. Thus, a simple rollback model indicates that even after attainment of a 1-hour maximum of 0.09 ppm, significantly elevated 8-hour averages could remain.

Therefore, based on both the evidence from the studies examining the concentrations of ozone at which adverse health effects have been observed and

on the existing relation between 1- and 8-hour averages at existing monitoring sites, it is reasonable to recommend standards for both 1- and 8-hour averages. Such standards would ensure that the public is protected from both single and multi-hour concentrations of concern. We recommend that the form of the standard continue as “not to be exceeded.”

## **B.7 Recommended Concentrations**

### **B.7.1 Considerations for the Margin of Safety**

Both the California Health & Safety Code (section 39606) and the federal Clean Air Act (section 109) refer to an adequate margin of safety, although neither includes a specific legislative definition of this term. The Children’s Environmental Health Protection Act [Senate Bill 25, Escutia; Stats. 1999, Ch731, sec. 3; Health & Safety Code section 39404(d)(2)] requires a standard that “*adequately* protects the health of the public, including infants and children, *with an adequate margin of safety.*” (emphasis added) Given the current state of the science, which is limited by uncertainties in the existing data sets and methods available to analyze the impacts of low-level exposures, it is not possible to set standards for ozone that absolutely protect all individuals.

The governing statutory language indicates that California’s ambient air quality standards should also protect other vulnerable populations, in addition to infants and children, and the general public [(Health & Safety Code sections 39606 (d)(2) and 39606 (d)(3)]. This legislative directive is consistent with historical practice in California, where ambient air quality standards have been formulated to protect identifiable susceptible subgroups, as well as the general population. Nonetheless, even with standards tailored to protect vulnerable populations, there may be exquisitely sensitive individuals who still have adverse responses.

As a result, OEHHA has recommended ozone standards that are somewhat below the lowest levels reported for statistically significant group mean decrements in lung functions as observed in the chamber studies. These studies have been given primary focus since both the dose and response are well characterized. A margin of safety was developed based on the following evidence: (1) chamber studies indicating variability in human response and the existence of particularly large individual responses; (2) chamber studies indicating, at higher ozone levels, both bronchial responsiveness and pulmonary inflammation; (3) animal toxicology studies supporting many of these findings and also suggesting the possibility of decreases in lung defense mechanism; and (4) epidemiologic studies reporting associations between ambient ozone and a suite of adverse outcomes including premature mortality, hospitalization, emergency room visits, school loss, respiratory symptoms and changes lung function. While it is difficult to use all of the latter set of studies quantitatively in developing a standard, the significant potential of adverse effects clearly should factor into the margin of safety considerations. Below, we provide the scientific rationale for the 1- and 8-hour standards.

### **B.7.2 One-hour Average**

We recommend that the current standard of 0.09 ppm, not to be exceeded, be retained. While there have been no new controlled chamber studies to indicate group-level effects at concentrations below 0.12 ppm for short (one to three hours) durations of exposure, the OEHHA recommendation is based on several factors.

First, at 0.12 ppm, in several studies 10 - 25% of the subjects experienced a decline of 10% or more in FEV1. In one study, these lung function changes were accompanied by increases in cough. At 0.24 ppm, increases were also observed in shortness of breath and pain on deep breath. These lung function and symptom outcomes have been demonstrated and replicated in several carefully controlled human exposure studies. The population at risk for these effects includes children engaged in active outdoor exercise and workers engaged in physical labor outdoors. Thus, a margin of safety is necessary to account for variability in human responses. In addition, the chamber studies, by design, do not include especially vulnerable populations (e.g., people with moderate to severe asthma, COPD, and heart disease), which may be incorporated in the epidemiologic studies.

Second, chamber studies indicate that bronchial responsiveness and pulmonary inflammation occur at 1-hour exposure to 0.18 to 0.20 ppm. The bronchial responsiveness is capable of aggravating pre-existing chronic respiratory disease. The ultimate impact of the inflammatory response is unclear but repeated exposures to high ozone levels may result in restructuring of the airways, fibrosis, and possibly permanent respiratory injury. These latter outcomes are supported by animal toxicology studies which also suggest the possibility of decreases in lung defense mechanism.

Third, there have been a plethora of epidemiological studies completed over the last 10 years indicating the potential for severe adverse health outcomes including premature mortality, hospitalizations, and emergency room visits. These studies include concentrations to which the public is currently being exposed. Some of the epidemiological associations have been reported for outcomes including cardiovascular mortality (likely to be observed among older individuals with pre-existing heart or lung disease) and hospital visits for children less than age two. Thus, it is possible that some of these associations are due to relatively short-term exposures of less than two hours in duration since these subgroups are unlikely to be engaged in multi-hour periods of moderate or heavy exercise outdoors. However, it is difficult to attribute these adverse outcomes to a specific ozone concentration or time. Likewise, because of the high temporal correlation of 1-, 8-, and 24-hour average ozone, the averaging time of concern cannot be discerned from these studies. Most of the studies used linear non-threshold models and did not explicitly test for thresholds. In addition, certain models, such as the time-series studies of mortality and hospitalization, suffer from problems of confounding from seasonal and weather factors and possibly co-pollutants. However, several of the studies of short-term exposure on mortality demonstrate effects only in the warmer months when ozone concentrations are

highest. This suggests either the importance of outdoor exposure, the possibility of thresholds (i.e., non-linear concentration-response functions, or both. These studies have annual averages for 1-hour daily maximum ozone of between 20 and 70 ppb (see Table 7.3).

