Air Resources Board

State of California

Governor Arnold Schwarzenegger

October 2005 Revision

Review of the California Ambient Air Quality Standard For Ozone

Volume I of IV

Chapters 1-2

Appendix A—Proposed Amendments

Staff Report

Initial Statement of Reasons for Proposed Rulemaking

October 27, 2005

California Environmental Protection Agency

Air Resources Board

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California Environmental Protection Agency

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Disclaimer

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This staff report, the Notice of Public Hearing to consider Amendments to Regulations for the State Ambient Air Quality Standard for Ozone, and all subsequent regulatory documents, including the FSOR, when completed, are available on the ARB Internet site for this rulemaking at www.arb.ca.gov/regact/ozone05/ozone05.htm
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Abbreviations and Definitions

abscission	the normal separation, involving a layer of specialized cells, of flowers, fruits and leaves of plants
AOT40	accumulated exposure over threshold of 40 ppb ozone
AQDA	air quality data action
ARB	Air Resources Board
AVG	aminoethoxyvinyl glycine
BSA	Broader Sacramento Area
Ca^{2+}	calcium ion
canopy	a cover of foliage that forms when the leaves on the branches trees in a forest overlap during the growing season
CEC	controlled environment chamber
CFR	Code of Federal Regulations
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
d	day
edaphic	the physical, chemical, and biological characteristics of soil
ESPACE	European Stress Physiology and Climate Experiment
FACE	Free Air Carbon Enrichment system, a chamber-free, open-air fumigation design
FEF25-75%	forced expiratory flow rate between 25 and 75% of forced vital capacity
FEM	federal equivalent method (for air monitoring)
FEV1	forced expiratory volume in one second
fine roots	roots with a diameter between 0.5 to 3 mm
foliar	of or referring to a plant leaf
FRM	federal reference method (for air monitoring)
full-sib	seedlings that have the same parents, but not necessarily from seed produced in the same year
FVC	forced vital capacity
g	gram
GBVAB	Great Basin Valleys Air Basin
gdw	gram dry weight
GIS	geographic information system

gfw	gram fresh weight
hr	hour
ha	hectare (= 10,000 m^2; an area that is 100 m x 100 m)
half-sib	seedlings that have one parent in common
hm	hourly mean
HNO_3	nitric acid
homeostasis	the tendency toward maintaining physiological stability within an organism (plant or animal)
H&SC	Health and Safety Code
IPM	Integrated Pest Management.
Jeffrey pine	*Pinus jeffreyi* Grev. and Balf.
k	allometric growth coefficient describing the distribution of dry weight gain between competing plant parts, defined as the ratio of the relative growth rates of the competing plant parts
K^+	potassium ion
kg	kilogram (= 1,000 g = 2.205 pounds)
km	kilometer (= 1,000 m = 0.6214 miles)
L	liter
LCAB	Lake County Air Basin
LST	local standard time
LTAB	Lake Tahoe Air Basin
m	meter (= 3.28 feet)
m^2	square meter, an area that is 1 m x 1 m
MCAB	Mountain Counties Air Basin
MDAB	Mojave Desert Air Basin
mesophyll cells	the internal cells of a leaf, distinct from cells at the leaf surface or from cell layers immediately adjacent to the leaf surface
mixed conifer	forests with a tree-layer dominated by a mixture of conifer species
montane	of or relating to a mountain or mountainous area
mRNA	messenger RNA (ribonucleic acid)
mycorrhizae	a biological association of a fungus (e.g., *Pisolithus tinctorius*) with the root cells of a plant (e.g., ponderosa pine tree)
mycorrhizal trees	trees with roots associated a mycorrhizae fungus

n	sample size
NARSTO	a public/private partnership to coordinate research in Canada, Mexico and the United States on tropospheric air pollution (formerly the North American Research Strategy for Tropospheric Ozone)
NCAB	North Coast Air Basin
NCCAB	North Central Coast Air Basin
NCLAN	National Crop Loss Assessment Network, a national study of ozone impacts on crops, undertaken during the 1980s
NEPAB	Northeast Plateau Air Basin
ng	nanogram (= 0.000000001 g = 10^{-9} g)
NH_4N_3	ammonium nitrate
nL	nanoliter (10^{-9} L)
nm	nanometer, or one billionth of a meter
NO	nitric oxide, the primary nitrogen-containing by-product of combustion
NO_2	nitrogen dioxide
NO_X	nitrogen oxides (or oxides of nitrogen)
ns	not statistically significant at p =0.05
O_3	ozone; triatomic oxygen
OII	ozone injury index
OTC	open top field exposure chamber
PAR	photosynthetically active radiation (400 – 700 nm)
phloem	the plant tissue through which sugars and other organic materials are transferred to different parts of the plant
photosynthesis	the production by green plants of organic compounds from water and carbon dioxide using energy absorbed from sunlight
Pisolithus tinctorius	a mycorrhizae-forming fungus that forms root-associations with a wide variety of pine and other tree species
ppb	parts per billion by volume
ppb-hr	parts per billion hours (i.e., sum of concentration times duration), a measure of exposure to ozone
ppm	parts per million by volume
ppm-hr	parts per million hours (i.e., sum of concentration times duration), a measure of exposure to ozone

process rates	the degree or amount at which specific actions or activities occur (e.g., water vapor loss from leaves of plants)
QAS	Quality Assurance Section (of ARB)
R:S	ratio of root biomass (dry weight) to shoot biomass
RGR	relative growth rate, defined as the difference in the dry weight of a plant or plant part over a time period, divided by the initial dry weight and the length of the time period
RH	relative humidity
RuBisCO	ribulose bisphosphate carboxylase-oxygenase
RuBP	ribulose bisphosphate
SCCAB	South Central Coast Air Basin
SCOIAS	Sierra Cooperative Ozone Impact Assessment Study
SDAB	San Diego Air Basin
senescence	the onset of aging -- a phase in plant development from maturity to the complete loss of organization and function in plants
SFBAAB	San Francisco Bay Area Air Basin
shoot	the aboveground portion of the plant (e.g., leaves, stems, flowers, and fruits)
sieve cells	the primary type of cell found in the phloem of plants
SIP	State Implementation Plan
SJVAB	San Joaquin Valley Air Basin
SoCAB	South Coast Air Basin
SSAB	Salton Sea Air Basin
sucrose	a disaccharide (with 12 carbon atoms) commonly found in plants
(sucrose) translocation	the movement of sucrose (or other soluble organic food materials) through plant tissues – most commonly from leaves to stems/roots
SUM06	an ozone exposure metric involving concentration weighting, defined as the sum of all hourly mean ozone concentrations equal to or greater than 70 ppb
terrain-effect winds	air currents influenced by the geographic features of the land that it passes over
TREEGRO	a physiologically based computer simulation model of tree growth and development

Ulmus americana	the scientific name for “American Elm”
UN-ECE	United Nations Economic Commission for Europe
USD	United States dollars
USDA	United States Department of Agriculture
USDI	United States Department of the Interior
USEPA	United States Environmental Protection Agency
USV	Upper Sacramento Valley
V_d	deposition velocity, defined as deposition flux of ozone divided by its concentration in air (usually in cm/s or m/s)
VPD	vapor pressure deficit, a measure of evaporative demand of air
whorl	the arrangement of leaves, petals, etc., at about the same place on a stem
wk	week
yr	year
ZAP	zonal application system, a chamber-free, open-air exposure system
µg	microgram (= 0.000001 g = 10^{-6} g)
µm	micrometer or micron (= 0.000001 m = 10^{-6} m)

1 Executive Summary

The California Health and Safety Code in section 39606, requires the Air Resources Board to adopt ambient air quality standards at levels that adequately protect the health of the public, including infants and children, with an adequate margin of safety. Ambient air quality standards are the legal definition of clean air. In December 2000, as a requirement of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Stats. 1999, Health and Safety Code 39606 (d)(1)), the Air Resources Board (ARB or Board), approved a report, "Adequacy of California Ambient Air Quality Standards" (ARB and OEHHA, 2000) that contained a brief review of all of the existing health-based California ambient air quality standards.

Following this review, the standard for ozone, currently set at 0.09 parts per million (ppm) for one hour, was prioritized to undergo full review after review of the standards for particulate matter and sulfates. Staff from ARB and the Office of Environmental Health Hazard Assessment (OEHHA) have reviewed the scientific literature on public exposure, atmospheric chemistry, health effects of exposure to ozone, and welfare effects. This Staff Report or Initial Statement of Reasons (Staff Report) presents the findings of the review and the staff recommendations to revise the ozone standard in order to adequately protect public health. The proposed amendments to the ambient air quality standard for ozone are based on the health effects review contained in Volume III of this Report and the recommendation of OEHHA, as required by Health and Safety Code section 39606(a)(2).

1.1 Summary of the Staff Report/Initial Statement of Reasons

1.1.1 Health Effects of Ozone

Controlled human exposure studies demonstrate that ozone exposure can result in reduced lung function, increased respiratory symptoms, increased airway hyperreactivity, and increased airway inflammation. Epidemiologic studies indicate that exposure to ozone is also associated with premature death, hospitalization for cardiopulmonary causes, emergency room visits for asthma, and restrictions in activity.

In controlled human exposure studies (see Chapter 9), exercising individuals exposed for 1 hour (hr) to an ozone concentration as low as 0.12 parts per million (ppm) or for 6.6 hours to a concentration as low as 0.08 ppm experienced lung function decrements and symptoms of respiratory irritation such as cough, wheeze, and pain upon deep inhalation. The lowest ozone concentrations at which airway hyperreactivity (an increase in the tendency of the airways to constrict in reaction to exposure to irritants) has been reported are 0.18 ppm ozone following 2-hour exposure in exercising subjects, 0.40 ppm following 2-hour exposure in resting subjects, and 0.08 ppm ozone in subjects exercising for 6.6 hr. Airway inflammation has been reported following 2-hour exposures to 0.20 ppm ozone and following 6.6-hour exposure to 0.08 ppm ozone.

Additional support for the exposure/response relationship for ozone health effects is derived from animal toxicological studies, which have shown that chronic ozone exposure can induce morphological (tissue) changes throughout the respiratory tract, particularly at the junction of the conducting airways and the gas exchange zone in the deep lung. In addition, the magnitude of ozone-induced effects is related to the inhaled dose (ozone concentration times breathing rate times exposure duration). Of these three factors ozone concentration is the most significant in predicting the magnitude of observed effects, followed by ventilation rate. Exposure duration has the least influence of the three factors.

Epidemiological studies (see Chapter 10) have shown positive associations between ozone levels and several health effects, including decreased lung function, respiratory symptoms, hospitalizations for cardiopulmonary causes, emergency room visits for asthma, and premature death. Children may be more affected by ozone than the general population due to effects on the developing lung and to relatively higher exposure than adults. There is little information available on the effects of ozone exposure on infants. Also, asthmatics may represent a sensitive sub-population for ozone. Since most California residents are exposed to levels at or above the current State ozone standard during some parts of the year, the statewide potential for significant health impacts associated with ozone exposure is large and wide-ranging.

1.1.2 Summary of Non-health Issues

The Staff Report contains reviews and discussions of non-health topics to provide a context for the health review and the staff recommendations for the State ozone standard. Almost all of the ozone in California's atmosphere results from reactions between substances emitted from sources including motor vehicles and other mobile sources, power plants, industrial plants, and consumer products. These reactions involve volatile organic compounds (VOC) and oxides of nitrogen (NO_X) in the presence of sunlight (Chapter 3). Ozone is a regional pollutant, as the reactions forming it take place over time, and downwind from the sources of the emissions. As a photochemical pollutant, ozone is formed only during daylight hours under appropriate conditions, but is destroyed throughout the day and night. Thus, ozone concentrations vary depending upon both the time of day and the location. Even in pristine areas there is some ambient ozone that forms from natural emissions that are not controllable (Chapter 4). This is termed "background" ozone. The average "background" ozone concentrations near sea level are in the range of 0.015 to 0.035 ppm, with a maximum of about 0.04 ppm.

The Staff Report includes an overview of statewide ozone precursor emissions that are involved in the formation of ozone (Chapter 5). The Staff Report also includes a discussion of the current ultraviolet photometry monitoring method, and a listing of approved samplers (Chapter 6). Although there are two measurement methods for ozone approved for use in the U.S. by the U.S. Environmental Protection Agency (USEPA), the method based on ultraviolet photometry is almost universally used in practice and is approved for use in California for state air quality standards.

The Staff Report includes a summary of current air quality in California, as well as long-term trends in statewide ozone concentrations (Chapter 7). Ozone is monitored continuously at approximately 175 sites in California. The highest number of exceedance days for both the State and federal 1-hour standards occurred in the San Joaquin Valley Air Basin and the South Coast Air Basin. Both areas had more than 115 State standard exceedance days and 31 or more federal standard exceedance days during each of the three years from 2001 through 2003. The Sacramento Metro Area, Mojave Desert Air Basin, and Salton Sea Air Basin all averaged more than 50 State standard exceedance days and averaged 6 or more federal standard exceedance days during 2001 through 2003. The remaining five areas (Mountain Counties Air Basin, San Diego Air Basin, San Francisco Bay Area Air Basin, South Central Coast Air Basin, and the Upper Sacramento Valley) averaged from 12 to 45 State standard exceedance days. The Upper Sacramento Valley area had no exceedances of the federal standard while the Mountain Counties Air Basin, San Diego Air Basin, San Francisco Bay Area Air Basin, and South Central Coast Air Basin each averaged 1 to 2 federal standard exceedance days for the three-year period.

The range of the measured maximum 1-hour concentrations tends to follow a similar pattern. The South Coast Air Basin showed the highest values, with measured concentrations of 0.169 ppm or higher during 2001 through 2003. The next highest 1-hour ozone concentrations occurred in the Salton Sea Air Basin and San Joaquin Valley Air Basin, which had concentrations of 0.149 ppm or higher during all three years. During 2001 through 2003, neither the State nor federal 1-hour standard was exceeded in the Lake County Air Basin, North Coast Air Basin, or Northeast Plateau Air Basin. Data for four additional areas, Great Basin Valleys Air Basin, Lake Tahoe Air Basin, North Central Coast Air Basin, and the Upper Sacramento Valley show exceedances of the State standard, but not the federal 1-hour standard (as described earlier, representative data for the Northeast Plateau Air Basin and Great Basin Valleys Air Basin are available for 2002 and 2003 only). Both the State and federal 1-hour standards were exceeded during at least two of the three years in all other areas.

Californians' indoor and personal exposures to ozone are largely determined by the outdoor ozone concentrations in their community. Nonetheless, some Californians experience a substantial exposure to ozone indoors, due to the increasing use of certain types of appliances and equipment that emit ozone. Children and those who are employed in outdoor occupations or exercise heavily outdoors, experience substantially greater exposures to ozone than the rest of the population, because they spend time outdoors during peak ozone periods.

A review of welfare effects, including effects of ozone on forest trees, agricultural crops, and materials is also discussed in this report (Chapter 8). Elevated concentrations of ozone can cause adverse effects on agricultural crops, forest trees and materials at current ambient levels, and the proposed health-based ozone standards should also provide protection to crops, forests and materials. In broad terms, impacts to crops are generally more severe than for forest trees owing to their inherently more vigorous rates of growth. Discussed in the

subsection on crops and the methods used to expose plants to ozone. This is followed by an examination of the physiological basis of ozone damage to plants, with special emphasis on carbon metabolism and the resulting impacts on crop growth and yield. Data collected since the 1950s on mixed conifer forests in the San Bernardino Mountains and the Sierra Nevada indicate that increasing numbers of ponderosa and Jeffrey pines exhibit ozone-specific needle damage due to the pollutant's cumulative effects. Also discussed are the impacts of ozone on materials, including building materials, rubber, paint, and fabrics. Although the proposed ozone standards are based on human health effects, progress toward attaining the proposed standards will provide welfare benefits.

1.2 Staff Recommendations for the Ozone Standard

California ambient air quality standards are defined in the Health and Safety Code section 39014, and 17 Cal. Code Regs. section 70101, and comprise four elements: (1) a definition of the air pollutant, (2) an averaging time, (3) a pollutant concentration, and (4) a monitoring method to determine attainment of the standard. The current California ambient air quality standard for ozone is 0.09 ppm averaged over one hour and was set by the Board in 1988. The data indicate that the current standard alone is not sufficiently protective of human health. Based on the review of the scientific literature and recommendations by OEHHA, the staff recommends that the following revisions be made to the California ambient air quality standard for ozone:

1. Ozone will continue to be the pollutant addressed by the standard.
2. Ozone 1-hour-average Standard – retain the current 1-hour-average standard for ozone at **0.09 ppm, not to be exceeded**.
3. Ozone 8-hour-average Standard – establish a new 8-hour-average standard for ozone at **0.070 ppm**, **not to be exceeded.**
4. Ozone Monitoring Method: retain the current monitoring method for ozone which uses the ultraviolet (UV) photometry method for determining compliance with the State ambient air quality standard for ozone. Incorporate by reference (17 Cal. Code Regs. section 70101) all federally approved UV methods (i.e., samplers) for ozone as "California Approved Samplers". This will result in no change in air monitoring equipment practices, but will align state monitoring requirements with federal requirements.

These recommendations are based on the following findings:

a. Reduced lung function and increased respiratory or ventilatory symptoms following 1-hour exposure to 0.12 ppm ozone with moderate to heavy exercise.

b. Increased airway hyperreactivity following 2-hour exposure to 0.18 ppm in exercising subjects.

c. Airway inflammation following 2-hour exposure to 0.20 ppm ozone in exercising subjects

d. Reduced lung function, increased respiratory and ventilatory symptoms, increased airway hyperreactivity, and increased airway inflammation following 6.6 to 8-hour exposure to 0.08 ppm ozone.

e. Evidence from epidemiological studies of several health endpoints at current ambient concentrations of ozone including premature death, hospitalization, respiratory symptoms, and restrictions in activity and lung function.

f. Evidence from epidemiological studies of emergency room visits for asthma suggesting a possible threshold concentration between 0.075 and 0.11 ppm from analyses based on a 1-hour averaging time, and a possible threshold concentration between 0.070 and 0.10 ppm from analyses based on an 8-hour averaging time.

g. There is no evidence that children and infants respond to lower ozone concentrations than adults. Their risk is primarily related to their greater ventilation rate and greater exposure duration.

h. The dose-rate of ozone inhalation influences the magnitude of observed effects.

The staff recommendations for revision of the California ambient air quality standard for ozone are primarily based on controlled human exposure studies. Epidemiologic data contributed to development of the margin of safety.

1.3 Other Recommendations

In light of the adverse health effects observed at current ambient concentrations and the lack of a demonstrated effect threshold for the population as a whole, staff makes the following comments:

1. Fund additional research investigating the responses of human subjects to multi-hour exposures to ozone concentrations between 0.04 and 0.08 ppm.
2. The standards should be revisited within five years, in order to re-evaluate the evidence regarding the health effects associated with ozone exposure.
3. In any air basin in California that currently attains the ambient air quality standards for ozone, air quality should not be degraded from present levels.

1.4 Estimated Health Impacts of Ozone Exposure

Exposure to ozone at current ambient levels has substantial health impacts, including, but not limited to, death, hospitalization, emergency room visits, and symptoms of respiratory irritation. Staff estimates that the annual health impact of exposure to ozone at current levels, compared to attainment of the proposed State 8-hour and 1-hour ozone standards throughout California includes:

- 630 (310 – 950 probable range) premature deaths for all ages.

- 4,200 (2,400 - 5,800, 95% confidence interval (CI)) hospitalizations due to respiratory diseases for all ages.
- 660 (400 – 920, 95% CI) emergency room visits for asthma for children under 18 years of age.
- 4.7 million (1,200,000 – 8,600,000, 95% CI) school absences for children 5 to 17 years of age.
- 3.1 million (1.3 million – 5.0 million, 95% CI) minor restricted activity days for adults above 18 years of age.

For comparison purposes, we also estimated the health impacts o current ozone levels compared to attainment of the federal 8-hour ozone standard of 0.08 ppm, and the health impacts of current ozone levels compared to attainment of the State 1-hour ozone standard alone. Specifically, we estimate that 360 (180 – 550, probable range) premature deaths annually are related to current ozone levels, compared to statewide attainment of the federal 8-hour standard, and about 540 (270 – 810, probable range) premature deaths annually are related to current ozone levels, compared to statewide attainment of the State 1-hour standard. Results for other health endpoints are in Appendix B.