Additional uncertainties with these studies exist due to issues related to measurement of exposure and biological mechanisms. Concerning exposure assessment, Sarnat et al (Sarnat et al. 2001) demonstrated a very low and statistically non-significant association between personal exposure to ozone and ambient ozone in Baltimore. In addition, evidence clearly indicates only low to moderate levels of indoor ozone associated with outdoor ozone. Finally, the limited number of chamber studies to date indicate that individuals with asthma, COPD or hypertension do not, in general, have proportionately greater responses to short-term exposures to ozone than healthy individuals. However, since such individuals' baseline health status is already compromised to some extent, on an absolute basis some ozone-associated effects would likely carry more significant clinical implications for this group. Therefore, the mortality effects should be viewed as suggestive until additional epidemiologic studies are undertaken that carefully control for season, weather and confounding by other pollutants, most importantly, particulate matter. Additional research on potential biological mechanisms is required, as well as some further reconciliation of the longer-term impacts of repeated ozone-induced inflammation. However, the existing evidence from the chamber and epidemiologic studies clearly argue for a significant margin of safety below the effect level of 0.12 ppm level of effect observed in the 1-hour chamber studies.

Only one set of epidemiological studies, those time-series studies examining emergency room visits for asthma, has more systematically examined the shape of the concentration-response function for possible non-linearities and thresholds. We have reviewed these studies and attempted to determine the likely interval of concentrations in each study where associations are clearly demonstrable (Figure 7.1). Taken together these studies suggest that the low end of the interval has a range of 0.060 to 0.115 ppm ozone averaged over one hour. The lowest value comes from the study of (Weisel et al. 1995) which did not include any analysis of daily PM<sub>10</sub>, PM<sub>2.5</sub> or sulfate, all of which have been demonstrated to exacerbate asthma. Thus it is difficult to attribute the results strictly to ozone. Dropping this study suggests a lower bound of the interval of 0.075 ppm. However, this is not the same as a "lowest observable effects level" since the actual concentrations at which statistically significant associations emerge are between 0.075 ppm and 0.16 ppm. In fact, three of the studies suggest that significant associations occur at around 0.11 ppm 1-hour ozone. We also can make some inferences about no effects levels from negative studies which rarely have values above 1-hour concentrations of 0.080 ppm. Again, it is difficult to determine the actual averaging time of concern from these studies given their high correlations. In addition, emergency room visits for asthma are a fairly serious indicator of ozone toxicity and other less severe outcomes may have lower thresholds, if any. Thus, the evidence suggesting associations

between emergency room visits and 1-hour ozone concentrations at or below 0.11 ppm needs to be incorporated into the margin of safety.

Finally, a large margin of safety (relative to the 1-hour 0.12 ppm from the chamber studies) may be necessary to account for the possibility of adverse impacts of long-term (i.e., one year or more) exposures to ozone. For example, modest associations have been reported between long-term summertime exposure to ozone and cardiovascular mortality (Pope et al. 2002). Also, long-term exposure to ozone, particularly prior to age 6 has been associated with impairment of the small airways (Kunzli et al. 1997; Galizia and Kinney 1999). The application of a safety margin reducing the standard below the level of effect of 0.12 ppm observed in the chamber studies to a concentration of 0.09 ppm would succeed in lowering the entire distribution of daily exposures at all durations. Therefore, this standard will afford some increased degree of protection from longer-term exposures. Specifically, our analysis indicates that when a 1-hour standard of 0.09 is attained, the annual mean of daily 1-hour maxima for the years 1999 - 2001 for monitors in California cities with populations above 100,000 will range from 0.023 to 0.052 ppm, with most of the cities in the range of 0.33 to 0.48 ppm, with an average of around 0.04 ppm (see Table 7.3).

In 1987, the Department of Health Services recommended a 1 hour standard of 0.08 ppm. The primary basis of the 1987 DHS recommendation were the chamber studies conducted for 1-2 hours in humans which showed effects on the group mean decrements in lung function and symptoms measurements in healthy young exercising adults at an ozone concentration of 0.12 ppm. At the time, there was little information on the effects of repeated or prolonged exposures to ozone. Experimental evidence in animals indicated concern for repeated or prolonged ozone exposure. Thus, DHS recommended a 1-hour standard of 0.08 ppm in order to incorporate some margin of safety from the chamber studies in humans. This was the only averaging time for which a standard was recommended.

Viewing all of the evidence, OEHHA recommends a retention of the 1-hour standard of 0.09 ppm, not to be exceeded, as being protective of public health with an adequate margin of safety. Our current recommendation is made in conjunction with an 8 hour standard which together with the 1-hour standard provides an adequate protection of public health. However, if the Board does not adopt the 8-hour standard then OEHHA recommends a 1-hour standard of 0.08, not to be exceeded, in consideration of the concern regarding repeated or prolonged exposures to ozone.

### **B.7.3 Eight-hour Standard**

We recommend an 8-hour standard of 0.070 ppm, not to be exceeded. Our recommendation for the 8-hour standard is based primarily on the chamber studies that have been conducted over the last 15 years, supported by the important health outcomes reported in many of the epidemiologic studies. At a 6.6 -to 8-hour concentration of 0.08 ppm, several studies have reported statistically significant group effects on lung function changes, respiratory

symptoms, and airway hyperresponsiveness among healthy, exercising individuals. A substantial fraction of subjects in these studies exhibited particularly marked responses in lung function and symptoms, effects that are labeled as “adverse” by ATS. As a result, a concentration of 0.08 ppm ozone for an 8 hour averaging time is not sufficiently protective of public health. The one published multi-hour study investigating a concentration below 0.08 ppm showed no statistically significant group mean decrement in lung function or symptoms at 0.04 ppm compared to a baseline of clear air. In addition, all subjects had changes in FEV<sub>1</sub> of less than 10%. One unpublished multi-hour study at 0.06 ppm (Adams 1998) reported no statistically significant group mean changes, relative to clean air, in either lung function or symptoms including pain on deep inspiration and total symptom score. Therefore, OEHHA has recommended an 8-hour level of 0.070 ppm, not to be exceeded, to ensure a minimal number of days of ozone-related, significant respiratory effects for children and adults.

Many of the studies, and issues and concerns associated with the epidemiological studies listed above concerning the 1-hour standard are also relevant to the 8-hour standard. As discussed above, it may be that these health effects, often correlated with 1-hour exposures in the epidemiologic studies, are actually associated with 8-hour (or other) average exposures. Evidence for this possibility is provided by the stronger response, in terms of effects on both lung function and symptoms, observed in multi-hour exposures at concentrations that do not elicit responses after only 1-hour exposures. Therefore, these epidemiologic studies need to be reflected in the margin of safety for the 8-hour average.

Regarding the level of effect from the studies of emergency room visits for asthma, the lower bound of 0.11 1-hour is generally associated with an 8-hour concentration of 0.09 ppm (using a general ratio of 1-hour to 8-hour of 1.20 in California). The one study of emergency room visits using the exposure intervals that examined 8-hour ozone (Tolbert et al. 2000) reported elevated risks within the interval of 0.070 to 0.10 ppm with a more consistent response in the interval from 0.090 to 0.10 ppm and statistical significance attained for the interval between 0.10 and 0.113 8-hour ozone.

Finally, as discussed concerning the 1-hour standard above, our recommendation recognizes that attainment of an 8-hour standard of 0.070 ppm would significantly reduce the entire distribution of daily ozone concentrations (Table 7.3). Based on information on current distributions of daily 8-hour averages, we would expect to see relatively few days in the 0.060 to 0.070 ppm 8-hour range in most major cities in California. Thus, we would expect to find few days were sensitive individuals would experience large changes in lung function.

#### **B.7.4 Consideration of Infants and Children in Recommending the Ozone Standards**

As noted above, children breathe more per body weight at rest and when active than adults, and tend to be outside more and more active than adults. Thus, by virtue of physiology and behavior, they are likely to be disproportionately more

highly exposed than the general population. However, the chamber studies of exercising children suggest that they have responses generally similar to adults. Similarly, those epidemiologic studies that have been able to examine both children and adults do not show clear evidence for greater sensitivity in children. Studies in animals at high exposure concentrations (0.5 ppm and higher, 8 hrs/day for several consecutive days) indicate that developing lungs of infant animals are adversely affected by ozone. The recommended standards are well below that level of exposure. Two studies have shown evidence of lower lung function in young adults raised in high ozone areas (Kunzli et al. 1997; Galizia and Kinney 1999). For the study by Kunzli et al. (1997), exposure to ozone prior to age 6 was an important variable. Examination of data for the Los Angeles basin from the early 1980s, show summer averages of the 1-hour maximum to be above 0.10 ppm. This is considerably above present levels and above the recommended one hour standard. There is also evidence that children who play three or more sports are at higher risk of developing asthma if they also live in high ozone communities in Southern California. This study needs to be repeated before the effect can be attributed to ozone exposure with greater certainty, but the finding is of concern. The warm season daily 8-hour maximum concentrations of ozone measured in these high ozone areas, over the four years of study, was 0.084 ppm. The proposed 8-hour standard of 0.070 ppm, therefore, should protect most children from asthma induction associated with exposure to ozone.