The differences between the results are the “incremental” impacts of not attaining the State 1-hour and 8-hour standards, compared to the federal 8-hour ozone standard. However, it is more reasonable to consider attainment of the two State standards together, compared to current ozone levels, since it is unlikely that control strategies will be geared to fist attain one standard and then the other. Nonetheless, the current impact of not attaining the federal 8-hour standard is about 360 premature deaths annually, with an additional 270 deaths associated with not attaining the proposed State 8-hour standard, making the total estimated impact of not attaining both standards 630 deaths. Similarly, the current impact of not attaining the State 1-hour standard is about 540 premature deaths annually, with an additional 90 deaths associated with not attaining the proposed 8-hour standard, making the total estimated impact of not attaining both the State 1-hour and 8-hour ozone standards 630 premature deaths. More detailed discussion of this analysis is available in Appendix B.

As discussed in Appendix B, there are a several important assumptions and uncertainties in this analysis. Some have to do with study design, statistical methods, and choice of epidemiological studies used to develop the concentration-response (CR) functions used in the analysis. Few studies have investigated the shape of the CR function, or whether there is a population response threshold for health endpoints other than emergency room visits for asthma. Further uncertainty is added by assumptions in the statewide exposure assessment. It should also be noted that since several health effects related to acute exposure, and effects of chronic ozone exposure, are not included in the estimates, the health benefits associated with lowering ozone exposure are likely underestimated.

1.5 Public and Peer Review of the Staff Recommendations

The draft version of this Staff Report was released to the public on June 21, 2004 and presented for review and comment at public workshops during 2004 on July 14 in Sacramento, July 15 in El Monte, July 16 in Fresno, and August 25 in Sacramento.

The draft Staff Report was peer reviewed by the Air Quality Advisory Committee (AQAC). AQAC is a scientific peer review committee, appointed by the University of California, to independently evaluate the scientific basis of staff findings and recommendations in the draft Staff Report for revising the California ambient air quality standard for ozone. The AQAC held a public meeting to discuss its review of the draft Staff Report, comments submitted by the public, and staff responses to those comments. AQAC concluded that the report was well written and researched, and that the proposed revision to the State ozone standard was adequately supported. AQAC findings, public comments, and staff responses can be found in Appendices C-E. Following the meeting of the Air Quality Advisory Committee (AQAC), staff revised the draft Staff Report based on comments received from AQAC and the public.

1.6 Environmental and Economic Impacts

The proposed ambient air quality standards will in and of themselves have no environmental or economic impacts. Standards simply define clean air. Once adopted, local air pollution control or air quality management districts are responsible for the adoption of rules and regulations to control emissions from stationary sources to assure their achievement and maintenance. The ARB is responsible for adoption of emission standards for mobile sources and consumer products. A number of different implementation measures are possible, and each could have its own environmental or economic impact. These impacts must be evaluated when the control measure is proposed. Any environmental or economic impacts associated with the imposition of future measures will be considered if and when specific measures are proposed.

1.7 Environmental Justice Considerations

State law defines environmental justice as the fair treatment of people of all races, cultures, and incomes with respect to the development, adoption, implementation, and enforcement of environmental laws, regulations, and policies. The available literature suggests there appears to be no special vulnerability related to race, ethnicity or income level, although there may be higher exposure. Ambient air quality standards define clean air; therefore, all of California's communities will benefit from the proposed health-based standards.

1.8 Comment Period and Board Hearing

Release of this Staff Report opens the official 45-day public comment period required by the Administrative Procedure Act prior to the public meeting of the Air Resources Board to consider the staff's recommendations. Please direct all comments to either the following postal or electronic mail address:

Clerk of the Board
Air Resources Board
1001 "I" Street, 23rd Floor
Sacramento, California 95814
ozone05@listserve.arb.ca.gov

To be considered by the Board, written submissions not physically submitted at the hearing must be received at the ARB no later than 12:00 noon, April 27, 2005. Public workshops will be scheduled for April 2005 to present the final staff recommendations and receive public input on the Staff Report. Information on these workshops, as well as summaries of the presentations from past workshops and meetings are available by calling 1-916-445-0753 or at the following ARB website:
http://www.arb.ca.gov/research/aaqs/ozone-rs/ozone-rs.htm.

An oral report summarizing the staff recommendations for revising the ozone standard will be presented to the Board at a public hearing scheduled for April 28, 2005.

The staff recommends that the Board adopt the proposed amendments to the ambient air quality standards for ozone as stated above. The proposed amendments and their basis are described in detail in this Staff Report, which contains the findings of ARB and OEHHA staff's full review of the public health, scientific literature, and exposure pattern data for ozone in California. Due to the extensive nature of the literature review and the hundreds of studies reviewed, the Staff Report is divided into four volumes. Volume I contains the Executive Summary, Overview and Staff Recommendations, and Appendix A, the proposed amendments to the California Code of Regulations (amended regulatory text). Volumes II through IV present more detailed discussions of the material that is summarized in Volume I. Volume II includes background material on non-health topics, including chemistry of ozone formation and deposition, ozone precursor sources and emissions, ozone exposure and background levels, measurement methods, and welfare effects of ozone exposure. Volume III contains a summary of ozone health effects and an in-depth discussion of the basis for the staff recommendation. Volume IV includes several appendices, including an analysis of the estimated health benefits associated with attainment of the proposed standards, summaries of Air Quality Advisory Committee and public comments and staff responses, and supplemental animal toxicologic data.

1.9 References

Air Resources Board and Office of Environmental Health Hazard Assessment (2000). Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act. Staff Report. Sacramento, CA. Available at http://www.arb.ca.gov/ch/programs/sb25/airstandards.htm.

2 Overview and Staff Recommendations

Ozone (O_3) can damage human cells upon contact, and has been implicated in a variety of adverse health effects. Scientific studies show that exposure to ozone can result in reduced lung function, increased respiratory symptoms, increased airway hyperreactivity, and airway inflammation. Exposure to ozone is also associated with premature death, hospitalization for cardiopulmonary causes, emergency room visits for asthma, and restrictions in activity. Ozone forms in the atmosphere as the result of reactions involving sunlight and two classes of directly emitted precursors. One class of precursors includes nitric oxide (NO) and nitrogen dioxide (NO_2), collectively referred to as nitrogen oxides or NO_X. The other class of precursors includes volatile organic compounds (VOCs, also called reactive organic gases or ROG), such as hydrocarbons. Ozone forms in greater quantities on hot, sunny, calm days. In metropolitan areas of California and areas downwind, ozone concentrations frequently exceed existing health-protective standards in the summertime. The current California ambient air quality standard for ozone is 0.09 ppm for one hour.

The sources of ozone precursor emissions within California have been grouped into three major categories: point sources, which are distinct facilities such as power plants and factories; mobile sources, which includes cars, trucks, and off-road mobile equipment; and area-wide sources, which include agricultural and construction activities, and consumer products. VOCs are emitted from vehicles, factories, fossil fuels combustion, evaporation of paints, and many other sources. NO_X is emitted from high-temperature combustion processes, such as at power plants or in motor vehicle exhaust .

The concentrations of ozone measured in the air vary both regionally and seasonally throughout California. For example, the Los Angeles area and the San Joaquin Valley experience highest ozone levels in the state. Ozone concentrations are typically higher during the summer months than the winter months.

To help understand which sources contribute to high ozone levels, the ARB has developed and maintains detailed facility and source specific estimates of the overall estimated ozone precursor emissions. Only the precursor gases are estimated. As a complement to emission inventory and routinely collected air quality monitoring data, the ARB conducts atmospheric modeling, using these precursor emission inventories and other appropriate information, to estimate ozone levels

2.1 Setting California Ambient Air Quality Standards

Ambient air quality standards (AAQS) represent the legal definition of clean air. They specify concentrations and durations of exposure to air pollutants that reflect the relationships between the intensities and composition of air pollution and undesirable effects (Health and Safety Code section 39014). The objective of an AAQS is to provide a basis for preventing or abating adverse health or welfare effects of air pollution (17 Cal. Code Regs. section 70101).

Health and Safety Code section 39606(a)(2) authorizes the Air Resources Board (Board) to adopt standards for ambient air quality "in consideration of public health,

safety, and welfare, including, but not limited to, health, illness, irritation to the senses, aesthetic value, interference with visibility, and effects on the economy." Standards represent the highest pollutant concentration for a given averaging time that is estimated to be without adverse effects for most people. Standards are set to ensure that sensitive population sub-groups are protected from exposure to levels of pollutants that may cause adverse health effects. A margin of safety is added to account for possible deficiencies in the data and measuring methodology. Health-based standards are based on the recommendation of the Office of Environmental Health Hazard Health Assessment (OEHHA).

Recent legislation requires that infants and children be given special consideration when ambient air quality standards are adopted. As part of its recommendation to the ARB, the statute requires OEHHA to use current principles, practices, and methods used by public health professionals to assess the following considerations for infants and children:

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

The law also requires that the scientific basis or the scientific portion of the method used to assess these considerations be peer reviewed (Health and Safety Code section 39606(c)). The draft Staff recommendations and their bases, including OEHHA's assessment and recommendation, is peer reviewed by the Air Quality Advisory Committee (AQAC). AQAC is an external peer review committee established in accordance with section 57004 of the Health and Safety Code and appointed by the President of the University of California a University of California. The AQAC meets to independently evaluate the scientific basis of draft recommendations for revising the California ambient air quality standards.

Ambient air quality standards should not be interpreted as permitting, encouraging, or condoning degradation of present air quality that is superior to that stipulated in the standards. Rather, they represent the minimum acceptable air quality. An AAQS adopted by the Board is implemented, achieved, and maintained by numerous rules and regulations that limit pollution from specific sources of ozone precursors. These rules and regulations are primarily, though not exclusively, emission limitations established by the regional and local air pollution control and air quality management districts for stationary sources, and by the Board for vehicular sources and consumer products (see generally, Health and Safety Code sections 39002, 40000, and 40001).

2.2 Current California Ambient Air Quality Standard for Ozone

The current California ambient air quality standard for ozone, established in 1988, is 0.09 ppm (180 μg/m^3) for a one-hour average. This value is not to be exceeded. This standard was established based on the following most relevant effects, which are listed in the table of standards (17 Cal. Code Regs. section 70200):

a. Short-term exposures:

(1) Pulmonary function decrements and localized lung edema in humans and animals.

(2) Risk to public health implied by alterations in pulmonary morphology and host defence in animals.

b. Long-term exposures: Risk to public health implied by altered pulmonary morphology in animals after long-term exposures and pulmonary function decrements in chronically exposed humans.

c. Welfare effects:

(1) Yield loss in important crops and predicted economic loss to growers and consumers.

(2) Injury and damage to native plants and potential changes in species diversity and number.

(3) Damage to rubber and elastomers and to paints, fabric, dyes, pigments, and plastics.

The US EPA has set national ambient air quality standards, as noted in the table below. The federal one-hour standard will be phased out beginning in June 2005. The Federal Clean Air Act gives California authority to set its own ambient air quality standards in consideration of statewide concerns. California has the largest number of exceedances of the Federal 8-hour ozone standard in the United States, supporting California's need to address a significant statewide public health issue.

Current Ambient Air Quality Standards for Ozone

Averaging Time	California Standard	Federal Standard
1 Hour	0.09 ppm (180 μg/m^3)	0.12 ppm (235 μg/m^3)
8 Hour	—	0.08 ppm (157 μg/m^3)

2.3 History of Ozone/Oxidant Standards

The first state oxidant standard was set in December 1959 by the state Department of Public Health (DPH), which had the responsibility for setting air pollution standards before the creation of the ARB. This standard was set at 0.15 ppm, averaged for one hour. The standard was for oxidant, rather than ozone, because the monitoring method available at that time, the potassium iodide (KI) method, measured all ambient oxidant

gases, including ozone and other oxidants such as peroxyacetyl nitrate (PAN) nitrogen dioxide, photochemical aerosols, and other unknown oxidants.

In 1969, the newly-created ARB reviewed the oxidant standard set by DPH and revised the standard to a concentration of 0.10 ppm, averaged over one hour, not to be equaled or exceeded. The information considered by the Board in 1969 included adverse effects upon: (1) the health of humans and animals; (2) vegetation; (3) materials; and (4) visibility. Eye irritation was listed as the most relevant effect of oxidant.

In 1974, the Board introduced ultraviolet photometry as the monitoring method for the standard. However, since ultraviolet photometry measures only ozone, the Board changed the designation of the standard from “oxidant” to “oxidant (as ozone).” Because only ozone was to be measured, the Board changed the most relevant effect from: “eye irritation” (which is caused primarily by peroxyacyl nitrates or PANs) to “aggravation of respiratory disease” (which is caused primarily by ozone).

In 1988, the Board changed the designation of the standard from “oxidant (as ozone)” to "ozone", and revised the standard to a concentration of 0.09 ppm, averaged over one hour, to reflect that the listed relevant effects were related to ozone exposure, rather than to oxidants in general.

For comparison, in 2000, the World Health Organization established a guideline value for ozone in ambient air of 120 $\mu g/m^3$ (0.061 ppm) for a maximum period of 8 hours per day (WHO 2000).

2.4 Review of the California Ambient Air Quality Standards

The Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Stats. 1999, ch. 731) required the ARB, in consultation with the OEHHA, to evaluate all health-based standards by December 31, 2000, to determine whether the standards were adequately protective of the health of the public, including infants and children (Health and Safety Code section 39606 (d)). At its December 7, 2000 meeting, the Board approved a report, “Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act” (ARB, et al., 2000), prepared by ARB and OEHHA staffs. The Adequacy Report concluded that health effects may occur in infants and children and other potentially susceptible subgroups exposed to ozone at or near levels corresponding to the current standard. The report identified the standard for ozone as having the second highest priority for further detailed review and possible revision. The standard for PM10 (including sulfates) had the highest priority and was reviewed and revised in 2002, including establishment of a new standard for PM2.5.

2.5 Findings of the Standard Review

2.5.1 Chemistry and Physics

Most of the ozone in California’s air results from reactions between substances emitted from sources including motor vehicles, power plants, industrial plants, consumer products, and vegetation. These reactions involve volatile organic compounds (VOCs, which the ARB also refers to as reactive organic gases or ROG) and oxides of nitrogen (NO_X) in the presence of sunlight. Ozone is a regional pollutant, as the reactions forming it take place over time, and downwind from the precursor sources. As a

photochemical pollutant, ozone is formed only during daylight hours under appropriate conditions, but is destroyed throughout the day and night. Thus, ozone concentrations vary depending upon both the time of day and the location. Ozone concentrations are higher on hot, sunny, calm days. In metropolitan and downwind areas of California, ozone concentrations frequently exceed regulatory standards during the summer.

2.5.2 Ozone Background

Even in pristine areas there is some ambient ozone that forms from natural emissions that are not controllable. This is termed “background” ozone. Overall, it appears that “background” ozone in California is dominated by natural tropospheric and stratospheric processes. The effects of occasional very large biomass fires and anthropogenic emissions are secondary factors. The foregoing discussion indicates that average “natural background” ozone near sea level is in the range of 0.015 to 0.035 ppm, with a maximum of about 0.04 ppm. Exogenous enhancements to “natural” levels generally are small (about 0.005 ppm), and are unlikely to alter peak concentrations.

At altitudes above 2 km stratospheric intrusions can push peak ambient concentrations to 0.045 to 0.050 ppm. The timing, spatial extent, and chemical characteristics of stratospheric air mass intrusions makes these events recognizable in air quality records, providing that the affected region has a fairly extensive monitoring network and that multiple air quality parameters (CO, VOC, PM, RH) are being measured as well.

Intermittent episodes of “natural” ozone from very large biomass fires in boreal forests (Alaska, Canada, Siberia) can produce short-lived pulses of ozone up to 0.020 ppm that may arrive during the North American ozone season. Present understanding suggests that these are infrequent events at latitudes below about 50N. There are no data documenting such an event in California. Long range transport of anthropogenic ozone may grow as Asian energy consumption increases the continent’s NO_X emissions. Model studies indicate that the Asian ozone increment in North America could double over the next few decades. Assuming the temporal pattern of transport remains unchanged, such an impact could increase mean ozone concentrations by 0.002 to 0.006 ppm. The potential effect on peak transport events is unknown at this time.

2.5.3 Ozone Precursor Emissions

Ozone is an oxidant gas that forms photochemically in the atmosphere when nitrogen oxides (NO_X) and reactive organic gases (ROG) are present under appropriate atmospheric conditions (see Chapter 5). Carbon monoxide (CO) is also an ozone precursor. Both ROG and NO_X are emitted from mobile sources, point sources, and area-wide sources. ROG emissions from anthropogenic sources result primarily from incomplete fuel combustion, and from the evaporation of solvents and fuels, while NO_X and CO emissions result almost entirely from combustion processes.

2.5.4 Monitoring Method

Two measurement methods for ozone are approved for use in the U.S. by the USEPA: one is based on the chemiluminescence that occurs when ozone and ethylene react, and the other on the attenuation of ultraviolet (UV) radiation by ozone. The method based on UV spectrometry is almost universally used in practice. Specifications and criteria for both methods exist in federal regulation. The UV photometry-based method

is approved for use in California for state air quality standards. Both state and federal requirements are applied directly by the ARB and the air districts in the ozone monitoring network in California.

2.5.5 Exposure

During 2001 through 2003, neither the State nor federal 1-hour standard was exceeded in the Lake County Air Basin, North Coast Air Basin, or Northeast Plateau Air Basin. Data for four additional areas, Great Basin Valleys Air Basin, Lake Tahoe Air Basin, North Central Coast Air Basin, and the Upper Sacramento Valley show exceedances of the State standard, but not the federal 1-hour standard (as described earlier, representative data for the Northeast Plateau Air Basin and Great Basin Valleys Air Basin are available for 2002 and 2003 only). Both the State and federal 1-hour standards were exceeded during at least two of the three years in all other areas.

The highest 8-hour average values were found in the South Coast Air Basin and San Joaquin Valley Air Basin. Maximum 8-hour concentrations in the South Coast Air Basin ranged from 0.144 ppm to 0.153 ppm during 2001 through 2003, while maximum 8-hour concentrations in the San Joaquin Valley ranged from 0.120 ppm to 0.132 ppm during the same three-year period. Three other areas, the Mojave Desert Air Basin, the Sacramento Metro Area, and the Salton Sea Air Basin also had a maximum 8-hour concentration above 0.120 ppm during at least one of the three years.

With respect to the federal 8-hour ozone standard, Lake County Air Basin and North Coast Air Basin showed no exceedance days during 2001 through 2003. One area, the Lake Tahoe Air Basin, averaged only one exceedance day for the three-year period, while the North Central Coast Air Basin averaged three 8-hour exceedance days. In contrast, the San Joaquin Valley Air Basin showed the highest average number of exceedance days (123), followed by the South Coast Air Basin (99). The Sacramento Metro Area, Mojave Desert Air Basin, Mountain Counties Air Basin, and Salton Sea Air Basin each averaged between 42 and 68 exceedance days during 2001 through 2003. The remaining four areas averaged between 7 and 25 federal 8-hour exceedance days during the three-year period.

Californians' indoor and personal exposures to ozone are largely determined by the outdoor ozone concentrations in their community. Nonetheless, some Californians experience a substantial exposure to ozone indoors, due to the increasing use of certain types of appliances and equipment that emit ozone. Others, such as many children and those who are employed in outdoor occupations, may experience substantially greater exposures to ozone than the rest of the population, because they spend time outdoors during peak ozone periods.

2.5.6 Welfare Effects

A review of welfare effects, including effects of ozone on forest trees, agricultural crops, and materials is also discussed in this report (Chapter 8). Elevated concentrations of ozone can cause adverse effects on agricultural crops, forest trees and materials at current ambient levels, and the proposed health-based ozone standards should also provide protection to crops, forests and materials. In broad terms, impacts to crops are generally more severe than for forest trees owing to their inherently more vigorous rates

of growth. Discussed in the subsection on crops and the methods used to expose plants to ozone. This is followed by an examination of the physiological basis of ozone damage to plants, with special emphasis on carbon metabolism and the resulting impacts on crop growth and yield. Data collected since the 1950s on mixed conifer forests in the San Bernardino Mountains and the Sierra Nevada indicate that increasing numbers of ponderosa and Jeffrey pines exhibit ozone-specific needle damage due to the pollutant's cumulative effects. Also discussed are the impacts of ozone on materials, including building materials, rubber, paint, and fabrics. Although the proposed ozone standards are based on human health effects, progress toward attaining the proposed standards will provide welfare benefits.

2.5.7 Health Effects

Review of the controlled human exposure, animal toxicology and epidemiologic literature led to the following conclusions as to the health effects of ozone exposure:

1. The lowest ozone concentration at which reduced lung function and increased respiratory and ventilatory symptoms have been observed following 1-hour exposure is 0.12 ppm with moderate to heavy exercise.
2. The lowest ozone concentration at which increased airway hyperreactivity following 2-hour exposure has been reported is 0.18 ppm in exercising subjects.
3. The lowest ozone concentration at which airway inflammation following 2-hour exposure has been reported is 0.20 ppm ozone in exercising subjects
4. Reduced lung function, increased respiratory and ventilatory symptoms, increased airway hyperreactivity, and increased airway inflammation have been reported following 6.6- to 8-hour exposure to 0.08 ppm ozone.
5. Evidence from epidemiological studies of several health endpoints including premature death, hospitalization, respiratory symptoms, and restrictions in activity and lung function.
6. Evidence from epidemiological studies of emergency room visits for asthma suggests a possible threshold concentration between 0.075 and 0.11 ppm from analyses based on a 1-hour averaging time, and a possible threshold concentration between 0.070 and 0.10 ppm from analyses based on an 8-hour averaging time.
7. There is no evidence that children and infants respond to lower ozone concentrations than adults. Their risk is primarily related to their greater ventilation rate and greater exposure duration.
8. The dose-rate of ozone inhalation influences the magnitude of observed effects.

2.6 Summary of Recommendations

Following a detailed review of the scientific literature on the health and welfare effects of ozone, staff is proposing to revise the ambient air quality standard for ozone. The recommended ozone standards are based on scientific information about the health impacts associated with ozone exposure, recognizing the uncertainties in these data. The definition of California ambient air quality standards assumes a threshold below which effects do not occur. However, the extremely wide range of individual

responsiveness to ozone makes identification of a threshold on a population level somewhat problematic. In addition, the Children's Environmental Health Protection Act [Senate Bill 25, Escutia; Stats. 1999, Ch. 731, H&SC 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." Recognizing the uncertainties in the database, staff makes the following recommendations.

1. Ozone will continue to be the pollutant addressed by the standard.

2. <u>One-hour ambient air quality standard</u>: staff recommends retaining the current 1-hour ozone standard at a concentration of **0.09 ppm**, not to be exceeded, based on several factors. First, at 0.12 ppm, in several studies 10 - 25% of the subjects experienced a decline of 10% of 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 and adults 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 potentially vulnerable populations (e.g., people with moderate to severe asthma, Chronic Obstructive Pulmonary Disease or COPD, and heart disease) who may be incorporated in the epidemiologic studies.

   Second, chamber studies indicate that bronchial responsiveness and pulmonary inflammation occur with 1-hour exposure to 0.18 to 0.20 ppm. Bronchial responsiveness can aggravate 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 mechanisms.

   Third, epidemiological studies completed over the last 10 years indicate the potential for severe adverse health outcomes including premature death, hospitalizations, and emergency room visits. These studies include concentrations to which the public is currently being exposed. It is possible that some of these associations are due to relatively short-term exposures, for example less than two hours, since people at risk of experiencing these endpoints are unlikely to be engaged in multi-hour periods of moderate or heavy work or exercise outdoors. However, since there is high temporal correlation between 1-, 8-, and 24-hour average ozone concentrations, the averaging time of concern cannot be discerned from these studies.

   Viewing all of the evidence, staff recommends 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.

3. <u>Eight-hour ambient air quality standard</u>: We recommend establishing a new 8-hour average 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. With exposure for 6.6 to 8-hours to an ozone concentration of 0.08 ppm, several studies have reported statistically significant group effects on lung function changes, ventilatory and respiratory symptoms, airway hyperresponsiveness, and airway inflammation in healthy, exercising individuals. A substantial fraction of subjects in these studies exhibited particularly marked responses in lung function and symptoms. Consequently, a concentration of 0.08 ppm ozone for an 8-hour averaging time can not be considered adequately protective of public health, and does not include any margin of safety, based on the definitions put forth in State law. 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 individual subjects had changes in FEV1 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 inhalation and total symptom score. Therefore, staff has recommended an 8-hour concentration of 0.070 ppm. 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 the health effects, often correlated with 1-hour exposures in the epidemiologic studies, are actually associated with 8-hour (or other) average exposures. Therefore, these epidemiologic findings were factored into the margin of safety for the 8-hour average.

It should be noted that the recommended 8-hour average concentration has three rather than two decimal places. Staff initially considered selection of 0.07 ppm. However, rounding conventions applied to air quality data (see Section 7.1.4) are such that any measured value up to and including 0.074 ppm would round down to 0.07 ppm. The available data suggested that selection of 0.07 ppm would not include an adequate margin of safety, as required by State law. The one available study at 0.06 ppm did not find a group mean effect. Staff is recommending that the 8 hour average standard have three decimal places, 0.070 ppm, to ensure an adequate margin of safety. Section 6.3 discusses issues related to precision and accuracy of the monitored data.

4. Monitoring method for ozone: Staff recommends retention of the current monitoring method for ozone which uses the ultraviolet (UV) absorption method for determining compliance with the state Ambient Air Quality Standard for ozone. Incorporate by reference all federally approved UV methods for ozone as California Approved Samplers for ozone. This will not change current air monitoring practices, but will align state monitoring requirements with federal requirements.

2.6.1 Consideration of Infants and Children

The Children's Environmental Health Protection Act [Health and Safety Code section 39606 (b)] requires that air pollution effects on children and infants be specifically considered in selection of ambient air quality standards. Children have a higher ventilation rate relative to body weight at rest and during activity than adults. Children also tend to spend more time outside and be more active than adults. Consequently,

virtue of their higher ventilation rates and outdoor behavior patterns, they are likely to inhale larger total doses of ozone than the general population. However, the chamber studies of exercising children suggest that they have responses generally similar to adults, pointing to a similar degree of responsiveness. Epidemiologic studies that have examined 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). The study by Kunzli et al. (1997) suggested that exposure to ozone prior to age 6 was associated with lower attained lung function. 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 1-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 that may be associated with ozone exposure. Collectively, this body of evidence suggests that although children appear to be similarly responsive to a given dose of ozone as adults, they are at greater risk than adults of experiencing adverse responses to ozone by virtue of their higher level of outdoor activity, and consequently greater total exposure.

2.7 Estimated Health Impacts

Exposure to ozone at current ambient levels has substantial health impacts, including, but not limited to, death, hospitalization, emergency room visits, and symptoms of respiratory irritation. Staff estimates that the annual health impact of exposure to ozone at current levels, compared to attainment of the proposed State 8-hour and 1-hour ozone standards throughout California includes :

- 630 (310 – 950, probable range) premature deaths for all ages.
- 4,200 (2,400 – 5,400, 95% CI) hospitalizations due to respiratory diseases for all ages.
- 660 (400 – 920, 95% CI) emergency room visits for asthma for children under 18 years of age.
- 4.7 million (1,200,000 – 8,600,000, 95% CI) school absences for children 5 to 17 years of age.
- 3.1 million (1.3 million – 5.0 million, 95% CI) minor restricted activity days for adults above 18 years of age.

For comparison purposes, we also estimated the health impacts o current ozone levels compared to attainment of the federal 8-hour ozone standard of 0.08 ppm, and the health impacts of current ozone levels compared to attainment of the State 1-hour

ozone standard alone. Specifically, we estimate that 360 (180 – 550, probable range) premature deaths annually are related to current ozone levels, compared to statewide attainment of the federal 8-hour standard, and about 540 (270 – 810, probable range) premature deaths annually are related to current ozone levels, compared to statewide attainment of the State 1-hour standard. Results for other health endpoints are in Appendix B.

The differences between the results are the "incremental" impacts of not attaining the State 1-hour and 8-hour standards, compared to the federal 8-hour ozone standard. However, it is more reasonable to consider attainment of the two State standards together, compared to current ozone levels, since it is unlikely that control strategies will be geared to fist attain one standard and then the other. Nonetheless, the current impact of not attaining the federal 8-hour standard is about 360 premature deaths annually, with an additional 270 deaths associated with not attaining the proposed State 8-hour standard, making the total estimated impact of not attaining both standards 630 deaths. Similarly, the current impact of not attaining the State 1-hour standard is about 540 premature deaths annually, with an additional 90 deaths associated with not attaining the proposed 8-hour standard, making the total estimated impact of not attaining both the State 1-hour and 8-hour ozone standards 630 premature deaths. More detailed discussion of this analysis is available in Appendix B.

As discussed in Appendix B, there are a several important assumptions and uncertainties in this analysis. Some concern the study design, statistical methods, and choice of epidemiological studies used to develop the concentration-response (CR) functions used in the analysis. Few studies have investigated the shape of the CR function, or whether there is a population response threshold for health endpoints other than emergency room visits for asthma. Further uncertainty is added by assumptions in the statewide exposure assessment. It should also be noted that since several health effects related to acute exposure, and effects of chronic ozone exposure, are not included in the estimates noted above, the health benefits associated with lowering ozone exposure are likely underestimated.

2.8 Public Outreach and Review

A draft Staff Report containing staff's preliminary findings was released to the public on June 21, 2004 titled, "Review of California Ambient Air Quality Standard for Ozone". Public outreach for the standard review involved dissemination of information through various outlets to include the public in the regulatory process. In an ongoing effort to include the public in the review of the ozone standard, the ARB and OEHHA integrated outreach into public meetings, workshop presentations, electronic "list serve" notification systems, and various web pages. Notification of release of the Staff Report, the schedule for public meetings and workshops, and invitations to submit comments on the Staff Report were made through the "list serve" notification system. Public workshops on the proposed ozone standard were held on July 14 – 16, 2004 in Sacramento, El Monte, and Fresno. An additional public workshop was held on August 24, 2004 in Sacramento.

Individuals or parties interested in signing up for an electronic e-mail "list serve" notification on the ozone standards, as well as any air quality-related issue, may self-

enroll at the following location: www.arb.ca.gov/listserv/aaqs/aaqs.htm. Additional information on the standards review process is also available at the ozone standards review schedule website at: www.arb.ca.gov/research/aaqs/ozone-rs/ozone-rs.htm.

2.9 Air Quality Advisory Committee Review

The Air Quality Advisory Committee, an external scientific peer review committee that was appointed by the President of the University of California, met January 11 and 12, 2005, in Berkeley, California to review the initial Staff Report and public comments, and to ensure that the scientific basis of the recommendations for the ozone standard are based upon sound scientific knowledge, methods, and practices. The AQAC held a public meeting, which provided time for oral public comments, and discussed their review of the draft Staff Report and the draft recommendations, and provided comments for improving the draft Staff Report. Final findings were received on February 24, 2005.

The AQAC determined that the staff recommendations were well founded on the scientific literature, and voted to endorse them. The Committee made suggestions for minor changes to the draft Staff Report to increase clarity, requested more detailed discussion of several topics, and inclusion of several additional scientific papers. The AQAC findings are included in this Initial Statement of Reasons as Appendix C, in Volume IV.

2.10 Environmental and Economic Impacts

The proposed ambient air quality standards are scientific in nature, and will in and of themselves have no environmental or economic impacts. Standards simply define clean air. Once adopted, local air pollution control or air quality management districts are responsible for the adoption of rules and regulations to control emissions from stationary sources to assure their achievement and maintenance. The Board is responsible for adoption of emission standards for mobile sources. A number of different implementation measures are possible, and each could have its own environmental and/or economic impact. These impacts must be evaluated when the control measure is proposed. Any environmental or economic impacts associated with the imposition of future measures will be considered if and when specific measures are proposed.

2.11 Environmental Justice

State law defines environmental justice as the fair treatment of people of all races, cultures, and incomes with respect to the development, adoption, implementation, and enforcement of environmental laws, regulations, and policies (Senate Bill 115, Solis; Stats 1999, Ch. 690; Government Code §65040.12(c)). The Board established a framework for incorporating environmental justice into the ARB's programs consistent with the directives of State law (ARB, 2001). The policies developed apply to all communities in California, but recognize that environmental justice issues have been raised more in the context of low-income and minority communities, which sometimes experience higher exposures to some pollutants as a result of the cumulative impacts of air pollution from multiple mobile, commercial, industrial, areawide, and other sources.

Because ambient air quality standards simply define clean air, all of California's communities will benefit from the proposed health-based standards, as progress is made to attain the standards. Over the past twenty years, the ARB, local air districts, and federal air pollution control programs have made substantial progress towards improving the air quality in California. However, some communities continue to experience higher exposures than others as a result of the cumulative impacts of air pollution from multiple mobile and stationary sources and thus may suffer a disproportionate level of adverse health effects. Since the same ambient air quality standards apply to all regions of the State, these communities will benefit by a wider margin and receive a greater degree of health improvement from the revised standards than less affected communities, as progress is made to attain the standards. Moreover, just as all communities would benefit from new, stricter standards, alternatives to the proposed recommendations, such as not proposing an eight-hour ozone standard, would adversely affect many communities.

While it is possible that residents in environmental justice communities may be particularly sensitive to ozone, only one study investigated whether socioeconomic status (SES) alters responses to ozone exposure, and those results were difficult to explain. Hence, the study did not allow inferences as to whether socioeconomic status impacts on sensitivity to ozone. Moreover, other controlled studies investigating whether gender, ethnicity or environmental factors contribute to the responses to ozone exposure could not convincingly demonstrate a link with responsiveness. Therefore, the database is insufficient to conclude whether differences in ozone susceptibility exist in environmental justice communities. These studies are discussed in more detail in Section 9.6.8.

Once ambient air quality standards are adopted, the ARB and the local air districts will propose emission standards and other control measures designed to result in a reduction of ambient ozone levels. The environmental justice aspects of each proposed control measure will be evaluated in a public forum at this time.

As additional relevant scientific evidence becomes available, the ozone standards will be reviewed again to make certain that the health of the public is protected with an adequate margin of safety.

2.12 References

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.

Air Resources Board. Ambient Air Quality Standard for Ozone: Health and Welfare Effects. Staff Report. September 1987. Sacramento, CA.

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

Air Resources Board (2001). Policies and Actions for Environmental Justice, December 13, 2001.

Galizia A, Kinney PL. 1999. Long-term residence in areas of high ozone: associations with respiratory health n a nationwide sample of nonsmoking young adults. Environ Health Perspect 107:675-679.

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.

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 359:386-391.

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Appendix A

PROPOSED AMENDMENTS TO CALIFORNIA CODE OF REGULATIONS

AND

AIR MONITORING QUALITY ASSURANCE MANUAL VOLUME IV, PARTS A, B, & C (DOCUMENT INCORPORATED BY REFERENCE)

[PROPOSED] REGULATION ORDER

Section 70100. Definitions

~~(g) Oxidant. Oxidant is a substance that oxidizes a selected reagent that is not oxidizable by oxygen under ambient conditions. For the purposes of this section, oxidant includes ozone, organic peroxides, and peroxyacyl nitrates but not nitrogen dioxide. Atmospheric oxidant concentrations are to be measured with ozone as a surrogate by ultraviolet photometry, or by an equivalent method.~~

(g~~h~~) Carbon Monoxide …

(h~~i~~) Sulfur Dioxide …

(i~~j~~) Suspended Particulate Matter (PM10). Suspended particulate matter (PM10) refers to atmospheric particles, solid and liquid, except uncombined water as measured by a (PM10) sampler which collects 50 percent of all particles of 10 mm aerodynamic diameter and which collects a declining fraction of particles as their diameter increases and an increasing fraction of particles as their diameter decreases, reflecting the characteristics of lung deposition. ~~Suspended particulate matter (PM10) is to be measured by a California Approved Sampler (CAS) for PM10, for purposes of monitoring for compliance with the Suspended Particulate Matter (PM10) standards. Approved samplers, methods, and instruments are listed in Section 70100.1(a) below. A CAS for PM10 includes samplers, methods, or instruments determined by the Air Resources Board or the Executive Officer to produce equivalent results for PM10 with the Federal Reference Method (40 CFR, part 50, Appendix M, as published in 62 Fed. Reg. 38763, July 18, 1997).~~

(j~~k~~) Fine Suspended Particulate Matter (PM2.5). Fine suspended particulate matter (PM2.5) refers to suspended atmospheric particles solid and liquid, except uncombined water as measured by a PM2.5 sampler which collects 50 percent of all particles of 2.5 mm aerodynamic diameter and which collects a declining fraction of particles as their diameter increases and an increasing fraction of particles as their diameter decreases, reflecting the characteristics of lung deposition. ~~Fine suspended particulate matter (PM2.5) is to be measured by a California Approved Sampler (CAS) for PM2.5 for purposes of monitoring for compliamce with the Fine Particulate Matter (PM2.5) standards. Approved samplers, methods, and instruments are listed in Section 70100.1(b) below. A CAS for PM2.5 includes samplers, method, and instruments determined by the Air Resources Board or the Executive Officer to produce equivalent results for PM2.5 with the Federal Reference Method (40 CFR, part 50, Appendix L, as published in 62 Fed. Reg. 38763, July 18, 1997).~~

(k~~l~~) Visibility Reducing Particles …

(l~~m~~) Hydrogen Sulfide …

(m~~n~~) Nitrogen Dioxide …

(n~~o~~) Lead (particulate) …

(o~~p~~) Sulfates …

(p~~q~~) Vinyl Chloride …

(q~~r~~) Ozone …

(r~~s~~) Extinction Coefficient …

Section 70100.1. Methods, Samplers, and Instruments for Measuring Pollutants.

a) PM10 Methods. The method for determining compliance with the PM10 ambient air quality standard shall be the Federal Reference Method for the Determination of Particulate Matter as PM10 in the Atmosphere (40 CFR, Chapter 1, part 50, Appendix M, as published in 62 Fed. Reg., 38753, July 18, 1997). California Approved Samplers for PM10 are set forth in "Air Monitoring Quality Assurance Manual Volume IV, Part A: Monitoring Methods for PM10", adopted [insert date], which is incorporated by reference herein. Samplers, methods, or instruments determined in writing by the Air Resources Board or the Executive Officer to produce equivalent results for PM10 shall also be California Approved Samplers for PM10. These include those continuous samplers that have been demonstrated to the satisfaction of the Air Resources Board to produce measurements equivalent to the Federal Reference Method. ~~The following samplers, methods, and instruments are California Approved Samplers for PM10 for the purposes of monitoring for compliance with the Suspended Particulate Matter (PM10) standards:~~

~~(1) The specific samplers approved are:~~

~~(A) Andersen Model RAAS10-100 PM10 Single Channel PM10 Sampler, U.S. EPA Manual Reference Method RFPS-0699-130, as published in 64 Fed. Reg., 33481, June 23, 1999.~~

~~(B) Andersen Model RAAS10-200 PM10 Single Channel PM10 Audit Sampler, U.S. EPA Manual Reference Method RFPS-0699-131, as published in 64 Fed. Reg., 33481, June 23, 1999.~~

~~(C) Andersen Model RAAS10-300 PM10 Multi Channel PM10 Sampler, U.S. EPA Manual Reference Method RFPS-0669-132, as published in 64 Fed. Reg., 33481, June 23, 1999.~~

~~(D) Sierra (currently known as Graseby) Andersen/GMW Model 1200 High Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-063, as published in 52 Fed. Reg., 45684, December 1, 1987 and in 53 Fed. Reg., 1062, January 15, 1988.~~

~~(E) Sierra (currently known as Graseby) Andersen/GMW Model 321B High Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-064, as published in 52 Fed. Reg., 45684, December 1, 1987 and in 53 Fed. Reg., 1062, January 15, 1988.~~

~~(F) Sierra (currently known as Graseby) Andersen/GMW Model 321-C High Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-065, as published in 52 Fed. Reg., 45684, December 1, 1987.~~

~~(G) BGI Incorporated Model PQ100 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-124, as published in 63 Fed. Reg., 69624, December 17, 1998.~~

~~(H) BGI Incorporated Model PQ200 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-125, as published in 63 Fed. Reg., 69624, December 17, 1998.~~

~~(I) Rupprecht & Patashnick Partisol Model 2000 Air Sampler, U.S. EPA Manual Reference Method RFPS-0694-098, as published in 59 Fed. Reg., 35338, July 11, 1994.~~