### **B.8 Summary of OEHHA Recommendation:**

- 1) Ozone continues to be the indicator for oxidant air pollutants.
- 2) An averaging time for standards of both 1- and 8-hours.
- 3) Retain the 1-hour average standard of 0.09 ppm, not to be exceeded and add an 8-hour standard of 0.070 ppm, not to be exceeded. If the Board does not adopt an 8-hour standard, then the 1-hour standard should be lowered to 0.08 ppm, not to be exceeded. Such a standard would protect against both 1-hour concentrations and repeated or multi-hour exposures to ozone.

## B.9 References

Abbey DE, Nishino N, McDonnell WF, Burchette RJ, Knutsen SF, Lawrence Beeson W, Yang JX. 1999. Long-term inhalable particles and other air pollutants related to mortality in nonsmokers. *Am J Respir Crit Care Med* 159:373-82.

Adams WC. 1998. Dose-response effects of varied equivalent minute ventilation rates on pulmonary function responses during exposure to ozone. Final Report to the American Petroleum Institute. Washington, D. C.

Adams WC. 2002. Comparison of chamber and face-mask 6.6-hour exposures to ozone on pulmonary function and symptoms responses. *Inhal Toxicol* 14:745-64.

American Thoracic Society. 1985. Guidelines as to what constitutes an adverse respiratory health effect, with special reference to epidemiologic studies of air pollution. 131. 131:666-8.

American Thoracic Society. 2000. What constitutes an adverse health effect of air pollution? 161. 161:665-73.

Anderson HR, Spix C, Medina S, Schouten JP, Castellsague J, Rossi G, Zmirou D, Touloumi G, Wojtyniak B, Ponka A, Bacharova L, Schwartz J, Katsouyanni K. 1997. Air pollution and daily admissions for chronic obstructive pulmonary disease in 6 European cities: results from the APHEA project. *Eur Respir J* 10:1064-71.

Bates DV, Baker-Anderson M, Sizto R. 1990. Asthma attack periodicity: a study of hospital emergency visits in Vancouver. *Environ Res* 51 :51-70.

Brauer M, Blair J, Vedal S. 1996. Effect of ambient ozone exposure on lung function in farm workers. *Am J Respir Crit Care Med* 154:981-7.

Burnett RT, Cakmak S, Brook JR, Krewski D. 1997. The role of particulate size and chemistry in the association between summertime ambient air pollution and hospitalization for cardiorespiratory diseases. *Environ Health Perspect* 105:614-20.

California Air Resources Board and Office of Environmental Health Hazard Assessment. 2000. Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act.

California Department of Health Services. 1987. Recommendation for an Ambient Air Quality Standard for Ozone.

Catalano PJ, Rogus J, Ryan LM. 1995. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part X: Robust composite scores based on median polish analysis. *Res Rep Health Eff Inst* :1-57; discussion 59-64.

Dominici F. 2003. Shape of the Exposure-Response Relation and Mortality Displacement in the NMMAPS Database. Health Effects Institute Special Report. Health Effects Institute Special Report:91-6.

Drechsler-Parks DM, Bedi JF, Horvath SM. 1987. Pulmonary function responses

of older men and women to ozone exposure. *Exp Gerontol* 22:91-101.

Drechsler-Parks DM, Bedi JF, Horvath SM. 1989. Pulmonary function responses of young and older adults to mixtures of O<sub>3</sub>, NO<sub>2</sub> and PAN . *Toxicol Ind Health* 5:505-17.

Emmons K, Foster WM. 1991. Smoking cessation and acute airway response to ozone. *Arch Environ Health* 46:288-95.

Evans MJ, Fanucchi MV, Baker GL, Van Winkle LS, Pantle LM, Nishio SJ, Schelegle ES, Gershwin LJ, Miller LA, Hyde DM, Sannes PL, Plopper CG. 2003. Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. *Am J Physiol Lung Cell Mol Physiol* 285:L931-9.

Galizia A, Kinney PL. 1999. Long-term residence in areas of high ozone: associations with respiratory health in a nationwide sample of nonsmoking young adults. *Environ Health Perspect* 107:675-9.

Gauderman WJ, McConnell R, Gilliland F, London S, Thomas D, Avol E, Vora H, Berhane K, Rappaport EB, Lurmann F, Margolis HG, Peters J. 2000. Association between air pollution and lung function growth in southern California children. *Am J Respir Crit Care Med* 162:1383-90.

Gent JF, Triche EW, Holford TR, Belanger K, Bracken MB, Beckett WS, Leaderer BP. 2003. Association of low-level ozone and fine particles with respiratory symptoms in children with asthma. *JAMA* 290:1859-67.

Gilliland FD, Berhane K, Rappaport EB, Thomas DC, Avol E, Gauderman WJ, London SJ, Margolis HG, McConnell R, Islam KT, Peters JM. 2001. The effects of ambient air pollution on school absenteeism due to respiratory illnesses. *Epidemiology* 12:43-54.

Goldberg MS, Burnett RT, Valois MF, Flegel K, Bailar JC 3rd, Brook J, Vincent R, Radon K. 2003. Associations between ambient air pollution and daily mortality among persons with congestive heart failure. *Environ Res* 91 :8-20.