~~(J) Rupprecht & Patashnick Partisol FRM Model 2000 PM10 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-126, as published in 63 Fed. Reg., 69625, December 17, 1998.~~

~~(K) Rupprecht & Patashnick Partisol Plus Model 2025 PM10 Sequential Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-127, as published in 63 Fed. Reg., 69625, December 17, 1998.~~

~~(L) Tisch Environmental Model TE-6070 PM10 High Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-0202-141, as published in 67 Fed. Reg., 15566, April 2, 2002.~~

~~(2) Continuous samplers:~~

~~(A) Andersen Beta Attenuation Monitor Model FH 62 C14 equipped with the following components: louvered PM10 inlet, volumetric flow controller, automatic filter change mechanism, automatic zero check, and calibration control foils kit*.~~

~~(B) Met One Beta Attenuation Monitor Model 1020 equipped with the following components: louvered PM10 size selective inlet, volumetric flow controller, automatic filter change mechanism, automatic heating system, automatic zero and span check capability*.~~

~~(C) Rupprecht & Patashnick Series 8500 Filter Dynamics Measurement System equipped with the following components: louvered PM10 size selective inlet, volumetric flow control, flow splitter (3 liter/min sample flow), sample equilibration system (SES) dryer, TEOM sensor unit, TEOM control unit, switching valve, purge filter conditioning unit, and palliflex TX40, 13 mm effective diameter cartridge*.~~

b) PM2.5 Methods. The method for determining compliance with the PM2.5 ambient air quality standard shall be the Federal Reference Method for the Determination of Particulate Matter as PM2.5 in the Atmosphere, 40 CFR, Chapter 1, part 50, Appendix L, as published in 62 Fed. Reg., 38714, July 18, 1997 and as amended in 64 Fed. Reg., 19717, April 22, 1999. The samplers listed in the Federal Reference Method must use either the WINS impactor or the U.S. EPA-approved very sharp cut cyclone (67 Fed. Reg., 15566, April 2, 2002) to separate PM2.5 from PM10. California Approved Samplers for PM2.5 are set forth in "Air Monitoring Quality Assurance Manual Volume IV, Part B: Monitoring

Methods for PM2.5", adopted [insert date], which is incorporated by reference herein. Samplers, methods, or instruments determined in writing by the Air Resources Board or the Executive Officer to produce equivalent results for PM2.5 shall also be California Approved Samplers for PM2.5. These include those continuous samplers that have been demonstrated to the satisfaction of the Air Resources Board to produce measurements equivalent to the Federal Reference Method. ~~The following samplers, methods, and instruments are California Approved Samplers for PM2.5 for the purposes of monitoring for compliance with the Fine Particulate Matter (PM2.5) standards:~~

~~(1) The specific samplers approved are:~~

~~(A) Andersen Model RAAS 2.5-200 PM2.5 Ambient Audit Air Sampler, U.S. EPA Manual Reference Method RFPS-0299-128, as published in 64 Fed. Reg., 12167, March 11, 1999.~~

~~(B) Graseby Andersen Model RAAS 2.5-100 PM2.5 Ambient Air Sampler, U.S. EPA Manual Reference Method RFPS-0598-119, as published in 63 Fed. Reg., 31991, June 11, 1998.~~

~~(C) Graseby Andersen Model RAAS 2.5-300 PM2.5 Sequential Ambient Air Sampler, U.S. EPA Manual Reference Method RFPS-0598-120, as published in 63 Fed. Reg., 31991, June 11, 1998.~~

~~(D) BGI Inc. Models PQ200 and PQ200A PM2.5 Ambient Fine Particle Sampler, U.S. EPA Manual Reference Method RFPS-0498-116, as published in 63 Fed. Reg., 18911, April 16, 1998.~~

~~(E) Rupprecht & Patashnick Partisol FRM Model 2000 Air Sampler, U.S. EPA Manual Reference Method RFPS-0498-117, as published in 63 Fed. Reg., 18911, April 16, 1998.~~

~~(F) Rupprecht & Patashnick Partisol Model 2000 PM-2.5 Audit Sampler, as described in U.S. EPA Manual Reference Method RFPS-0499-129, as published in 64 Fed. Reg., 19153, April 19, 1999.~~

~~(G) Rupprecht & Patashnick Partisol-Plus Model 2025 PM-2.5 Sequential Air Sampler, U.S. EPA Manual Reference Method RFPS-0498-118, as published in 63 Fed. Reg., 18911, April 16, 1998.~~

~~(H) Thermo Environmental Instruments, Incorporated Model 605 "CAPS" Sampler, U.S. EPA Manual Reference Method RFPS-1098-123, as published in 63 Fed. Reg., 58036, October 29, 1998.~~

~~(I) URG MASS100 Single PM2.5 FRM Sampler, U.S. EPA Manual Reference Method RFPS-0400-135, as published in 65 Fed. Reg., 26603, May 8, 2000.~~

~~(J) URG MASS300 Sequential PM2.5 FRM Sampler, U.S. EPA Manual Reference Method RFPS-0400-136, as published in 65 Fed. Reg., 26603, May 8, 2000.~~

~~(K) BGI Inc. Model PQ200 VSCC PM2.5 Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-142, as published in 67 Fed. Reg., 15567, April 2, 2002.~~

~~(L) BGI Inc. Model PQ200A-VSCC PM2.5 Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-142, as published in 67 Fed. Reg., 15567, April 2, 2002.~~

~~(M) Rupprecht & Patashnick Partisol-FRM Model 2000 PM2.5 FEM Air Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-143, as published in 67 Fed. Reg., 15567, April 2, 2002.~~

~~(N) Rupprecht & Patashnick Partisol Model 2000 PM2.5 FEM Audit Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-144, as published in 67 Fed. Reg., 15567, April 2, 2002.~~

~~(O) Rupprecht & Patashnick Partisol-Plus Model 2025 PM-2.5 FEM Sequential Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-145, as published in 67 Fed. Reg., 15567, April 2, 2002.~~

~~(2) Continuous samplers:~~

~~(A) Andersen Beta Attenuation Monitor Model FH 62 C14 equipped with the following components: louvered PM10 size selective inlet, very sharp cut or~~

~~sharp cut cyclone, volumetric flow controller, automatic filter change mechanism, automatic zero check, and calibration control foils kit*.~~

~~(B) Met One Beta Attenuation Monitor Model 1020 equipped with the following components: louvered PM10 size selective inlet, very sharp cut or sharp cut cyclone, volumetric flow controller, automatic filter change mechanism, automatic heating system, and automatic zero and span check capability*.~~

~~(C) Rupprecht & Patashnick Series 8500 Filter Dynamics Measurement System equipped with the following components: louvered PM10 size selective inlet, very sharp cut or sharp cut cyclone, volumetric flow control, flow splitter (3 liter/min sample flow), sample equilibration system (SES) dryer, TEOM sensor unit, TEOM control unit, switching valve, purge filter conditioning unit, and palliflex TX40, 13 mm effective diameter cartridge*.~~

~~*Instrument shall be operated in accordance with the vendor's instrument operation manual that adheres to the principles and practices of quality control and quality assurance as specified in Volume I of the "Air Monitoring Quality Assurance Manual", as printed on April 17, 2002, and available from the California Air Resources Board, Monitoring and Laboratory Division, P.O. Box 2815, Sacramento CA 95814, incorporated by reference herein.~~

(c) Ozone Methods. The method for determining compliance with the ozone ambient air quality standard shall be the Federal Equivalent Method for the Determination of Ozone in the Atmosphere (40 CFR, part 53). California Approved Samplers for ozone are set forth in "Air Monitoring Quality Assurance Manual Volume IV, Part C: Monitoring Methods for Ozone", as adopted [insert date]. Samplers, methods, or instruments determined in writing by the Air Resources Board or the Executive Officer to produce equivalent results for ozone shall also be California Approved Samplers for ozone.

NOTE

Authority cited: Sections 39600, 39601 and 39606, Health and Safety Code. Reference: Sections 39014, 39606, 39701 and 39703(f), Health and Safety Code.

Section 70200. Table of Standards ***

Substance	Concentration and Methods*	Duration of Averaging Periods	Most Relevant Effects	Comments
Ozone	0.09 ppm** 0.070 ppm** ultraviolet photometry using California Approved Sampler as set forth in section 70100.1 (c)	1 hour 8 hour	a. Short-term exposures: (1) ~~Pulmonary function decrements and localized lung edema in humans and animals.~~ One-hour and multi-hour exposures: lung function decrements, and symptoms of respiratory irritation such as cough, wheeze, and pain upon deep inhalation. (2) Multi-hour exposures: airway hyperreactivity and airway inflammation. ~~(2) Risk to public health implied by alterations in pulmonary morphology and host defence in animals.~~ (3) excess deaths, hospitalization, emergency room visits, asthma exacerbation, respiratory symptoms and restrictions in activity b. Long-term exposures: ~~Risk to public health implied by altered pulmonary morphology in animals after long-term exposures and pulmonary function decrements in chronically exposed humans.~~ Ozone can induce tissue changes in the respiratory tract, and is associated with decreased lung function and emergency room visits for asthma. c. Welfare effects: (1) Yield loss in important crops and predicted economic loss to growers and consumers. (2) Injury and damage to forests ~~native plants and potential changes in species diversity and number~~. ~~(3) Damage to rubber and elastomers and to paints, fabric, dyes, pigments, and plastics.~~	a. The standard is intended to prevent adverse human health effects. b. The standard, when achieved, will not prevent all injury to crops and other types of vege~~t~~tation, but is intended to place an acceptable upper limit on the amount of yield and economic loss, as well as on adverse environmental impacts.

Suspended Particulate Matter (PM10)	50 µg/m^3 PM10** 20 µg/m^3 PM10** using California Approved Sampler as set forth in section 70100.1(a)	24 hour sample 24 hour samples, annual arithmetic mean	Prevention of excess deaths, illness and restrictions in activity from short-and long-term exposures. Illness outcomes include, but are not limited to, respiratory symptoms, bronchitis, asthma exacerbation, emergency room visits and hospital admissions for cardiac and respiratory diseases. Sensitive subpopulations include children, the elderly, and individuals with pre-existing cardiopulmonary disease.	This standard applies to suspended mater as measured by PM10 sampler, which collects 50% of all particles of 10 µm aerodynamic diameter and collects a declining fraction of particles as their diameter increases, reflecting the characteristics of lung deposition.

* The list of California Approved Samplers may be obtained from the Air Resources Board, Monitoring and Laboratory Division, P.O. Box 2815, Sacramento, CA 95814. Any equivalent procedure which can be shown to the satisfaction of the Air Resources Board to give equivalent results at or near the level of the air quality standard may be used.

** These standards are violated when concentrations exceed those set forth in the body of the regulation. All other standards are violated when concentrations equal or exceed those set forth in the body of the regulation.

*** Applicable statewide unless otherwise noted.

****These standards are violated when particle concentrations cause measured light extinction values to exceed those set forth in the regulations.

NOTE

Authority cited: Sections 39600, 39601(a) and 39606, Health and Safety Code. Reference: Sections 39014, 39606, 39701 and 39703(f), Health and Safety Code; and Western Oil and Gas Ass'n v. Air Resources Bd. (1984) 37 Cal.3d 502.

HISTORY

1. Amendment filed 9-18-89; operative 10-18-89 (Register 89, No. 39). For prior history, see Register 88, No. 27.
2. Amendment filed 6-29-92; operative 7-29-92 (Register 92, No. 27).
3. Amendment filed 6-5-2003; operative 7-5-2003 (Register 2003, No. 23).

(1) The method for determining compliance with the State PM10 ambient air quality standard shall be the Federal Reference Method (FRM) for the Determination of Particulate Matter as PM10 in the Atmosphere (40 CFR, Chapter 1, part 50, Appendix M, as published in 62 Fed. Reg., 38753, July 18, 1997). When employed according to the FRM, the following are California Approved Samplers:

(A) Andersen Model RAAS10-100 PM10 Single Channel PM10 Sampler, U.S. EPA Manual Reference Method RFPS-0699-130, as published in 64 Fed. Reg., 33481, June 23, 1999.

(B) Andersen Model RAAS10-200 PM10 Single Channel PM10 Audit Sampler, U.S. EPA Manual Reference Method RFPS-0699-131, as published in 64 Fed. Reg., 33481, June 23, 1999.

(C) Andersen Model RAAS10-300 PM10 Multi Channel PM10 Sampler, U.S. EPA Manual Reference Method RFPS-0669-132, as published in 64 Fed. Reg., 33481, June 23, 1999.

(D) Sierra (currently known as Graseby) Andersen/GMW Model 1200 High-Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-063, as published in 52 Fed. Reg., 45684, December 1, 1987 and in 53 Fed. Reg., 1062, January 15, 1988.

(E) Sierra (currently known as Graseby) Andersen/GMW Model 321B High-Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-064, as published in 52 Fed. Reg., 45684, December 1, 1987 and in 53 Fed. Reg., 1062, January 15, 1988.

(F) Sierra (currently known as Graseby) Andersen/GMW Model 321-C High-Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-1287-065, as published in 52 Fed. Reg., 45684, December 1, 1987.

(G) BGI Incorporated Model PQ100 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-124, as published in 63 Fed. Reg., 69624, December 17, 1998.

(H) BGI Incorporated Model PQ200 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-125, as published in 63 Fed. Reg., 69624, December 17, 1998.

(I) Rupprecht & Patashnick Partisol Model 2000 Air Sampler, U.S. EPA Manual Reference Method RFPS-0694-098, as published in 59 Fed. Reg., 35338, July 11, 1994.

(J) Rupprecht & Patashnick Partisol-FRM Model 2000 PM10 Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-126, as published in 63 Fed. Reg., 69625, December 17, 1998.

(K) Rupprecht & Patashnick Partisol-Plus Model 2025 PM10 Sequential Air Sampler, U.S. EPA Manual Reference Method RFPS-1298-127, as published in 63 Fed. Reg., 69625, December 17, 1998.

(L) Tisch Environmental Model TE-6070 PM10 High-Volume Air Sampler, U.S. EPA Manual Reference Method RFPS-0202-141, as published in 67 Fed. Reg., 15566, April 2, 2002.

(2) The following continuous Californian Approved Samplers have been demonstrated to the satisfaction of the Air Resources Board to produce measurements equivalent to the FRM:

(A) Andersen Beta Attenuation Monitor Model FH 62 C14 equipped with the following components: louvered PM10 inlet, volumetric flow controller, automatic filter change mechanism, automatic zero check, and calibration control foils kit*.

(B) Met One Beta Attenuation Monitor Model 1020 equipped with the following components: louvered PM10 size selective inlet, volumetric flow controller, automatic filter change mechanism, automatic heating system, automatic zero and span check capability*.

(C) Rupprecht & Patashnick Series 8500 Filter Dynamics Measurement System equipped with the following components: louvered PM10 size selective inlet, volumetric flow control, flow splitter (3 liter/min sample flow), sample equilibration system (SES) dryer, TEOM sensor unit, TEOM control unit, switching valve, purge filter conditioning unit, and palliflex TX40, 13 mm effective diameter cartridge*.

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*Instrument shall be operated in accordance with the vendor's instrument operation manual that adheres to the principles and practices of quality control and quality assurance as specified in Volume I of the “Air Monitoring Quality Assurance Manual”, as printed on April 17, 2002, and available from the California Air Resources Board, Monitoring and Laboratory Division, P.O. Box 2815, Sacramento CA 95814, incorporated by reference herein.

(1) The method for determining compliance with the State PM2.5 ambient air quality standard shall be the Federal Reference Method (FRM) for the Determination of Particulate Matter as PM2.5 in the Atmosphere, 40 CFR, part 50, Appendix L, as published in 62 Fed. Reg., 38714, July 18, 1997 and as amended in 64 Fed. Reg., 19717, April 22, 1999. These must use either the WINS impactor or the U.S. EPA-approved very sharp cut cyclone (67 Fed. Reg., 15566, April 2, 2002) to separate PM2.5 from PM10. When employed according to the FRM, the following are California Approved Samplers:

(A) Andersen Model RAAS 2.5-200 PM2.5 Ambient Audit Air Sampler, U.S. EPA Manual Reference Method RFPS-0299-128, as published in 64 Fed. Reg., 12167, March 11, 1999.

(B) Graseby Andersen Model RAAS 2.5-100 PM2.5 Ambient Air Sampler, U.S. EPA Manual Reference Method RFPS-0598-119, as published in 63 Fed. Reg., 31991, June 11, 1998.

(C) Graseby Andersen Model RAAS 2.5-300 PM2.5 Sequential Ambient Air Sampler, U.S. EPA Manual Reference Method RFPS-0598-120, as published in 63 Fed. Reg., 31991, June 11, 1998.

(D) BGI Inc. Models PQ200 and PQ200A PM2.5 Ambient Fine Particle Sampler, U.S. EPA Manual Reference Method RFPS-0498-116, as published in 63 Fed. Reg., 18911, April 16, 1998.

(E) Rupprecht & Patashnick Partisol-FRM Model 2000 Air Sampler, U.S. EPA Manual Reference Method RFPS-0498-117, as published in 63 Fed. Reg., 18911, April 16, 1998.

(F) Rupprecht & Patashnick Partisol Model 2000 PM-2.5 Audit Sampler, as described in U.S. EPA Manual Reference Method RFPS-0499-129, as published in 64 Fed. Reg., 19153, April 19, 1999.

(G) Rupprecht & Patashnick Partisol-Plus Model 2025 PM-2.5 Sequential Air Sampler, U.S. EPA Manual Reference Method RFPS-0498-118, as published in 63 Fed. Reg., 18911, April 16, 1998.

(H) Thermo Environmental Instruments, Incorporated Model 605 “CAPS” Sampler, U.S. EPA Manual Reference Method RFPS-1098-123, as published in 63 Fed. Reg., 58036, October 29, 1998.

(I) URG-MASS100 Single PM2.5 FRM Sampler, U.S. EPA Manual Reference Method RFPS-0400-135, as published in 65 Fed. Reg., 26603, May 8, 2000.

(J) URG-MASS300 Sequential PM2.5 FRM Sampler, U.S. EPA Manual Reference Method RFPS-0400-136, as published in 65 Fed. Reg., 26603, May 8, 2000.

(K) BGI Inc. Model PQ200-VSCC PM2.5 Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-142, as published in 67 Fed. Reg., 15567, April 2, 2002.

(L) BGI Inc. Model PQ200A-VSCC PM2.5 Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-142, as published in 67 Fed. Reg., 15567, April 2, 2002.

(M) Rupprecht & Patashnick Partisol-FRM Model 2000 PM2.5 FEM Air Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-143, as published in 67 Fed. Reg., 15567, April 2, 2002.

(N) Rupprecht & Patashnick Partisol Model 2000 PM2.5 FEM Audit Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-144, as published in 67 Fed. Reg., 15567, April 2, 2002.

(O) Rupprecht & Patashnick Partisol-Plus Model 2025 PM-2.5 FEM Sequential Sampler, U.S. EPA Manual Equivalent Method EQPM-0202-145, as published in 67 Fed. Reg., 15567, April 2, 2002.

(2) The following continuous samplers have been demonstrated to the satisfaction of the Air Resources Board to produce measurements equivalent to the FRM:

(A) Andersen Beta Attenuation Monitor Model FH 62 C14 equipped with the following components: louvered PM10 size selective inlet, very sharp cut or sharp cut cyclone, volumetric flow controller, automatic filter change mechanism, automatic zero check, and calibration control foils kit*.

(B) Met One Beta Attenuation Monitor Model 1020 equipped with the following components: louvered PM10 size selective inlet, very sharp cut or sharp cut cyclone, volumetric flow controller, automatic filter change mechanism, automatic heating system, and automatic zero and span check capability*.

(C) Rupprecht & Patashnick Series 8500 Filter Dynamics Measurement System equipped with the following components: louvered PM10 size selective inlet, very sharp cut or sharp cut cyclone, volumetric flow control, flow splitter (3 liter/min sample flow), sample equilibration system (SES) dryer, TEOM sensor unit, TEOM control unit, switching valve, purge filter conditioning unit, and palliflex TX40, 13 mm effective diameter cartridge*.

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*Instrument shall be operated in accordance with the vendor's instrument operation manual that adheres to the principles and practices of quality control and quality assurance as specified in Volume I of the “Air Monitoring Quality Assurance Manual”, as printed on April 17, 2002, and available from the California Air Resources Board, Monitoring and Laboratory Division, P.O. Box 2815, Sacramento CA 95814, incorporated by reference herein.

Part C: Monitoring Methods for Ozone

The method for determining compliance with the State ozone ambient air quality standard shall be the Federal Equivalent Method (FEM) for the Determination of Ozone in the Atmosphere (40 CFR, part 53). The FEM (ultraviolet photometry) is considered equivalent to the Federal Reference Method (chemiluminescence) as described in 40 CFR, Chapter 1, Part 50, Appendix D as published in FR 62, 38895, July 18, 1997. When employed according to the FEM (40 CFR, part 53), the following are California Approved Samplers:

(A) Dasibi Models 1003-AH, 1003-PC, or 1003-RS Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0577-019, as published in FR 42, 28571, June 03, 1977.

(B) Dasibi Models 1008-AH, 1008-PC, or 1008-RS Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0383-056, as published in FR 48, 10126, March 10, 1983.

(C) DKK-TOA Corp. Model GUX-113E Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0200-134, as published in FR 65, 11308, March 02, 2000.

(D) Environics Series 300 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0990-078, as published in FR 55, 38386, September 18, 1990.

(E) Environnement S.A. Model $O_3$41M UV Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0895-105, as published in FR 60, 39382, August 02, 1995.

(F) Environnement S.A. Model $O_3$42M UV Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0206-148, as published in FR 67, 42557, June 24, 2002.

(G) Environnement S.A. SANOA Multigas Longpath Monitoring System, USEPA Automated Equivalent Method EQOA-0400-137, as published in FR 65, 26603, May 08, 2000.

(H) Horiba Instruments Models APOA-360 and APOA-360-CE Ozone Monitor, USEPA Automated Equivalent Method EQOA-0196-112, as published in FR 61, 11404, March 20, 1996.

(I) Monitor Labs/Lear Siegler Model 8810 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0881-053, as published in FR 46, 52224, October 26, 1981.

(J) Monitor Labs/Lear Siegler Models ML9810, ML9811, or ML9812, Monitors Labs Model ML9810B, or Wedding & Associates Model 1010 Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0193-091, as published in FR 58, 6964, February 03, 1993.

(K) Opsis Model AR 500 and System 300 Open Path Ambient Air Monitoring Systems for Ozone, USEPA Automated Equivalent Method EQOA-0495-103, as published in FR 60, 21518, May 02, 1995.

(L) PCI Ozone Corporation Model LC-12 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0382-055, as published in FR 47, 13572, March 31, 1982.

(M) Philips PW9771 O3 Analyzer, USEPA Automated Equivalent Method EQOA-0777-023, as published in FR 42, 38931, August 01, 1977; FR 42, 57156, November 01, 1977.

(N) Teledyne-Advanced Pollution Instrumentation, Inc. Model 400E Ozone Analyzer, Advanced Pollution Instrumentation, Inc. Model 400/400A Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0992-087, as published in FR 57, 44565, September 28; 1992, FR 63, 31992, June 11, 1998; FR 67, 57811, September 12, 2002.

(O) Thermo Electron/Thermo Environmental Instruments Models 49, 49C, USEPA Automated Equivalent Method EQOA-0880-047, as published in FR 45, 57168, August 27, 1980

Air Resources Board

State of California

Governor Arnold Schwarzenegger

October 2005 Revision

Review of the California Ambient Air Quality Standard For Ozone

Volume II of IV
Chapters 3-8

Staff Report
Initial Statement of Reasons for Proposed Rulemaking

October 27, 2005

California Environmental Protection Agency

Air Resources Board

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

Alan C. Lloyd, Ph.D., Secretary

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Abbreviations and Definitions

abscission	the normal separation, involving a layer of specialized cells, of flowers, fruits and leaves of plants
AOT40	accumulated exposure over threshold of 40 ppb ozone
AQDA	air quality data action
ARB	Air Resources Board
AVG	aminoethoxyvinyl glycine
BSA	Broader Sacramento Area
Ca^{2+}	calcium ion
canopy	a cover of foliage that forms when the leaves on the branches trees in a forest overlap during the growing season
CEC	controlled environment chamber
CFR	Code of Federal Regulations
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
d	day
edaphic	the physical, chemical, and biological characteristics of soil
ESPACE	European Stress Physiology and Climate Experiment
FACE	Free Air Carbon Enrichment system, a chamber-free, open-air fumigation design
FEF25-75%	forced expiratory flow rate between 25 and 75% of forced vital capacity
FEM	federal equivalent method (for air monitoring)
FEV1	forced expiratory volume in one second
fine roots	roots with a diameter between 0.5 to 3 mm
foliar	of or referring to a plant leaf
FRM	federal reference method (for air monitoring)
full-sib	seedlings that have the same parents, but not necessarily from seed produced in the same year
FVC	forced vital capacity
g	gram
GBVAB	Great Basin Valleys Air Basin
gdw	gram dry weight
GIS	geographic information system

gfw	gram fresh weight
hr	hour
ha	hectare (= 10,000 m^2; an area that is 100 m x 100 m)
half-sib	seedlings that have one parent in common
hm	hourly mean
HNO_3	nitric acid
homeostasis	the tendency toward maintaining physiological stability within an organism (plant or animal)
H&SC	Health and Safety Code
IPM	Integrated Pest Management.
Jeffrey pine	*Pinus jeffreyi* Grev. and Balf.
k	allometric growth coefficient describing the distribution of dry weight gain between competing plant parts, defined as the ratio of the relative growth rates of the competing plant parts
K^+	potassium ion
kg	kilogram (= 1,000 g = 2.205 pounds)
km	kilometer (= 1,000 m = 0.6214 miles)
L	liter
LCAB	Lake County Air Basin
LST	local standard time
LTAB	Lake Tahoe Air Basin
m	meter (= 3.28 feet)
m^2	square meter, an area that is 1 m x 1 m
MCAB	Mountain Counties Air Basin
MDAB	Mojave Desert Air Basin
mesophyll cells	the internal cells of a leaf, distinct from cells at the leaf surface or from cell layers immediately adjacent to the leaf surface
mixed conifer	forests with a tree-layer dominated by a mixture of conifer species
montane	of or relating to a mountain or mountainous area
mRNA	messenger RNA (ribonucleic acid)
mycorrhizae	a biological association of a fungus (e.g., *Pisolithus tinctorius*) with the root cells of a plant (e.g., ponderosa pine tree)
mycorrhizal trees	trees with roots associated a mycorrhizae fungus

n	sample size
NARSTO	a public/private partnership to coordinate research in Canada, Mexico and the United States on tropospheric air pollution (formerly the North American Research Strategy for Tropospheric Ozone)
NCAB	North Coast Air Basin
NCCAB	North Central Coast Air Basin
NCLAN	National Crop Loss Assessment Network, a national study of ozone impacts on crops, undertaken during the 1980s
NEPAB	Northeast Plateau Air Basin
ng	nanogram (= 0.000000001 g = 10^{-9} g)
NH_4N_3	ammonium nitrate
nL	nanoliter (10^{-9} L)
nm	nanometer, or one billionth of a meter
NO	nitric oxide, the primary nitrogen-containing by-product of combustion
NO_2	nitrogen dioxide
NO_X	nitrogen oxides (or oxides of nitrogen)
ns	not statistically significant at p =0.05
O_3	ozone; triatomic oxygen
OII	ozone injury index
OTC	open top field exposure chamber
PAR	photosynthetically active radiation (400 – 700 nm)
phloem	the plant tissue through which sugars and other organic materials are transferred to different parts of the plant
photosynthesis	the production by green plants of organic compounds from water and carbon dioxide using energy absorbed from sunlight
Pisolithus tinctorius	a mycorrhizae-forming fungus that forms root-associations with a wide variety of pine and other tree species
ppb	parts per billion by volume
ppb-hr	parts per billion hours (i.e., sum of concentration times duration), a measure of exposure to ozone
ppm	parts per million by volume
ppm-hr	parts per million hours (i.e., sum of concentration times duration), a measure of exposure to ozone

process rates	the degree or amount at which specific actions or activities occur (e.g., water vapor loss from leaves of plants)
QAS	Quality Assurance Section (of ARB)
R:S	ratio of root biomass (dry weight) to shoot biomass
RGR	relative growth rate, defined as the difference in the dry weight of a plant or plant part over a time period, divided by the initial dry weight and the length of the time period
RH	relative humidity
RuBisCO	ribulose bisphosphate carboxylase-oxygenase
RuBP	ribulose bisphosphate
SCCAB	South Central Coast Air Basin
SCOIAS	Sierra Cooperative Ozone Impact Assessment Study
SDAB	San Diego Air Basin
senescence	the onset of aging -- a phase in plant development from maturity to the complete loss of organization and function in plants
SFBAAB	San Francisco Bay Area Air Basin
shoot	the aboveground portion of the plant (e.g., leaves, stems, flowers, and fruits)
sieve cells	the primary type of cell found in the phloem of plants
SIP	State Implementation Plan
SJVAB	San Joaquin Valley Air Basin
SoCAB	South Coast Air Basin
SSAB	Salton Sea Air Basin
sucrose	a disaccharide (with 12 carbon atoms) commonly found in plants
(sucrose) translocation	the movement of sucrose (or other soluble organic food materials) through plant tissues – most commonly from leaves to stems/roots
SUM06	an ozone exposure metric involving concentration weighting, defined as the sum of all hourly mean ozone concentrations equal to or greater than 70 ppb
terrain-effect winds	air currents influenced by the geographic features of the land that it passes over
TREEGRO	a physiologically based computer simulation model of tree growth and development

Ulmus americana	the scientific name for “American Elm”
UN-ECE	United Nations Economic Commission for Europe
USD	United States dollars
USDA	United States Department of Agriculture
USDI	United States Department of the Interior
USEPA	United States Environmental Protection Agency
USV	Upper Sacramento Valley
V_d	deposition velocity, defined as deposition flux of ozone divided by its concentration in air (usually in cm/s or m/s)
VPD	vapor pressure deficit, a measure of evaporative demand of air
whorl	the arrangement of leaves, petals, etc., at about the same place on a stem
wk	week
yr	year
ZAP	zonal application system, a chamber-free, open-air exposure system
µg	microgram (= 0.000001 g = 10^{-6} g)
µm	micrometer or micron (= 0.000001 m = 10^{-6} m)

3 Physics and Chemistry of Ozone Air Pollution

3.1 Introduction

The ozone molecule consists of three oxygen atoms that are bound together (triatomic oxygen, or ozone), and for the purposes of this report, is measured and reported as parts per million (ppm) by volume. Ozone is a powerful oxidizing agent. It can react with gases (such as nitric oxide or NO), and with surfaces (such as dust particles). Ozone can also react with biological materials, such as leaves and cell membranes. These reactions can damage living cells, such as those present in the linings of the human lungs. Exposure has been associated with several adverse health effects, such as aggravation of asthma and decreased lung function.

Ozone was first observed in the Los Angeles area in the 1940s, but not identified as such until the early 1950s. The ozone that the ARB regulates as an air pollutant is mainly produced close to ground (tropospheric ozone), where people live, exercise, and breathe. This should not be confused with the layer of ozone high up in the atmosphere, called stratospheric ozone, that reduces the amount of ultraviolet light entering the earth's atmosphere. Without the protection of the stratospheric ozone layer, plant and animal life would be seriously harmed. In this document, 'ozone' refers to tropospheric ozone unless otherwise specified.

Most of the ozone in California's air results from reactions between substances emitted from vehicles, power plants, industrial plants, consumer products, and vegetation. These reactions involve volatile organic compounds (VOCs, which the ARB also refers to as reactive organic gases or ROG) and oxides of nitrogen (NO_X) in the presence of sunlight. As a photochemical pollutant, ozone is formed only during daylight hours under appropriate conditions, but is destroyed throughout the day and night. Thus, ozone concentrations vary depending upon both the time of day and the location. Ozone concentrations are higher on hot, sunny, calm days. In metropolitan and downwind areas of California, ozone concentrations frequently exceed regulatory standards during the summer.

From the 1950s into the 1970s, California had the highest ozone concentrations in the world, with hourly average concentrations in Los Angeles peaking over 0.5 ppm and triggering frequent "smog alerts". The smog alert system was designed in 1955 to prevent a possible air pollution disaster in Los Angeles County. In the early 1970s, the ARB initiated emission control strategies that provided for concurrent and continuing reductions of both NO_X and VOC from mobile sources and, in conjunction with the local air districts, stationary and area sources. Since then, peak ozone concentrations have decreased by more than 60 percent and smog alerts rarely occur in the Los Angeles area, despite more than a 35 percent increase in population and almost a doubling in vehicle miles traveled. However, most Californians still live in areas that do not attain the State's health-based standard (0.09 ppm for one hour) for ozone in ambient air. Section 7.2.8 of this review includes ozone trends by air basin since 1982.

This chapter discusses the processes by which ozone is formed and removed, background ozone, the role of weather, and spatial and temporal variations in ozone concentrations. In addition, this chapter includes discussions of research that the ARB has been conducting in the following areas that affect ozone concentrations: reactivity, weekend/weekday effect, and biogenic emissions. Subsequent sections of this chapter include ARB websites for more information. The ARB also conducts more general research in atmospheric processes that affect air pollution; information is available at

http://www.arb.ca.gov/research/apr/past/atmospheric.htm#Projects. For more extensive general information on the physics and chemistry of ozone, the reader is referred to Finlayson-Pitts and Pitts (2000), Seinfeld and Pandis (1998), and Whitten (1993).

3.2 Formation and Removal of Tropospheric Ozone

The formation of ozone in the troposphere is a complex process involving the reactions of hundreds of precursors.

3.2.1 Nitrogen Cycle and the Photostationary-State Relationship for Ozone

The formation of ozone in the troposphere results from only one known reaction: addition of atomic oxygen (O) to molecular oxygen (O_2) in the presence of a third "body" (M). [M is any "body" with mass, primarily nitrogen or oxygen molecules, but also particles, trace gas molecules, and surfaces of large objects. M absorbs energy from the reaction as heat; without this absorption, the combining of O and O_2 into ozone cannot be completed.]

$$O + O_2 + M \rightarrow O_3 + M \qquad (1)$$

The oxygen atoms are produced from photolysis of NO_2 by the ultraviolet portion (wavelength = 290 - 430 nm) of solar radiation ($h\nu$).

$$NO_2 + h\nu \rightarrow NO + O \qquad (2)$$

Reaction 3 converts ozone back to oxygen and NO back to NO_2, completing the "nitrogen cycle."

$$O_3 + NO \rightarrow NO_2 + O_2 \qquad (3)$$

Reactions 1 and 3 are comparatively fast. Therefore, the slower photolysis reaction 2 is usually the rate-limiting reaction for the nitrogen cycle and the reason why ozone is not formed appreciably at night. It is also one of the reasons why ozone concentrations are high during the summer months, when temperatures are high and solar radiation is intense. The cycle time for the three reactions described above is only a few minutes. Ozone accumulates over several hours, depending on emission rates and meteorological conditions. Therefore, the nitrogen cycle operates fast enough to maintain a close approximation to the following photostationary-state equation derived from the above reactions.

$$[O_3]_{photostationary\text{-}state} = (k_2/k_3) \times [NO_2]/[NO]$$

(the brackets denote concentration)

The ratio of the rate constants for reactions 2 and 3, (k_2/k_3), is about 1:100. Assuming equilibrium could be reached in the ambient air and assuming typical urban pollution concentrations, a NO_2 to NO ratio of 10:1 would be needed to generate about 0.1 ppm of ozone (a violation of the state one-hour ozone standard [0.09 ppm]). In contrast, the NO_2 to NO emission ratio is approximately 1:10; therefore, the nitrogen cycle by itself does not generate the high ozone concentrations observed in urban areas. The net effect of the nitrogen cycle is neither to generate nor destroy ozone molecules. Therefore, for ozone to accumulate according to the photostationary-state equation, an additional pathway is needed to convert NO to NO_2; one that will not destroy ozone. The photochemical oxidation of VOCs, such as hydrocarbons and aldehydes, provides that pathway.

3.2.2 The VOC Oxidation Cycle

Hydrocarbons and other VOCs are oxidized in the atmosphere by a series of reactions, to ultimately form carbon monoxide (CO), carbon dioxide (CO_2) and water (H_2O). Intermediate steps in this overall oxidation process typically involve cyclic stages driven by hydroxyl radical (OH) attack on the parent hydrocarbon, on partially oxidized intermediate compounds, and on other VOCs. Hydroxyl radical is ubiquitous in the ambient air; it is formed by photolysis from ozone in the presence of water vapor, and also from nitrous acid, hydrogen peroxide, and other sources. In the sequence shown below, R can be hydrogen or virtually any organic fragment. The oxidation process usually starts with reaction 4, from OH attack on a hydrocarbon or other VOC:

$$RH + OH \rightarrow H_2O + R \qquad (4)$$

This is followed by reaction with oxygen in the air to generate the peroxy radical (RO_2).

$$R + O_2 + M \rightarrow RO_2 + M \qquad (5)$$

The key reaction in the VOC oxidation cycle is the conversion of NO to NO_2. This takes place through the fast radical transfer reaction with NO.

$$RO_2 + NO \rightarrow NO_2 + RO \qquad (6)$$

R can also be generated by photolysis, which usually involves only VOCs with molecules containing the carbonyl (C=O) bond. The simplest VOC molecule that contains the carbonyl bond is formaldehyde (HCHO). Because formaldehyde enters into several types of reactions of importance for understanding ozone formation and removal, we will use it to help illustrate these reactions. The oxidation cycle for formaldehyde can be written in the following sequence of reactions.

$$OH + HCHO \rightarrow H_2O + HCO \qquad (7)$$

$$HCO + O_2 \rightarrow HO_2 + CO \qquad (8)$$

$$HO_2 + NO \rightarrow NO_2 + OH \qquad (9)$$

Hydroperoxyl radical (HO_2) is generated by reaction 8, and the hydroxyl radical (consumed in reaction 7) returns in reaction 9 to complete the cycle. In addition, reaction 9 produces the NO_2 required for ozone formation, as described above. Also, the carbon monoxide (CO) generated by reaction 7 can react like an organic molecule to yield another hydroperoxyl radical.

$$OH + CO \rightarrow H + CO_2 \qquad (10)$$

$$H + O_2 + M \rightarrow HO_2 + M \qquad (11)$$

Another component that formaldehyde provides for smog formation is a source of hydrogen radicals.

$$HCHO + h\nu \rightarrow H + HCO \qquad (12)$$

The hydrogen atom (H) and formyl radical (HCO) produced by this photolysis reaction yield two hydroperoxyl radicals via reaction with oxygen, as shown in reactions 8 and 11.

The reactions above comprise the simplest VOC oxidation cycle. Actually, hundreds of VOC species participate in thousands of similar reactions.

3.2.3 The Nitrogen Dioxide and Radical Sink Reaction

Another reaction is central to a basic understanding of ozone formation: the NO_2 plus radical sink reaction that forms nitric acid.

$$NO_2 + OH + M \rightarrow HNO_3 + M \qquad (13)$$

The previous discussion can be used to explain the typical pattern of ozone concentrations found in the urban atmosphere. Nitric oxide concentrations are relatively high in the early morning because the free radicals needed to convert the NO_X emissions (which are primarily NO) to NO_2 are not yet present in sufficient quantities. After sunrise, photolysis of formaldehyde (reaction 12) and other compounds starts the VOC oxidation cycle for the hundreds of organic gases present in the atmosphere. Subsequent NO to NO_2 conversion by the peroxy radical reaction 6 results in NO_2 becoming the dominant NO_X species. When the NO_2 to NO ratio becomes large enough, ozone builds up. In the South Coast Air Basin (Los Angeles area), the highest ozone concentrations are observed in the San Bernardino Mountains, many miles downwind from the highest concentration of emission sources (freeways, power generating facilities, and oil refineries along the coast), because the reactions involving the organic gases are relatively slow. Meanwhile, NO_2 concentrations decrease via the sink reaction 13.

Winds disperse and dilute both NO_X and ozone. During the day, NO_X is also diluted by the diurnal rising of the inversion layer, allowing for more mixing (see Section 3.5 for further discussion). For ozone, however, the deepening mixing layer may cause its concentration to decrease on some days and increase on others. Although increased mixing almost always dilutes NO_X, the effect of increased mixing on ozone concentrations depends upon whether higher concentrations of ozone are present aloft. Ozone that is trapped above the

inversion layer overnight is available to increase the concentrations of ozone generated by the following day's emissions.