Goldberg MS, Burnett RT. 2003. Revised analysis of the Montreal time-series study. Health Effects Institute Special Report. Health Effects Institute Special Report 113-32.

Gong H Jr, Bradley PW, Simmons MS, Tashkin DP. 1986. Impaired exercise performance and pulmonary function in elite cyclists during low-level ozone exposure in a hot environment. *Am Rev Respir Dis* 134:726-33.

Harkema JR, Plopper CG, Hyde DM, St George JA, Wilson DW, Dungworth DL. 1993. Response of macaque bronchiolar epithelium to ambient concentrations of ozone. *Am J Pathol* 143:857-66.

Hoek G, Brunekreef B, Verhoeff A, van Wijnen J, Fischer P. 2000. Daily mortality and air pollution in The Netherlands. *J Air Waste Manag Assoc* 50:1380-9.

Horstman DH, Folinsbee LJ, Ives PJ, Abdul-Salaam S, McDonnell WF. 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* 142:1158-63.

Kinney PL, Lippmann M. 2000. Respiratory effects of seasonal exposures to ozone and particles. *Arch Environ Health* 55:210-6.

Kinney PL, Nilsen DM, Lippmann M, Brescia M, Gordon T, McGovern T, El-Fawal H, Devlin RB, Rom WN. 1996. Biomarkers of lung inflammation in recreational joggers exposed to ozone. *Am J Respir Crit Care Med* 154:1430-5.

Kunzli N, Lurmann F, Segal M, Ngo L, Balmes J, Tager IB. 1997. Association between lifetime ambient ozone exposure and pulmonary function in college freshmen--results of a pilot study. *Environ Res* 72:8-23.

Larson SD, Schelegle ES, Walby WF, Gershwin LJ, Fanuccihi MV, Evans MJ, Joad JP, Tarkington BK, Hyde DM, Plopper CG. 2004. Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. *Toxicol Appl Pharmacol* 194:211-20.

Last JA, Gelzleichter TR, Harkema J, Hawk S. 1994. Consequences of prolonged inhalation of ozone on Fischer-344/N rats: collaborative studies. Part I: Content and cross-linking of lung collagen. *Res Rep Health Eff Inst* :1-29; discussion 31-40.

Linn WS, Shamoo DA, Anderson KR, Peng R-C, Avol EL, Hackney JD, Gong H Jr. 1996. Short-term air pollution exposure and responses in Los Angeles area schoolchildren. *J Exp Anal Environ Epidemiol* 6:449-471.

McConnell R, Berhane K, Gilliland F, London SJ, Islam T, Gauderman WJ, Avol E, Margolis HG, Peters JM. 2002. Asthma in exercising children exposed to ozone: a cohort study. *Lancet* 359:386-91.

McDonnell WF, Horstman DH, Hazucha MJ, Seal E Jr, Haak ED, Salaam SA, House DE. 1983. Pulmonary effects of ozone exposure during exercise: dose-response characteristics. *J Appl Physiol* 54:1345-52.

McDonnell WF, Kehrl HR, Abdul-Salaam S, Ives PJ, Folinsbee LJ, Devlin RB, O'Neil JJ, Horstman DH. 1991. Respiratory response of humans exposed to low levels of ozone for 6.6 hours. *Arch Environ Health* 46: 145-50.

McDonnell WF 3rd, Chapman RS, Leigh MW, Strobe GL, Collier AM. 1985a. Respiratory responses of vigorously exercising children to 0.12 ppm ozone exposure. *Am Rev Respir Dis* 132:875-9.

McDonnell WF 3rd, Horstman DH, Abdul-Salaam S, House DE. 1985b. Reproducibility of individual responses to ozone exposure. *Am Rev Respir Dis* 131:36-40.

Moolgavkar SH, Luebeck EG, Anderson EL. 1997. Air pollution and hospital admissions for respiratory causes in Minneapolis-St. Paul and Birmingham. *Epidemiology* 8:364-70.

National Asthma Education and Prevention Program . 2002. National Asthma

Education and Prevention Program Expert Panel Report: guidelines for the diagnosis and management of asthma update on selected topics. 110. 110((5 pt 2)): S141-219.

Peters JM, Avol E, Gauderman WJ, Linn WS, Navidi W, London SJ, Margolis H, Rappaport E, Vora H, Gong H Jr, Thomas DC. 1999. A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. *Am J Respir Crit Care Med* 159:768-75.

Pinkerton KE, Menache MG, Plopper CG. 1995. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part IX: Changes in the tracheobronchial epithelium, pulmonary acinus, and lung antioxidant enzyme activity. *Res Rep Health Eff Inst* :41-98; discussion 99-110.

Pinkerton KE, Weller BL, Menache MG, Plopper CG. 1998. Consequences of prolonged inhalation of ozone on F344/N rats: collaborative studies. Part XIII. A comparison of changes in the tracheobronchial epithelium and pulmonary acinus in male rats at 3 and 20 months. *Res Rep Health Eff Inst* :1-32; discussion 33-7.