During the night, NO and ozone combine to form NO_2 and oxygen via reaction 3 until either the NO or ozone is consumed. Nitrous acid or HONO is also present at night in polluted ambient air in California. Nitrous acid is produced from NO_2 and water, and is also emitted from various combustion sources. Its levels are low during the day because sunlight breaks it down rapidly. At sunrise, sunlight causes gas-phase HONO to react rapidly to provide NO and OH, two key reactants in the formation of ozone. In this way, they help initiate ozone formation in the morning by being available to react with VOCs as soon as their emissions increase due to an increase in human activity.

Nitric acid (HNO_3) was once thought to be a permanent sink for NO_X and for radicals. However, nitric acid on surfaces may react with NO to regenerate NO_2, which would increase the ozone-forming potential of NO_X emissions.

3.2.4 Ratio of Volatile Organic Compounds to Nitrogen Oxides in Ambient Air

Although VOCs are necessary to generate high concentrations of ozone, NO_X emissions can be the determining factor in the peak ozone concentrations observed in many locations (Chameides, 1992; National Research Council, 1991). VOCs are emitted from both natural and anthropogenic sources. Statewide, natural VOC sources dominate, primarily from vegetation. However, in urban and suburban areas, anthropogenic VOC emissions dominate and, in conjunction with anthropogenic NO_X emissions, lead to the peak concentrations of ozone observed in urban areas and areas downwind of major urban areas.

The relative balance of VOCs and NO_X at a particular location helps to determine whether the NO_X behaves as a net ozone generator or a net ozone inhibitor. When the VOC/NO_X ratio in the ambient air is low (NO_X is plentiful relative to VOC), NO_X tends to inhibit ozone formation. In such cases, the amount of VOCs tends to limit the amount of ozone formed, and the ozone formation is called "VOC-limited". When the VOC/NO_X ratio is high (VOC is plentiful relative to NO_X), NO_X tends to generate ozone. In such cases, the amount of NO_X tends to limit the amount of ozone formed, and ozone formation is called " NO_X -limited". The VOC/NO_X ratio can differ substantially by location and time-of-day within a geographic area. Furthermore, the VOC/NO_X ratio measured near the ground might not represent the ratio that prevails in the air above the ground where most of the tropospheric ozone is generated.

3.2.5 Reactivity

Photochemical reactivity, or reactivity, is a term used in the context of air quality management to describe a VOC's ability to react (participate in photochemical reactions) to form ozone in the atmosphere. Different VOCs react at different rates. The more reactive a VOC, the greater potential it has to form ozone. Examples of the more reactive VOCs in California’s atmosphere include propene, *m*-xylene, ethene, and formaldehyde. The ARB has helped to pioneer

an approach to ozone control that considers the reactivity of each VOC constituent. In California's urban areas, ozone formation tends to be limited by the availability of VOCs. Therefore, the reactivity-based regulatory approach has been applied in conjunction with reduction of NO_X emissions. Reactivity-based regulations promote the control of those VOCs that form ozone most effectively, thereby guiding the affected industries (such as manufacturers of motor vehicle and consumer product formulators that use solvents) to choose the most cost-effective processes and designs to reduce VOC emissions.

A complete table of reactivity values is available at the ARB website, http://www.arb.ca.gov/regact/mir2003/appa.pdf. Further information on photochemical reactivity is available from the ARB website, http://www.arb.ca.gov/research/reactivity/reactivityresearch.htm.

3.3 Background Ozone Concentrations in California

Contributions to background ground-level ozone concentrations include downward mixing of ozone from the stratosphere, and ozone formation due to photochemical reactions of locally emitted natural precursors. Lightning, wildfires, and transport are additional factors. This topic, "background ozone concentrations" is discussed in detail in Chapter 4.

Although little mixing occurs between the troposphere and stratosphere, stratospheric ozone intrusion occasionally causes localized ozone increases, especially at high mountain locations. Most of this intrusion is due to "tropopause folding", which results from strong storms that draw stratospheric air down into the troposphere. In California, this tends to occur in spring. Because stable, stagnant conditions are necessary to support high ozone concentrations in urban California, this process generally does not contribute significantly to peak ozone concentrations. Stratospheric ozone intrusion is also due to general stratospheric subsidence. On a global basis, California is particularly prone to springtime stratospheric ozone intrusion from this process. However, this process is a relatively minor contributor to surface ozone concentrations in California, especially in the summer when ozone concentrations tend to be highest.

Another process leading to ground-level ozone arises from photochemical reactions involving natural precursors. Plants emit VOCs (see Section 3.4), and soil microbes produce NO_X that is vented into the air. (Small amounts of NO_X are also emitted from crops, apparently related to fertilizer application.) Natural precursors may react with anthropogenic precursors to produce ozone concentrations that are of ambiguous origin. Where vegetation produces large amounts of VOCs, if anthropogenic NO_X is also present, significant amounts of ozone can be produced.

Lightning contributes to the formation of ozone by heating and ionizing the air along the path of the discharge, thus forming the ozone precursor NO_X. However, lightning tends to occur when meteorological conditions are not conducive to high ozone concentrations. Wildfires also contribute to ozone formation by producing NO_X from combustion, and by distilling VOCs from vegetation. However, wildfires in California are not a major contributor to ozone pollution.

Finally, transport from outside of California contributes to in-state ozone concentrations. Cities in neighboring states and Mexico emit ozone precursors that impact California. In addition, urban plumes can be lofted high enough into the atmosphere to be entrained in global circulation and transported thousands of miles. In particular, ozone due to emissions in Asia reaches California in springtime. However, this transport is not a major contributor to peak ozone concentrations in California because downward mixing of Asian ozone to the surface is precluded by the strong surface inversion usually present during high ozone episodes. Also, periods of effective long-range transport are generally restricted to spring, while high ozone concentrations due to local sources in California tend to occur in late summer and fall.

3.4 Effect of Vegetation on Ozone Concentrations

California's varied ecosystems interact with emissions related to human activity to influence ozone concentrations. Certain desert species, oaks, and pines emit substantial amounts of highly reactive VOCs, called biogenic emissions. Vegetation can either increase or decrease the ambient ozone concentration as the result of complex processes briefly described below.

Vegetation can reduce ozone concentrations by providing cooling and by removing pollutants. The shade provided by trees lowers ozone concentrations in several ways. It reduces the pollutant emissions from many sources (such as less evaporation of fuel from cooler parked vehicles). By cooling homes and offices, tree shade lowers emissions associated with electricity generation because less power is needed for air conditioning. In addition, cooling reduces the speed of chemical reactions in ambient air that lead to the formation of ozone.

Vegetation can also enhance the removal of ozone through deposition on plant surfaces. The surfaces of leaves and pine needles allow for deposition of ozone and NO_2. Several different factors affect pollutant removal, such as how long a parcel of air is in contact with the leaf, and the total leaf area available for deposition. Also, rain tends to reduce ambient ozone concentrations by washing out atmospheric gases as well as gases deposited on leaves and needles.

Other processes involving vegetation can lead to higher concentrations of ozone. For example, trees and other types of vegetation emit biogenic VOCs, such as isoprene, pinenes, and terpenoid compounds. These biogenic VOCs can react with NO_X emitted from sources such as cars and power plants to form ozone. Many biogenic VOCs are highly reactive (i.e., especially efficient in reacting to form ozone); some VOCs are even more efficient in forming ozone than those emitted from cars and power plants. In addition, VOCs can be emitted from decomposing leaves.

To help understand the complex mechanisms by which vegetation influences ambient ozone concentrations, the ARB established a “Biogenic Working Group” (BWG). The BWG has developed vegetation maps, leaf biomass databases, emission factors, and a California-specific “biogenic emissions inventory through geographic information systems” (BEIGIS) that has satisfactorily accounted for

observed ambient ozone concentrations. The information developed by the BWG will help the ARB to better model ozone formation, and to better determine the relative importance of VOC and NO_X control. Additional information is available from the ARB website,
http://www.arb.ca.gov/research/ecosys/biogenic/biogenic.htm.

3.5 Role of Weather in Ozone Air Quality

In the troposphere, the air is usually warmest near the ground. Warm air has a tendency to rise and cold air to sink, causing the air to mix, which disperses ground-level pollutants. However, if cooler air gets layered beneath warm air, no mixing occurs -- the air is stable or stagnant. The region in which temperature is so inverted is called an inversion layer. One type of inversion occurs frequently several thousand feet above the ground and limits the vertical dispersion of pollutants during the daytime. Another type of inversion occurs on most evenings very near the ground and limits the vertical dispersion of pollutants to a few hundred feet during the night. Pollutants released within an inversion tend to get trapped there. When the top of the daytime inversion is especially low, people can be exposed to high ozone concentrations. Mountain chains, such as those downwind of California's coastal cities and the Central Valley, help to trap air and enhance the air quality impact of inversions. Cooler air draining into the state's valleys and 'air basins' also enhances inversion formation.

The direction and strength of the wind also affect ozone concentrations. Based on worldwide climate patterns, western coasts at California's latitude tend to have high-pressure areas over them, especially in summer. By preventing the formation of storms, and by promoting the sinking of very warm air, these high-pressure areas are associated with light winds and temperature inversions, both of which limit dispersion of pollutants.

On a daily basis, the highest ozone concentrations tend to occur in the afternoon. This is because ozone forms as a result of photochemical reactions involving other pollutants; these reactions generally require a few hours (see Section 3.2) after the emissions of substantial amounts of VOCs. Also, these photochemical reactions are most effective when sunlight is intense and air temperatures are warm. Therefore, ozone concentrations in California are usually highest in the summer, although the ozone season in some areas of the State can include spring and fall. The prevailing daytime winds in summer are on-shore, bringing relatively clean air from over the ocean to the immediate coastal areas, but carrying emissions of ozone precursors further inland. With the climatically favored clear skies and temperature inversions that limit the vertical dispersion of pollutants, these emissions are converted into ozone, with the highest concentrations tending to occur at distances a few tens of miles downwind of urban centers (ARB 2002).

During the periods of the year when the sunlight is most intense, much of California experiences a high frequency of inversions, relatively low inversion heights, and low wind and rainfall. As a result, no other State has more days per

year with such a high meteorological potential for unhealthy ozone concentrations.

Additional information on the effects of weather on air pollution is available from the following textbooks:

Ahrens, C.D. (1994), *Meteorology Today*, West Publishing Co., St. Paul, MN.

Neiburger, M., Edinger, J.G., and Bonner, W.D. (1982), *Understanding our Atmospheric Environment*, W.H. Freeman & Co., San Francisco, CA.

3.6 Spatial and Temporal Variations of Ozone Concentrations

3.6.1 Spatial Variations of Ozone Concentrations

Ambient ozone concentrations can vary from non-detectable near combustion sources, where nitric oxide (NO) is emitted into the air, to several hundred parts per billion (ppb) in areas downwind of VOC and NO_X emissions. In continental areas far removed from direct anthropogenic effects, ozone concentrations are generally 20-40 ppb. In rural areas downwind of urban centers, ozone concentrations are higher, typically 50-80 ppb, but occasionally 100-200 ppb. In urban and suburban areas, ozone concentrations can be high (well over 100 ppb), but peak for at most a few hours before deposition and reaction with NO emissions cause ozone concentrations to decline (Finlayson-Pitts and Pitts 2000, Chameides et al. 1992, Smith et al. 1997).

Ozone concentrations vary in complex ways due to its photochemical formation, its rapid destruction by NO, and the effects of differing VOC/NO_X ratios in air. A high ratio of NO_X emissions to VOC emissions usually causes peak ozone concentrations to be higher and minimum concentrations to be lower, compared to background conditions. Peak ozone concentrations are usually highest downwind from urban centers. Light winds carry ozone from urban centers, and photochemical reactions create ozone from urban emissions of VOC and NO_X Also, away from sources of NO_X emissions, less NO is available to destroy ozone. Due to the time needed for transport, these peak ozone concentrations in downwind areas tend to occur later in the day compared to peak ozone concentrations in urban areas.

Due to the lack of ozone-destroying NO, ozone in rural areas tends to persist at night, rather than declining to the low concentrations (<30 ppb) typical in urban areas and areas downwind of major urban areas, that have plenty of fresh NO emissions. Ratios of peak ozone to average ozone concentrations are typically highest in urban areas and lowest in remote areas (ARB 2002). Within the ground-based inversions that usually persist through the night, ozone concentrations can be very low. In urban areas, emissions of NO near the ground commonly reduce ozone below 30 ppb. In rural areas, however, NO emissions are less prevalent and nighttime ozone may persist well above 30 ppb.

3.6.2 Temporal Variations in Ozone Concentrations

Ambient ozone concentrations tend to vary temporally in phase with human activity patterns, magnifying the resulting adverse health and welfare effects.

Ambient ozone concentrations increase during the day when formation rates exceed destruction rates, and decline at night when formation processes are inactive. This diurnal variation in ozone depends on location, with the peaks being very high for relatively brief periods of time (an hour or two duration) in urban areas, and being low with relatively little diurnal variation in remote regions. In urban areas, peak ozone concentrations typically occur in the early afternoon, shortly after solar noon when the sun's rays are most intense, but persist into the later afternoon, particularly where transport is involved. Thus, the peak urban ozone period of the day can correspond with the time of day when people, especially children, tend to be active outdoors.

In addition to varying during the day, ozone concentrations vary during the week. In the 1960s, the highest ozone concentrations at many urban monitoring sites tended to occur on Thursdays. This pattern was believed to be due to the carryover of ozone and ozone precursors from one day to the next, resulting in an accumulation of ozone during the workweek. In the 1980s, the highest ozone concentrations at many sites tended to occur on Saturdays and the "ozone weekend effect" became a topic of discussion. Since then, the weekend effect has become prevalent at more urban monitoring locations and the peak ozone day of the week has shifted to Sunday. Although ozone concentrations have declined on all days of the week in response to emission controls, they have declined faster on weekdays than on weekends. Thus, the peak ozone period of the week now tends to coincide with the weekend, when more people tend to be outdoors and active than during the week.

The causes of the ozone weekend effect and its implications regarding ozone control strategies have not yet been resolved. Almost all of the available data represent conditions at ground level, where the destruction of ozone by fresh emissions of NO is a major factor controlling ozone concentrations. However, most ozone is formed aloft, and the air quality models used to analyze ozone formation have not demonstrated the ability to represent the ozone-forming system aloft with sufficient realism. In addition, several potentially significant photochemical processes are yet to be fully incorporated in simulation models. These deficiencies leave unresolved this fundamental question: does the ozone weekend effect occur because more ozone is formed (aloft) on weekend, because more ozone is destroyed (at the surface) on weekdays, or because ozone formation is more efficient on weekends? More information may be obtained from the ARB website, http://www.arb.ca.gov/aqd/weekendeffect/weekendeffect.htm, and from a review article in EM magazine (Croes et al., 2003)

Ozone concentrations also vary seasonally. Ozone concentrations tend to be highest during the summer and early fall months. In areas where the coastal marine layer (cool, moist air) is prevalent during summer, the peak ozone season tends to be in the early fall. Additionally, as air pollution controls have reduced the emissions of ozone precursors and the reactivity of VOCs, ozone concentrations have declined faster during times of the year when temperatures and the amount of sunlight are less than during the summer. Thus, the peak

ozone season corresponds with the period of the year when people tend to be most active outdoors.

Also, ozone concentrations can vary from year to year in response to meteorological conditions such as El Niño and other variations in global pressure systems that promote more or less dispersion of emissions than typical. Although peak ozone concentrations vary on a year-to-year basis, peak ozone concentrations in southern California have been declining on a long-term basis, as anthropogenic emissions of VOC and NO_X have declined. However, since the advent of the industrial revolution, global background concentrations of ozone appear to be increasing (Finlayson-Pitts and Pitts, 2000). This increase has implications regarding the oxidative capability of the atmosphere and potentially global warming processes (ozone is a strong greenhouse gas but is present at relatively low concentrations). Further discussion of these topics is beyond the scope of this document.

3.7 References

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4 “Policy-Relevant Background” Ozone in California

Many “pollutant” chemicals do not originate solely from controllable sources such as industrial emissions or automobile tailpipes. The fractions of the ambient concentrations of pollutant species that are not due to regulated emissions are commonly referred to as “background;” and, sometimes erroneously, as “natural background.” The notion of “background” pollutant concentrations derives from laboratory parlance in which “background” in controlled experiments (or modeling exercises) is a constant value used to keep track of material distinct from that being studied or manipulated. In the open environment, even under “natural” conditions, ambient concentrations of most pollutant species vary with location, time of day, and weather, making it impossible to specify a single “background” level, and rendering the usage of the term “background” confusing at best.

Earth’s “natural” ozone regime prevailed prior to the industrial revolution, but there is little direct evidence of actual ozone concentrations at that time. Bojkov (1986) resurrected a smattering of historical measurements from Europe and the American Midwest taken in the period 1870 through the 1900, and Lisac and Grubisic (1991) report ozone data from Zagreb, Yugoslavia for the period 1888 to 1900. There are no known measurement records for other regions. Bojkov's data show a strong seasonal trend, peaking in spring, and typical daily maxima in the range of 30 to 50 ppb. The Zagreb data show a similar but narrower range of monthly mean values (30-35 ppb, peaking in April and May). “Preindustrial” ozone concentrations reconstructed through modeling (Lelieveld and Dentener, 2000; Hauglustaine and Brasseur, 2001) are in rough agreement, and show that ozone concentrations were highly variable under “pre-industrial” conditions.

Ozone in the modern world has multiple origins and concentrations may change for many reasons: ozone produced by “natural” causes may be raised or lowered by reaction with anthropogenic emissions; natural ozone precursors may react with anthropogenic precursors to produce ozone concentrations that are of ambiguous origin; and ozone may be transported from one region to another.

From a regulatory perspective, the important distinction is not between “natural” and “anthropogenic” ozone, but between ozone produced by controllable emissions and ozone due to emissions beyond the reach of regulation. In a policy context, anthropogenic ozone produced outside the jurisdiction of an agency and transported into a control region is functionally indistinguishable from that due to natural processes. Within the range of concentrations due to such external or uncontrollable sources, those concentrations that may impact determinations of compliance with air quality standards or limit the potential air quality improvements due to control programs are the “policy-relevant background.”

The following sections present a brief overview of atmospheric ozone creation and summarize current understanding of “policy-relevant background” ozone concentrations impacting California. A full treatment of ozone chemistry and

atmospheric transport far exceeds the scope of this discussion; the reader needing such information is directed to the section of this report dealing with ozone chemistry and the references cited in both sections.

4.1 "Natural" Ozone

Under preindustrial conditions, ozone at ground level is largely the result of three processes: meteorologically regulated downward mixing of ozone from the stratosphere, local boundary layer ozone formation due to photochemical reactions of natural precursors, and regional to continental scale impacts of large biomass fires producing episodic releases of large volumes of ozone precursors (VOCs and NO_X). Since there are only a few ozone measurements available from preindustrial times, global distributions of "natural" concentrations must be inferred from chemical kinetics and atmospheric modeling (Lelieveld and Dentener, 2000). In this regard, ARB staff disagrees with the assumption embedded in many published papers on "background" ozone, that observed elevated ozone in non-urban, non-industrial sites can be presumed to be due to natural causes (*e.g.* Lefohn et al., 2001). ARB staff believe that such observations need to be supported by chemical and meteorological data that preclude anthropogenic influences if the measurements are to be accepted as "natural."

4.1.1 Stratospheric Ozone

Earth's atmosphere is layered and ozone is not uniformly distributed vertically or horizontally. The energy that drives atmospheric processes comes from the sun, and most sunlight penetrates the atmosphere to the ground or ocean where solar energy goes into surface heating and evaporation of water. Air in contact with the heated planetary surface becomes warmer than the overlying air and then rises, carrying heat and water vapor into the atmosphere. Rising air expands and cools with decreasing pressure, thus air temperature in the lower atmosphere tends to be cooler with increasing altitude. The bottom 85 percent of the atmosphere, where circulation is driven by surface heating and energy stored and released by evaporation and condensation of water, is termed the troposphere (Levine, 1985).