Pope CA 3rd, Burnett RT, Thun MJ, Calle EE, Krewski D, Ito K, Thurston GD. 2002a. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 287:1132-41.

Reiser KM, Tyler WS, Hennessy SM, Dominguez JJ, Last JA. 1987. Long-term consequences of exposure to ozone. II. Structural alterations in lung collagen of monkeys. *Toxicol Appl Pharmacol* 89:314-22.

Sarnat JA, Schwartz J, Catalano PJ, Suh HH. 2001. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environ Health Perspect* 109:1053-61.

Schelegle ES, Miller LA, Gershwin LJ, Fanucchi MV, Van Winkle LS, Gerriets JE, Walby WF, Mitchell V, Tarkington BK, Wong VJ, Baker GL, Pantle LM, Joad JP, Pinkerton KE, Wu R, Evans MJ, Hyde DM, Plopper CG. 2003a. Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. *Toxicol Appl Pharmacol* 191:74-85.

Seal E Jr, McDonnell WF, House DE, Salaam SA, Dewitt PJ, Butler SO, Green J, Raggio L. 1993. The pulmonary response of white and black adults to six concentrations of ozone. *Am Rev Respir Dis* 147:804-10.

Simpson RW, Williams G, Petroeschovsky A, Morgan G, Rutherford S. 1997. Associations between outdoor air pollution and daily mortality in Brisbane, Australia. *Arch Environ Health* 52:442-54.

Stieb DM, Burnett RT, Beveridge RC, Brook JR. 1996. Association between ozone and asthma emergency department visits in Saint John, New Brunswick, Canada. *Environ Health Perspect* 104:1354-60.

Szarek JL, Stewart NL, Zhang JZ, Webb JA, Valentovic MA, Catalano P. 1995. Contractile responses and structure of small bronchi isolated from rats after 20 months' exposure to ozone. *Fundam Appl Toxicol* 28:199-208.

Tager IB, Kunzli N, Lurmann F, Ngo L, Segal M, Balmes J. 1998. Methods development for epidemiologic investigations of the health effects of prolonged ozone exposure. Part II. An approach to retrospective estimation of lifetime ozone exposure using a questionnaire and ambient monitoring data (California sites). *Res Rep Health Eff Inst* :27-78; discussion 109-21.

Tolbert PE, Mulholland JA, MacIntosh DL, Xu F, Daniels D, Devine OJ, Carlin BP, Klein M, Dorley J, Butler AJ, Nordenberg DF, Frumkin H, Ryan PB, White MC. 2000. Air quality and pediatric emergency room visits for asthma in Atlanta, Georgia, USA. *Am J Epidemiol* 151:798-810.

Tyler WS, Tyler NK, Last JA, Gillespie MJ, Barstow TJ. 1988. Comparison of daily and seasonal exposures of young monkeys to ozone. *Toxicology* 50:131-44.

Vedal S, Brauer M, White R, Petkau J. 2003. Air pollution and daily mortality in a city with low levels of pollution. *Environ Health Perspect* 111:45-52.

Weisel CP, Cody RP, Lioy PJ. 1995. Relationship between summertime ambient ozone levels and emergency department visits for asthma in central New Jersey. *Environ Health Perspect* 103 Suppl 2:97-102.

White MC, Etzel RA, Wilcox WD, Lloyd C. 1994. Exacerbations of childhood asthma and ozone pollution in Atlanta. *Environ Res* 65:56-68.

**Table B-1** Predicted values of daily maximum 1-hour ozone at a time in the future when an 8-hour standard of 0.080 ppm is attained statewide at selected monitor sites.

| SITE                              | COUNTY | BASIN | DISTRICT | 1-hr EPDC |
|-----------------------------------|--------|-------|----------|-----------|
| Calexico-Ethel Street             | IMP    | SS    | IMP      | 0.111     |
| Livermore-Old 1st Street          | ALA    | SFB   | BA       | 0.102     |
| Concord-2975 Treat Blvd           | CC     | SFB   | BA       | 0.098     |
| Westmorland-W 1st Street          | IMP    | SS    | IMP      | 0.097     |
| Gilroy-9th Street                 | SCL    | SFB   | BA       | 0.097     |
| Calexico-Grant Street             | IMP    | SS    | IMP      | 0.095     |
| Santa Clarita                     | LA     | SC    | SC       | 0.095     |
| Livermore-793 Rincon Avenue       | ALA    | SFB   | BA       | 0.094     |
| El Centro-9th Street              | IMP    | SS    | IMP      | 0.094     |
| Parlier                           | FRE    | SJV   | SJV      | 0.093     |
| Cool-Highway 193                  | ED     | MC    | ED       | 0.093     |
| San Martin-Murphy Avenue          | SCL    | SFB   | BA       | 0.093     |
| Pinnacles National Monument       | SBT    | NCC   | MBU      | 0.093     |
| Calexico-East                     | IMP    | SS    | IMP      | 0.092     |
| Clovis-N Villa Avenue             | FRE    | SJV   | SJV      | 0.091     |
| Palm Springs-Fire Station         | RIV    | SS    | SC       | 0.091     |
| Simi Valley-Cochran Street        | VEN    | SCC   | VEN      | 0.090     |
| Sloughhouse                       | SAC    | SV    | SAC      | 0.090     |
| Fremont-Chapel Way                | ALA    | SFB   | BA       | 0.089     |
| Folsom-Natoma Street              | SAC    | SV    | SAC      | 0.089     |
| Echo Summit                       | ED     | LT    | ED       | 0.089     |
| Phelan-Beekley Road & Phelan Road | SBD    | MD    | MD       | 0.089     |
| South Lake Tahoe-Sandy Way        | ED     | LT    | ED       | 0.088     |
| Lancaster-W Pondera Street        | LA     | MD    | AV       | 0.088     |
| Edison                            | KER    | SJV   | SJV      | 0.088     |
| Bethel Island Road                | CC     | SFB   | BA       | 0.088     |
| Fairfield-Bay Area AQMD           | SOL    | SFB   | BA       | 0.088     |
| Alpine-Victoria Drive             | SD     | SD    | SD       | 0.088     |
| Arvin-Bear Mountain Blvd          | KER    | SJV   | SJV      | 0.087     |