Conversely, the upper layers of the atmosphere are heated from above by the small amount of sunlight that is absorbed. At altitudes above about 100km the atmosphere is highly modified by intense extreme ultraviolet (EUV) radiation, X-rays, and cosmic rays from the sun and outer space; this flux ionizes and heats the outer layers (collectively termed the ionosphere). Radiation absorption by the ionosphere shades the lower layers from the effects of interplanetary radiation, such that the common atmospheric molecules (N_2, O_2, CO_2, etc.) can survive (Levine, 1985).

Below about 90km the solar UV flux is still strong enough to heat the air from above; this temperature gradient suppresses convective mixing, hence this zone is termed the stratosphere. The base of the stratosphere, at the level of equilibrium between the influence of convective surface heating from below and direct solar heating from above, is the tropopause; its height varies with location

and season – highest in the tropics, lowest over the poles, higher in summer than in winter. Over California, the tropopause ranges from 10 to 20 km above the surface (Levine, 1985).

The presence of ozone in the stratosphere was determined by Hartley using sun spectrometry in the 1880s; a photochemical mechanism of ozone formation was first proposed by Chapman in the 1930s, and modified by Turco, Crutzen, and others in the 1970s (Turco, 1985): Intense solar ultraviolet (UV) radiation causes oxygen molecules to split into free oxygen atoms (photolysis); the singlet oxygen atoms quickly bond with diatomic oxygen, forming ozone; ozone is recycled back into diatomic oxygen by UV photolysis and reaction with hydroxyl radical, oxides of nitrogen, and various trace constituents in the air. Thus stratospheric ozone concentrations represent a dynamic equilibrium between ozone creation and destruction, and ozone concentration varies seasonally and geographically with changes in UV radiation intensity and concentrations of reactive trace chemicals (Turco, 1985).

Since ozone itself absorbs ultraviolet light, it shades the lower atmosphere in UV bands and weakens ozone production lower in the atmosphere, resulting in an equilibrium ozone concentration appearing as a distinct layer near 20 km altitude. The stratospheric ozone layer contains about 90 percent of total atmospheric ozone (Turco, 1985).

Of course, ozone in the stratosphere, isolated far above the ground, is only "policy relevant" if it can be transported to lower altitudes where it may impact terrestrial organisms (especially humans).

4.1.2 Ozone Exchange Across the Tropopause

Because ozone concentrations in the lower atmosphere are high near the tropopause, it was long believed that downward diffusion of ozone from the troposphere was the major source of tropospheric ozone. More recently, new three-dimensional chemical modeling and better understanding of tropospheric chemistry indicates that much of the ozone in the troposphere, even in nonindustrial regions, is generated in situ (Lelieveld and Dentener, 2000). Nonetheless, stratospheric ozone intrusions do cause localized ozone increases.

Under stable meteorological conditions, the troposphere and stratosphere do not mix, so stratospheric ozone generally remains isolated far above the ground. Strong storms in the troposphere can occasionally cause stratospheric air to be drawn downward into the lower atmosphere. Such stratospheric intrusions or "tropopause folding events" can bring high concentrations of ozone far down into the troposphere. In the midlatitudes of the northern hemisphere these events tend to occur in spring accompanying deep pressure "troughs." When present, stratospheric ozone will be greatest at high mountain locations; effects near sea level are usually small (Lelieveld and Dentener, 2000).

The dynamics of stratospheric ozone intrusion are not fully understood, and are still a focus of active research, but there are two general aspects of the phenomenon that are not in dispute. First, stratospheric ozone transport requires

strong, deep tropospheric circulation to draw ozone-laden air downward, such that ozone intrusions are associated with strong midlatitude cyclonic storms and general midlatitude subsidence. Second, warmer temperatures and strong sunlight promote both tropospheric and stratospheric ozone formation processes. Taken together, these factors make stratospheric ozone intrusions greatest in late winter and spring, when strong latitudinal temperature gradients promote strong circulation and high solar zenith angles promote abundant ozone formation (Lelieveld and Dentener, 2000).

Ozone intrusion has recently been the subject of intensive investigation and model development (STACCATO, 2003). There are two mechanisms of stratospheric ozone intrusion: tropopause folding events (TFEs), which are rapid (a day or two) and somewhat localized (a few hundred km in extent), and general stratospheric subsidence (SS), which is more or less continuous and of continental or larger scope.

4.1.2.1 Tropopause Folding Events

A two year ozone lidar study of tropopause folding over Europe provides a picture of "typical" conditions (Galani et al., 2003). Meteorological modeling was used to anticipate potential folding events for study – 45 potential events were noted during the two years. About half (24) were successfully probed with lidar, and of those about 1/3 actually produced the expected elevated ozone concentrations, generally at altitudes greater than 3km. Only one of the 45 events produced a clear ozone pulse at a low altitude surface measurement site (850m elevation); on that occasion (in March 2001) surface ozone reached 75 ppb. Most events produced elevated (5-6 km) ozone layers with concentrations in the range of 50-75 ppb, and ozone pulses around 50 to 60 ppb at mountain monitoring sites (above 3km elevation). ARB staff believes that the European observations are representative processes acting throughout the northern hemisphere. This is reinforced by the global assessments of Lelieveld and Dentener (2000), Lawrence et al. (2003), and the record of ozonesonde observations reported by Newchurch et al. (2003).

From a regulatory perspective, TFEs are likely to be easily recognized as such because they do not replicate "typical" anthropogenic ozone events. Seasonally, TFEs tend to occur in winter and early spring, outside the anthropogenic ozone season. Geographically, TFEs will manifest themselves with higher concentrations at elevated sites than lowland sites, and will not be constrained by "air basin" distribution patterns. Meteorologically, TFEs will exhibit very low relative humidity, since stratospheric air is dry to begin with, and will be warmed by significant adiabatic heating in the descent from the tropopause to the surface. Chemically, TFEs will exhibit high ozone without the accompanying markers for anthropogenic emissions such as volatile organic compounds (VOCs), carbon monoxide (CO), and oxides of nitrogen (NO_X). Since California has a fairly large network of ozone monitoring sites, many of which also collect other gas data such as CO and NO_X, suspect ozone events should be amenable to retrospective analysis using archived meteorological data and data from surrounding monitoring stations.

4.1.2.2 Stratospheric Subsidence

Globally there is a slow turnover between the troposphere and the stratosphere. Very strong convective lofting in the intertropical convergence zone, the warmest areas of the western Pacific, and the poleward ends of major warm ocean currents (Japan Current, Gulf Stream, etc.) injects tropospheric air into the stratosphere where it is globally dispersed. The upward flux into the stratosphere is balanced by persistent subsidence in the midlatitudes. Together these processes comprise the Brewer-Chapman circulation (Turco, 1985).

Recent global circulation modeling exercises (Wernli and Bourqui, 2002; Lelieveld and Dentener, 2000) indicate that global-scale stratospheric down-mixing is concentrated near the polar jet stream, such that ozone intrusion is strongest and most frequent in bands between 30 and 50 degrees latitude in both hemispheres. In the Northern Hemisphere there are zones of stronger stratospheric ozone intrusion in the eastern and western North Pacific, the western North Atlantic, and the Mediterranean Sea. California is particularly prone to springtime stratospheric ozone intrusion (Wernli and Borqui, 2002; Seo and Bowman, 2002; Fiore et al., 2002). The mean stratospheric contribution to the middle troposphere (5km altitude) varies from about 20 ppb in march to about 5 ppb in August (Lelieveld and Dentener, 2000). Surface concentrations due to the Brewer-Chapman circulation would be somewhat lower.

From a regulatory perspective, long term contributions of stratospheric subsidence to observed high surface concentrations are small in winter and trivial in summer. Fiore et al. (2002) calculated that, in summer, no more than 2-ppb ozone at the surface could be ascribed to stratospheric origin.

Direct evidence of stratospheric subsidence would be seen in routine ozonesonde observations. Newchurch et al., (2003) reported analysis of weekly ozonesonde releases from 1997 through 2002 at Trinidad Head in northwestern California. They observed seasonal variation in ozone consistent with the general pattern described above, but noted that surface ozone at Trinidad Head was consistently low, especially in summer. This pattern is due to the prevention of mixing of air from aloft to the surface due to the persistent cool, stable marine layer along the California coast. The authors contrast this with the stronger mixing seen at continental sites or on the east coast of the United States. It is reasonable to extrapolate from the Trinidad Head data that, since all of coastal California experiences the effects of the cool California current and the associated marine layer, stratospheric down-mixing is suppressed throughout coastal California.

The data and models discussed here reflect the peculiar dynamics of the California climate. Average “natural background” ozone near sea level in the coastal zone is in the range of 15 – 35 ppb, with a maximum monthly mean of about 40 ppb at low altitude inland sites. At altitudes above 2 km stratospheric intrusions can infrequently push peak “natural background” concentrations to 45 –50 ppb. Extreme downmixing of stratospheric ozone as in “tropopause folding events” (TFEs) can raise ozone well above these levels, but such events

only very rarely reach the surface (there has been one documented case in California since ozone monitoring began) and ARB has in place an "exceptional events" policy to exempt such events from regulatory consideration as exceedances of air quality standards.

4.1.3 Natural Tropospheric Ozone

4.1.3.1 Lightning

Lightning heats and ionizes air along the path of the discharge, forming NO_X which reacts with biogenic VOCs (including CO) to form ozone (Lelieveld and Dentener, 2000). This process accounts for 10 to 40 percent of present day tropospheric ozone (20 – 42 percent under preindustrial conditions), depending on local humidity, precursor emissions, and sunlight. Recent work suggests that lightning is the source of much of the vertical increase in ozone in the troposphere (Lelieveld and Dentener, 2000; STACCATO, 2003).

4.1.3.2 Photochemistry of Biogenic Emissions

Natural volatile organic compounds (VOCs) are emitted by plants and NO_X is emitted by soil microbiota. Emission rates are highly variable across plant species and ecosystems. VOC emissions are generally greater during the growing season, but instantaneous emission rates are governed less by season than specific conditions such as sunlight and air temperature. Biogenic NO_X is produced by microbial processes in soils and vented to the atmosphere by diffusion. Just as in urban areas, the combination of sunlight, VOCs, and NO_X works to produce ozone. The strength of natural VOC emissions is usually limited by type and density of vegetation, and total ozone formation is usually constrained by low natural NO_X flux (Lelieveld and Dentener, 2000).

Surface ozone production in areas remote from anthropogenic precursor sources has been observed to be uniformly limited to concentrations well below the level of the proposed standard. Data on 19th century ozone concentrations measured in Europe and the U.S. (Bojkov, 1986; Lisac and Grubisic, 1991) show that spring peak ozone partial pressures were about 4 ± 1 mPa (30-50 ppb) in the Midwestern U.S. and ranged from 2-3 mPa (20-30 ppb) in Europe. Although the 19th century data are somewhat uncertain, these values are supported by a report by Derognat et al. (2003) that model computations of the modern biogenic fraction of ground level ozone in central France peak at 40 ppb.

A detailed modeling study of summertime ozone formation over the United States mainland (Fiore et al., 2002) estimates that natural precursors in the local troposphere react to produce about 50 percent of observed ozone (15 – 25 ppb), and the balance is advected from other regions.

In locations where vegetation produces large amounts of VOCs, ozone may be enhanced or diminished. Fiore et al. (2002) estimate that 10-20 percent of ozone destruction in rural areas in the eastern U.S. is due to reaction with biogenic hydrocarbons.

Conversely, when anthropogenic NO_X is available (e.g. downwind of an urban area), reaction with biogenic VOCs can create significant amounts of ozone.

Excellent demonstrations of this process have been reported in both NO_X- and VOC- limited cases. In a NO_X-limited case, St. John, Chameides and Saylor (1998) reported total ozone was similar in a VOC-poor powerplant plume to that in the VOC-rich Nashville urban plume. In a VOC-limited case reported by Dreyfus, Schade, and Goldstein (2002) the Sacramento CA urban plume's encounter with oak forests enhanced ozone by 20 to 70 ppb (40 to 100 percent) through reaction of urban NO_X emissions with biogenic isoprene.

The regulatory policy implications of ozone formed by reactions involving biogenic precursors hinge on a definitional dilemma: some is clearly "natural", but some exists by virtue of reaction with anthropogenic precursors. This can be resolved by revisiting the definition of "policy relevance:" concentrations beyond regulatory control. Viewed in this perspective, truly "natural" ozone - for which the precursors are themselves beyond regulatory control (e.g. the 15-25 ppb identified by Fiore et al. (2002) – are part of the uncontrollable background. Enhanced ozone such as that observed east of Sacramento (e.g. as reported by Dreyfus, Schade, and Goldstein, 2002) is controllable since the NO_X input to the reaction is anthropogenic and thus potentially amenable to regulatory action. In other words, ozone produced by reaction of natural and anthropogenic precursors may be an unwelcome complication in ozone control, but it is not "background."

4.1.3.3 Biomass Burning

The processes discussed above tend to operate whenever the sun shines, with modulation of ozone concentration due to meteorological conditions, change of season, etc. Some ozone production is much more sporadic, depending on infrequent precursor emissions.

Biomass fires accelerate natural ozone formation by distilling large amounts of VOCs out of plant material and producing CO and NO_X as products of combustion. Ozone photochemistry in smoke plumes is somewhat different from that under clear-sky conditions. The large amount of carbonaceous aerosol reduces solar UV flux, slowing photochemical processes near the fire, but the very high concentrations of combustion gases and long lifetimes of very large smoke plumes cause ozone formation far downwind by oxidation of CO and methane (Crutzen, 1995). Very large fires observed by satellite have been shown to have high ozone concentrations accompanying their smoke plumes far downwind (Jenkins and Ryu, 2003). Ozone concentrations associated with fires are highly variable, but large tropical fire plumes have been observed to commonly have 70-ppb ozone or more in the middle troposphere, but ground level impacts are much less, typically in the range of 15-25 ppb (Jenkins and Ryu, 2003).

Although less studied than tropical fires, boreal forest fires in northern Canada and Siberia have recently been identified as strong but highly variable sources of very large biomass smoke plumes that have the potential to reach the western United States. Modeling by Fiore et al. (2002) found limited biomass fire ozone impact in North America, with the summer average ozone impact of biomass fires

over Canada in the range of 4-8 ppb for 1995. Modeling supported by surface and aircraft measurements reported by Jaegle et al. (2003) looked at springtime (March-May, 2001) transport of pollutants due to Asian sources. The model showed less than 1 ppb ozone due to Asian biomass burning (SE Asia in this season) arriving at the surface in the Pacific Northwest.

Global modeling by Galanter, Levy and Carmichael (2000) indicates that biomass burning contributes half or more of surface and about a quarter of mid-troposphere ozone in the tropics and over the southern oceans. Conversely, the limited burn season and irregular nature of boreal fires limits their average impact to about ¼ of surface ozone in Alaska, western Canada and Siberia in summer and near zero the rest of the year.

Despite the limited long-term mean impact of biomass fires, occasional strong fire impacts are possible. New reports based on ground, aircraft, and transport modeling (Jaffe, et al., 2003a, 2004; Bertschi, et al., 2004; Weiss-Penzias, et al., 2003) indicate that ground level CO, ozone, and particulate pollution in the Puget sound region is occasionally enhanced by smoke plumes from very large Siberian fires. Preliminary data on the impact of one event (June 2-4, 2003) show peak ozone concentration aloft (up to 6km) reached 100 ppb; surface monitoring data showed possible enhancement of suburban ozone by about 10 – 20 ppb. Such events appear to be rare (once in an entire summer of observations in a year of unusually large Siberian fire activity). If further study demonstrates that this phenomenon occurs with some regularity (i.e. more than once in three years) it would be prudent for regulators to develop a methodology for recognizing such an event (e.g. merging back trajectories, satellite fire data, and observed atmospheric chemistry) to assure recognition as an “unusual event”.

In California, the potential for Siberian fire ozone impacts enhancing urban ozone appears to be significantly lower than that for the Pacific Northwest. This is due to a combination of reduced potential transport at the lower latitudes of California’s major urban areas and stronger local inversions during ozone episodes. This view is supported by the modeling of Galanter, Levy, and Carmichael (2000) which shows a strong decreasing gradient of boreal biomass fire ozone along the coast of North America, from a peak in interior Alaska to no impact in southern California.

Although much of California’s natural vegetation is prone to frequent burning, there is little evidence that local fires significantly impact measured ozone in the state. Small fires produce much the same mix of ozone precursors as do large ones, however plumes from small fires tend to disperse more readily, and thus precursor concentrations are lower and there is less time for ozone to form. Although there has been little scientific research on the ozone effects of California fires, the general nature of burning in California suggests it is not large. California’s aggressive fire suppression policies sharply restrict the frequency of very large fires, and keep most small fires from spreading or lasting more than a few hours. The degree of fire suppression is evident in statistics compiled by the California Department of Forestry and Fire Protection (CDFFP, 2004a,b). The year 2003 was a very bad fire year, with enormous damage due to an

unprecedented group of fires in southern California in October – despite that, annual statistics for 2003 show that, of over 7600 fires reported, only about 120 exceeded 300 acres (121 hectares). Air Pollution measurements influenced by large fires are generally recognized as exceptional events and excluded from consideration for standards compliance, and regulators should be aware of major fires in proximity to monitoring sites. Large fires are inherently low frequency events in any particular area, since it takes many years for fuel to accumulate after a major fire. Taken together, these considerations keep local biomass burning from being considered a significant contributor to California's ozone pollution.

4.2 Transported Anthropogenic Ozone

Because ozone is highly chemically reactive it was long believed that its influence on downwind air quality was limited to local transport of concentrated urban plumes. In California, ozone due to precursor emissions outside the State's jurisdiction were thought to be limited to border cities such as Tijuana, Mexico. Although technically exogenous to California, ozone from such sources is well-understood and subject to indirect control through international agreements and bi-national control programs.

Research during the last decade has changed perception of the spatial scale of impact for urban/industrial ozone. Urban plumes lofted high enough into the atmosphere to be entrained in global circulation can maintain considerable chemical integrity and effectively transport ozone thousands of kilometers. Individual pollution plumes from urban / industrial areas have recently been traced from the eastern United States across the Atlantic Ocean to Europe (Trickl et al., 2003), from Europe to Asia (Pochanart et al., 2003), Asia to the north Pacific Ocean (Thouret, et al., 2001), from Asia to North America (Jaffe, McKendry, Anderson, and Price, 2003), and from all the industrialized regions of the Northern Hemisphere to the Arctic (Browell et al., 2003).

Globally, injection of anthropogenic ozone precursors (especially NO_X) into the free troposphere has been estimated to enhance tropospheric ozone by 12 percent (Lawrence, et al., 2003).

Ozone due to urban and industrial emissions in east Asian megacities (Beijing, Tokyo, Hong Kong, Taipei, Shanghai, *etc.*) has been observed to reach western North America in springtime (Jaffe et al., 1999; Jaffe et al., 2003b; Jaffe, McKendry, Anderson, and Price, 2003). Modeling exercises aimed at determining the Asian air pollution contribution in western North America have consistently shown frequent enhancement of carbon monoxide and aerosols, but ozone effects have been harder to discern. This may be, in part, due to recent shifts in the balance of ozone chemistry over the Pacific Ocean. Pacific basin and global ozone chemical dynamics modeling (Jaegle et al., 2003; Lawrence et al., 2003) indicates that increasing anthropogenic NO_X emissions are pushing tropospheric chemistry over the northeastern Pacific Ocean from net ozone destruction to net ozone formation. Jaegle et al. (2003) show that about half of CO observed at Cheeka Peak, WA is Asian, while only 10-20 percent of ozone is Asian. This

implies that the Asian CO signal would be expected to be detected earlier in the growth of Asian emissions, with positive ozone impacts delayed until Asian NO_X emissions shift the balance for eastern Pacific ozone formation. Jacob, Logan and Murti (1999) presented model results that are consistent with this hypothesis, indicating mean Asian ozone impact in western North America would increase from around 1 ppb in 1985 to as much as 7 ppb in 2010 if Asian NO_X emissions continue to grow at late 20th century rates (4-5 percent/year). Berntsen, Karlsdottir and Jaffe (1999) modeled transPacific transport to Cheeka Peak, WA, with model validation based on aircraft and ground measurements; they reported a mean springtime Asian ozone increment of 4 ppb, with peak events (48 hours) reaching 7.5 ppb. Jaffe et al. (2003b) report statistical analyses of ozone records from rural sites from northern California to the Olympic Peninsula from the mid-1980s to 2002 that show a broad regional increase in "background" ozone. All sites show a statistically significant increase in springtime ozone (about 4 ppb/decade) for days selected for oceanic influence, with stronger gradients for higher elevation sites. This is consistent with the vertical gradient pattern of Asian aerosol impacts (VanCuren and Cahill, 2002), ozonesonde observations (Newchurch, et al., 2003), and increased transport exposure above the marine boundary layer shown in transport modeling (Jaegle et al., 2003).