**Table B-2 Predicted 8-hour EPDC after rollback to 1-hour maximum of 0.09 ppm at design monitors throughout the state.**

| SITE NAME                          | 8-hour EPDC |
|------------------------------------|-------------|
|                                    |             |
| Mammoth Lakes-Gateway HC           | 0.087       |
| Echo Summit                        | 0.083       |
| Alpine-Victoria Drive              | 0.082       |
| Simi Valley-Cochran Street         | 0.082       |
| Death Valley Natl Monument         | 0.082       |
| Crestline                          | 0.081       |
| Cool-Highway 193                   | 0.080       |
| Livermore-Old 1st Street           | 0.080       |
| Pinnacles National Monument        | 0.080       |
| Fresno-Sierra Skypark #2           | 0.079       |
| Redlands-Dearborn                  | 0.079       |
| Folsom-Natoma Street               | 0.078       |
| Phelan-Beekeley Road & Phelan Road | 0.078       |
| Clovis-N Villa Avenue              | 0.078       |
| Arvin-Bear Mountain Blvd           | 0.077       |
| San Bernardino-4th Street          | 0.077       |
| Parlier                            | 0.077       |
| Upland                             | 0.077       |
| South Lake Tahoe-Sandy Way         | 0.077       |
| Hesperia-Olive Street              | 0.077       |
| Santa Clarita                      | 0.077       |
| Gilroy-9th Street                  | 0.077       |
| Ojai-Ojai Avenue                   | 0.076       |
| Placerville-Gold Nugget Way        | 0.076       |
| Grass Valley-Litton Building       | 0.076       |
| White Cloud Mountain               | 0.076       |
| Lancaster-W Pondera Street         | 0.076       |
| Fresno-1st Street                  | 0.075       |
| Fontana-Arrow Highway              | 0.075       |

|                                     |       |
|-------------------------------------|-------|
| Auburn-Dewitt-C Avenue              | 0.075 |
| Perris                              | 0.075 |
| Piru-3301 Pacific Avenue            | 0.075 |
| Palm Springs-Fire Station           | 0.075 |
| Edison                              | 0.075 |
| Mojave-923 Poole Street             | 0.075 |
| Sequoia & Kings Canyon Np           | 0.075 |
| Westmorland-W 1st Street            | 0.075 |
| Jerseydale - 6440 Jerseydale        | 0.074 |
| Merced-S Coffee Avenue              | 0.074 |
| Healdsburg-Municipal Airport        | 0.074 |
| Glendora-Laurel                     | 0.074 |
| Calexico-Ethel Street               | 0.074 |
| Bakersfield-5558 California Avenue  | 0.074 |
| Sloughhouse                         | 0.073 |
| San Martin-Murphy Avenue            | 0.073 |
| San Andreas-Gold Strike Road        | 0.073 |
| Jackson-Clinton Road                | 0.073 |
| Five Mile Learning Center           | 0.073 |
| Riverside-Rubidoux                  | 0.073 |
| Ventura County-W Casitas Pass Road  | 0.073 |
| Hanford-S Irwin Street              | 0.073 |
| Camp Pendleton                      | 0.073 |
| Tuscan Butte                        | 0.073 |
| Joshua Tree-National Monument       | 0.073 |
| Lakeport-Lakeport Blvd              | 0.073 |
| Hollister-Fairview Road             | 0.072 |
| Sequoia National Park-Lookout Point | 0.072 |
| Rocklin-Rocklin Road                | 0.072 |
| Victorville-14306 Park Avenue       | 0.072 |
| Twentynine Palms-Adobe Road #2      | 0.072 |
| Sacramento-Del Paso Manor           | 0.072 |
| Banning Airport                     | 0.072 |
| Roseville-N Sunrise Blvd            | 0.072 |
| Maricopa-Stanislaus Street          | 0.072 |

|                                    |       |
|------------------------------------|-------|
| Azusa                              | 0.072 |
| Livermore-793 Rincon Avenue        | 0.072 |
| Fresno-Drummond Street             | 0.072 |
| Concord-2975 Treat Blvd            | 0.072 |
| Escondido-E Valley Parkway         | 0.072 |
| Shafter-Walker Street              | 0.071 |
| Barstow                            | 0.071 |
| Oildale-3311 Manor Street          | 0.071 |
| Bethel Island Road                 | 0.071 |
| Fairfield-Bay Area AQMD            | 0.071 |
| Visalia-N Church Street            | 0.071 |
| San Diego-Overland Avenue          | 0.071 |
| Lake Elsinore-W Flint Street       | 0.071 |
| Calexico-East                      | 0.071 |
| Shaver Lake - Perimeter Road       | 0.070 |
| Yosemite Natl Park-Turtleback Dome | 0.070 |
| Thousand Oaks-Moorpark Road        | 0.070 |
| Los Gatos                          | 0.070 |
| North Highlands-Blackfoot Way      | 0.070 |
| Calexico-Grant Street              | 0.070 |

**Table B-3 Annual mean of daily 1-hour maximum for 1999-2001 associated with attainment of 0.09 ppm 1-hour ozone for monitors in cities greater than 100,000 population.**

| City – Monitoring Site       | 2000 Population | Annual mean of daily 1-hr maximum for 1999-2001 |
|------------------------------|-----------------|---|
| Los Angeles-651 Mott St      | 3,694,820       | 0.0393  |
| Los Angeles-North Main St    | 3,694,820       | 0.0374  |
| San Diego-12th Ave           | 1,223,400       | 0.0408  |
| San Diego-Logan Ave          | 1,223,400       | 0.0369  |
| San Diego-Overland Ave       | 1,223,400       | 0.0437  |
| San Jose-4th St              | 894,943         | 0.0323  |
| San Jose-935 Piedmont Rd     | 894,943         | 0.0349  |
| San Francisco-Arkansas St    | 776,733         | 0.0295  |
| North Long Beach             | 461,522         | 0.0359  |
| Fresno-1st St                | 427,652         | 0.0471  |
| Fresno-Drummond St           | 427,652         | 0.0461  |
| Fresno-Sierra Skypark #2     | 427,652         | 0.0524  |
| Sacramento-3801 Airport Rd   | 407,018         | 0.0409  |
| Sacramento-Del Paso Manor    | 407,018         | 0.0427  |
| Sacramento-T St              | 407,018         | 0.0382  |
| Oakland-Alice St             | 399,484         | 0.0242  |
| Oakland-6701 Intern Blvd     | 399,484         | 0.0228  |
| Anaheim-Harbor Blvd          | 328,014         | 0.0380  |
| Riverside-Rubidoux           | 255,166         | 0.0446  |
| Bakersfield-5558 Cal. Ave    | 247,057         | 0.0485  |
| Bakersfield-GS Hway          | 247,057         | 0.0463  |
| Stockton-E Mariposa          | 243,771         | 0.0436  |
| Stockton-Hazelton St         | 243,771         | 0.0369  |
| Fremont-Chapel Way           | 203,413         | 0.0349  |
| Modesto-14th St              | 188,856         | 0.0407  |
| San Bernardino-4th St        | 185,401         | 0.0446  |
| Chula Vista                  | 173,556         | 0.0453  |
| Oceanside-Mission Ave        | 161,029         | 0.0423  |
| Santa Clarita                | 151,088         | 0.0489  |
| Santa Clarita-Cnty Fire Stat | 151,088         | 0.0417  |

|                             |         |        |
|-----------------------------|---------|--------|
| Salinas-High School         | 151,060 | 0.0380 |
| Salinas-Natividad Road #2   | 151,060 | 0.0357 |
| Pomona                      | 149,473 | 0.0336 |
| Santa Rosa Island           | 147,595 | 0.0427 |
| Santa Rosa-5th St           | 147,595 | 0.0328 |
| Hayward-La Mesa             | 140,030 | 0.0385 |
| Pasadena-S Wilson Ave       | 133,936 | 0.0398 |
| Sunnyvale-910 Ticonderoga   | 131,760 | 0.0304 |
| Fontana-Arrow Highway       | 128,929 | 0.0413 |
| Concord-2975 Treat Blvd     | 121,780 | 0.0388 |
| Lancaster-43301 Division St | 118,718 | 0.0368 |
| Lancaster-W Pondera St      | 118,718 | 0.0481 |
| Thousand Oaks-Moorpark Rd   | 117,005 | 0.0475 |
| Vallejo-304 Tuolumne St     | 116,760 | 0.0326 |
| Simi Valley-Cochran St      | 111,351 | 0.0508 |
| Burbank-W Palm Ave          | 100,316 | 0.0400 |

**Figure B-1 Intervals of 1-hr Ozone Indicating Likely Effect Levels for Emergency Room Visits for Asthma**