4.3 Exogenous Impacts on California Ozone Air Quality

4.3.1 Present Conditions

The exogenous ozone sources discussed above are generally not major contributors to observed peak ozone concentrations in California. This is due to the meteorology of the processes that transport ozone on continental and hemispheric scales, and to a seasonal mismatch between peak transport and local ozone maxima.

Stratospheric ozone intrusions generally occur as a result of large scale atmospheric disturbances – conditions that are inimical to the stable, stagnant conditions necessary to support buildup of pollutants in an urban area, thus the probability of stratospheric ozone adding to a high ozone concentration due to anthropogenic emissions is low. In addition, stratospheric ozone events can be recognized by unique atmospheric chemistry – very dry air, low aerosol concentrations, a general lack of anthropogenic precursor gases (VOCs and NO_X), and very low carbon monoxide (CO) concentrations compared to typical urban plumes. This chemical signature should make it possible to recognize stratospheric intrusion and to classify associated ozone concentrations as "exceptional events".

Long-range transport of anthropogenic ozone is similarly restricted to particular meteorological conditions. Jacob, Logan and Murti (1999) report that, although the lofting of Asian precursors is associated with unstable air along frontal boundaries, the movement of ozone downward to impact the surface in North America is dominated by subsidence in the middle and lower troposphere, sometimes assisted by dry convection in the lower layers of the atmosphere. In California, weak subsidence is associated with strong surface inversions in the

heavily populated coastal zone and Central Valley, while strong subsidence is associated with “Santa Ana” conditions. Downward mixing of Asian ozone to the surface is not possible when a strong surface inversion is present, thus locally generated high ozone concentrations are unlikely to be enhanced by long range transport. Conversely, Santa Ana conditions are generally associated with low ozone concentrations in the coastal zone as locally generated ozone precursors are swept out to sea; under such conditions transported ozone would tend to replace, rather than supplement locally generated ozone.

There is also a seasonal mismatch between the peak of long range transport and California’s ozone seasons. Periods of effective long range transport are generally restricted to late winter and spring (Berntsen, Karlsdottir and Jaffe, 1999), while high ozone due to local sources in California tend to occur in late summer and fall.

4.3.2 Past and Future Trends

Ozone delivered by long range transport may be contributing to a small increase in ozone concentration on days that would otherwise have low ozone. Statistical analyses of U.S. ozone data (Lin et al., 2000) indicate that “background” effects appear to be driving an increase in minimum ozone concentrations at rural locations in the U.S. at the some time as peak ozone concentrations are declining due to U.S. emission controls. They estimate a mean increase in U.S. “background” ozone of 3 – 5 ppb between 1980 and 1998.

Model studies indicate that long-range transport of ozone to the western U. S. may increase as global energy use increases, especially as it drives NO_X emissions in Asia. Jacob, Logan and Murti explored the change in Asian ozone impact in the United States for a base case of Asian NO_X emissions for 1985 and a tripling of the 1985 level projected to occur around 2010. The growth scenario caused Asian ozone contributions along the U.S. west coast to develop a spring “bubble” rising from 3-4 ppb in March to a peak between 5 and 6 ppb in May, then dropping gradually through the summer to return to about 2-3 ppb in August.

4.4 Total “Background” Ozone in California.

Fiore et al*.,* (2002) modeled summer ozone over the United States using a global air quality model and retrieved from the results separate signals for “natural” ozone from all sources, total ozone due to non-U.S. sources (natural + Asia, Europe), and total ozone including all sources.

“Natural” ozone is highly sensitive to insolation, thus the desert southwest showed the highest values, 20- 25 ppb mean afternoon maxima for the season (June-August). The model identified a gradient along the coast, ranging from 15 ppb over the ocean to about 20 ppb in interior California. Transported ozone from outside the U.S. follows a similar pattern. Due to the combination of strong sunlight and atmospheric subsidence, the desert southwest again is the “hot spot” with mean seasonal afternoon ozone in the range of 30 – 35 ppb, and there is there is a gradient from the ocean inland, ranging from 20-25 ppb over the ocean to about 30 ppb in interior California.

The difference between these two cases gives an estimate of the transported anthropogenic increment of background. The peak summer afternoon impact of 7 ppb falls in the northern Great Basin (eastern Oregon, southern Idaho, northern Nevada). The entire intermountain west and interior California lie in the area receiving 5 or more ppb, while the Pacific coast shows a weak gradient from about 4 ppb near the Mexican border to about 5 near the Canadian border.

However, it does provide a basis for exploring the relationship between background and peak ozone concentrations in general. Statistics from the model runs show that the mean enhancement to summer ozone levels is low (about 2 ppb) when total ambient ozone is low (<30 ppb) or high (>80 ppb). Exogenous enhancement rises to about 5 (range 1-10) ppb when ambient ozone concentrations are moderate (40-65 ppb). This result is consistent with the observations of Lin et al. (2000), supporting the view that exogenous ozone impacts are not driving peak concentrations, but probably are increasing long term averages by a few ppb.

4.5 Summary - Estimating the "Policy Relevant " Background

Overall, it appears that "background" ozone in California is dominated by natural tropospheric and stratospheric processes. The effects of occasional very large biomass fires and anthropogenic emissions are secondary factors. The foregoing discussion indicates that average "natural background" ozone near sea level is in the range of 15 – 35 ppb, with a maximum of about 40.

Exogenous enhancements to "natural" levels generally are small (about 5 ppb), and are unlikely to alter peak concentrations.

At altitudes above 2km stratospheric intrusions can push peak ambient concentrations to 45 – 50 ppb. The timing, spatial extent, and chemical characteristics of stratospheric air mass intrusions makes these events recognizable in air quality records, providing that the affected region has a fairly extensive monitoring network and that multiple air quality parameters (CO, VOC, PM, RH) were being measured as well.

Intermittent episodes of "natural" ozone from very large biomass fires in boreal forests (Alaska, Canada, Siberia) can produce short-lived pulses of ozone up to 20 ppb that may arrive during the North American ozone season. Present understanding suggests that these are infrequent events at latitudes below about 50N. There are no data documenting such an event in California.

Long range transport of anthropogenic ozone may grow as Asian energy consumption increases the continent's NO_X emissions. Model studies indicate that the Asian ozone increment in North America could double over the next few decades. Assuming the temporal pattern of transport remains unchanged, such an impact could increase mean ozone concentrations by 2-6 ppb. The potential effect on peak transport events is unknown at this time.

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5 Ozone Precursor Sources and Emissions

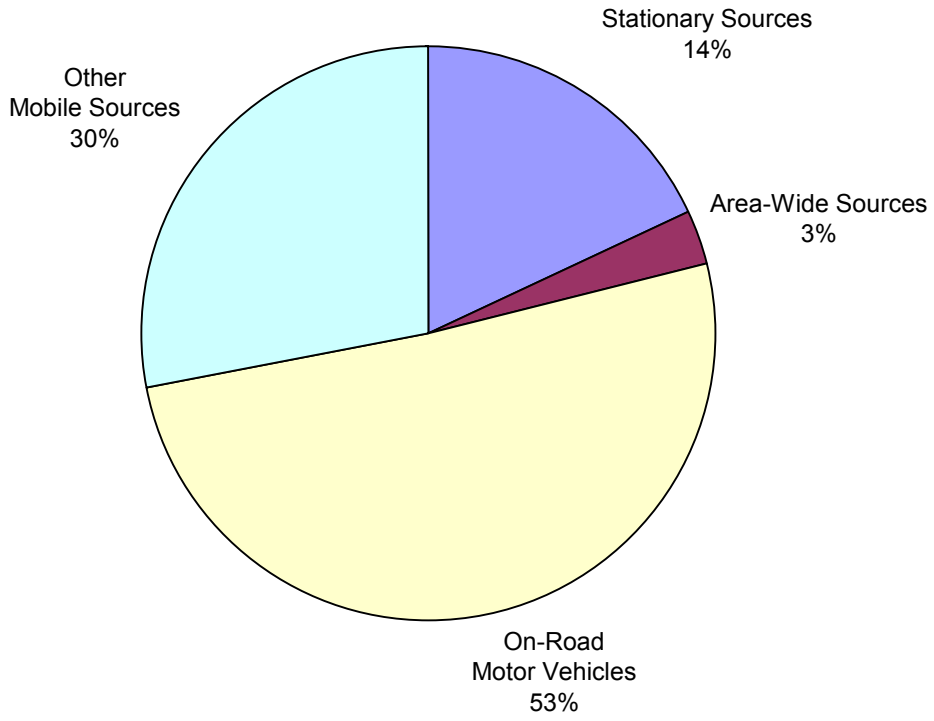
5.1 Ozone Precursor Sources

Ozone is an oxidant gas that forms photochemically in the atmosphere when nitrogen oxides (NO_X) and reactive organic gases (ROG) are present under appropriate atmospheric conditions (see Chapter 3). Carbon monoxide (CO) is also an ozone precursor. Both ROG and NO_X are emitted from mobile sources, point sources, and area-wide sources. ROG emissions from anthropogenic sources result primarily from incomplete fuel combustion, and from the evaporation of solvents and fuels, while NO_X emissions result almost entirely from combustion processes.

5.2 Sources of NO_X Emissions

NO_y is defined as a group of gaseous compounds of nitrogen and oxygen, many of which contribute to the formation of ozone. For example, NO_X includes nitric oxide (NO) and nitrogen dioxide (NO_2), which result from the combustion of fuels. Mobile sources (including on-road and others) made up about 83 percent of the total statewide NO_X emissions in 2003. The category of other mobile sources includes emissions from aircraft, trains, ships, recreational boats, industrial and

NOx emissions (tons/day, annual average)



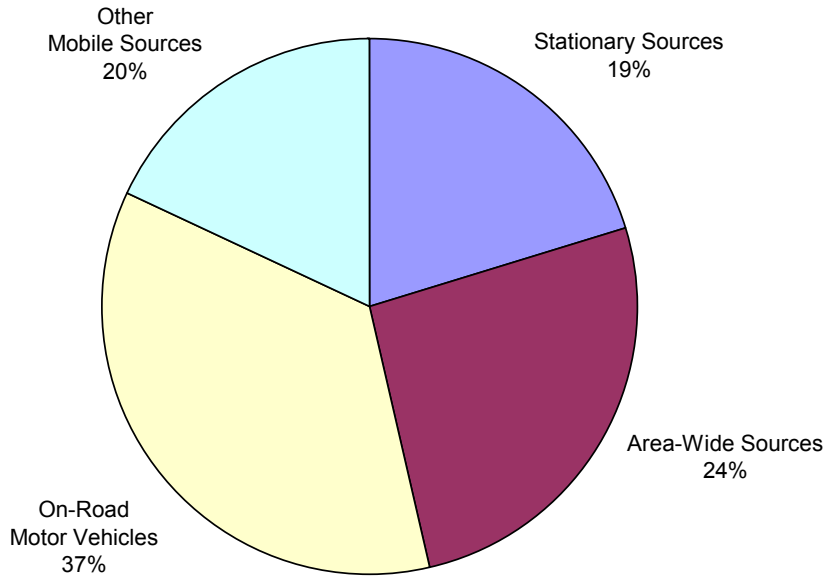
construction equipment, farm equipment, off-road recreational vehicles, and other equipment. Stationary sources of NO_X include both internal and external combustion processes in industries such as electric utilities, which contribute approximately 2% of Statewide NO_X emissions, manufacturing, food processing, and petroleum refining. Area-wide sources, which include residential fuel combustion, waste burning, and fires, contribute only a small percent of the total NO_X emissions.

5.3 Sources of ROG Emissions

ROG consists of volatile organic compounds that are photochemically reactive and contribute to the formation of ozone. These emissions result primarily from incomplete fuel combustion and the evaporation of chemical solvents and fuels. Mobile sources (including on-road and others) made up approximately 57 percent of total statewide ROG emissions in 2003. This category includes emissions from cars, trucks, and motorcycles powered by gasoline and diesel fuels. Stationary sources of ROG emissions include processes that use solvents (such as dry cleaning, degreasing, and coating operation) and petroleum-related processes (such as petroleum refining and marketing and oil and gas extraction). Electricity generation is estimated to contribute about 1% of Statewide ROG emissions. Area-wide ROG sources include consumer products, pesticides, aerosol and architectural coatings, asphalt paving and roofing, and other evaporative emissions.

To determine the amount of ROG in the emissions, speciation profiles are used. These profiles provide estimates of the chemical composition of emissions, and are used in the emission inventory and air quality models. The ARB maintains and updates estimates of the chemical composition and reactive fractions of total organic gases (TOG), for a variety of emission source categories.

ROG emissions (tons/day, annual average)



5.4 Regional Differences in Ozone Precursor Emissions

Emissions from different types of sources vary regionally in California. The following table shows 2003 ozone precursor emissions from mobile sources statewide and regionally for selected areas. The emissions are expressed as a percentage of total emissions for each area.

Percentage of Ozone Precursor Emissions From Mobile Sources

Area	*NO_X (%)*	*ROG (%)*
Statewide	*83*	*57*
Sacramento Valley	84	49
San Diego	95	56
San Francisco Bay Area	85	61
San Joaquin Valley	75	44
South Coast	90	64

5.5 Temporal Differences in Ozone Precursor Emissions

Generally speaking, emissions of ozone precursors will be higher in warmer months than in cooler ones. However, the emissions for individual source categories may further vary with time, season and place. For example, ROG emissions from on-road motor vehicles are significantly higher in warmer months because of the effect of higher ambient temperatures on evaporative emissions. Stationary source emissions of ROG and NO_X may vary based on the nature of the source. Power plants typically have higher emissions of ROG and NO_X in the summer because of widespread use of air conditioning in homes and workplaces, while emissions associated with oil extraction will fluctuate based on the price of crude oil and other factors. Emissions from manufacturing facilities are subject to great variation. They may be consistent year-round for one business or may vary seasonally for another business because of the product being manufactured. Area-wide source emissions may likewise vary. For example, ROG emissions associated with the application of exterior paint increase in the summer because it is more practical to apply paint in warm and dry weather.

5.6 Natural Source Emissions

Natural source emissions include a variety of compounds and occur as a result of geologic or meteorological activity (such as petroleum seeps or wildfires), or living processes by flora and fauna (such as vegetation foliage or soil microbes). Natural source emissions are strongly affected by seasonal influences on factors such as temperature and moisture conditions, or wind regimes. Estimates of

natural source emissions are calculated and used as inputs to climate research and photochemical modeling.

5.6 References

Planning and Technical Support Division, Air Resources Board. The 2003 California Almanac of Emissions and Air Quality.

Emission Inventory Branch, Air Resources Board. May 1996.Emission Inventory Procedure Manual, Volume I, Inventory Development Process.

6 Measurement of Ozone

6.1 Introduction

Ozone is a highly reactive, colorless gas. Any method used to measure ozone in an atmosphere must consider these properties. Ozone must be measured at the sampling location, as samples cannot be taken back to a laboratory for analysis.

Two measurement methods for ozone are approved for use in the U.S. by the USEPA: one is based on the chemiluminescence that occurs when ozone and ethylene react, and the other on the attenuation of ultraviolet (UV) radiation by ozone. The method based on UV spectrometry is almost universally used in practice. Specifications and criteria for both methods exist in federal regulation. The UV photometry-based method is approved for use in California for state air quality standards. Both state and federal requirements are applied directly by the ARB and the air districts in the ozone monitoring network in California.

6.2 Existing Monitoring Methods

The USEPA has developed design and performance criteria for methods used to measure tropospheric ozone. The federal reference method (FRM) is based on gas-phase chemiluminescence (40 CFR Part 50, Appendix D), and the federal equivalent methods (FEMs) are based on UV photometry (40 CFR Part 53, and McElroy et al., 1997), with the exception of one method based on gas-solid chemiluminescence (USEPA, 1996).

The state ambient air quality standard for ozone (California Code of Regulations, Title 17, section 70200) stipulates that ultraviolet photometry is the method to be used to measure ozone. The standard also allows an equivalent method to be used, as described in the first footnote to the “Table of Standards” in section 70200:

“Any equivalent procedure which can be shown to the satisfaction of the ARB to give equivalent results at or near the level of the air quality standard may be used.”

To implement the general requirement of a UV photometry-based measurement method, ARB and air district staff employ specific UV photometry-based methods and procedures as prescribed by the USEPA. These UV methods must be operated in accordance with the manufacturers’ instructions and instrument-specific ARB standard operating procedures, both of which are consistent with the requirements.

Below are general descriptions of the chemiluminescence and UV methods for ozone measurement.

6.2.1 Gas-Phase Chemiluminescence Methods

The most common chemiluminescence method for ozone is direct gas phase reaction of ozone with olefin to produce electronically excited products, which decay with the emission of light. When ozone reacts with ethylene gas, an olefin,

electronically excited formaldehyde is produced. As this excited species returns to the ground state, it gives off light in a band centered at 430 nanometers (nm) in proportion to the amount of ozone present. This chemiluminescence can be measured using a photomultiplier tube, and the concentration of ozone is calculated (ARB, 1987, and USEPA, 1996).

The USEPA has identified specific monitoring instruments based on gas-phase chemiluminescence as FRMs for ozone measurement. Any other measurement method or instrument must be compared against the reference method, and must perform on a par with the reference method to be deemed equivalent. A detailed discussion of the measurement method principle and the calibration procedures for chemiluminescence-based instruments is given in the Code of Federal Regulations (CFR) 40 CFR, Part 50, Appendix D, and USEPA, 1996.

Humidity causes a positive bias in chemiluminescence methods. This can be compensated for by using humidified air for instrument calibration.

Because the reference method uses a potentially flammable gas as a reactant, and because there is an equivalent method for directly measuring ozone, chemiluminescence-based monitors are seldom used for routine air monitoring. In areas of high humidity, the interference has been problematic and the debate continues as to the instrument's complete reliability for such an important purpose.

6.2.2 Ultraviolet Photometry Methods

Ozone exhibits a strong absorption band in the ultraviolet region at 254 nm. This feature is the basis of the photometric measurement method for ozone. Many commercially-available UV instruments meet U.S. EPA equivalency criteria as set forth in 40 CFR Part 53, § 53.32. A discussion of the principle of the UV spectrometric method for ozone is given in ARB (1987), McElroy et al. (1997), and U.S. EPA (1996). Calibration techniques and other quality control and quality assurance methods and practices for the state method are described in the ARB Air Monitoring Quality Assurance Manual Volume II (Appendix A, 1995); see the on-line copy of the manual at:
http://www.arb.ca.gov/aaqm/qmosqual/qamanual/qamanual.html

Other species present in the atmosphere such as aromatic hydrocarbons also absorb at or near 254 nm, and so represent potential interferences to the method. The commercially-available instruments compensate for this possible interference by comparing the absorbance of the sample with the absorbance of air in which the ozone has been catalytically reduced to molecular oxygen (O_2); consequently attenuation of the UV light due to non-ozone species is taken into account. However, negative interference due to high humidity and positive interference due to high hydrocarbon concentrations have been reported. For details on this, the reader is referred to McElroy et al. (1997).

6.3 Precision and Significant Figures

It is important to consider the number of significant figures when reporting ambient air quality data to avoid overstating, or understating; the preciseness of

measured concentrations. By definition, significant figures are those digits in a number that are known with certainty, *plus* the first uncertain digit. In other words, the last digit (rightmost digit) is an estimated digit.

The precision of a measurement method refers to measurement repeatability, or variability, and is expressed as the sample variance, or standard deviation. A simple evaluation of method precision determines the number of appropriate significant figures that should be used to record and report measurement data for that method.

For a grouping of monitors, such as a network, one way to evaluate *method* precision is to look at the performance of all monitors in the group or *network*. In effect, you are determining the *network precision*. For purposes of this report, the evaluation of network precision was made by examination of ozone performance audit data. Performance audits are conducted by testing an ozone monitor with know concentrations of ozone. Each audit consists of testing the monitor at the high, mid and low range of the monitor. Since the level of interest for this report (0.070 ppm) is closest to the low audit level, only low concentration audit data (from 0.030 to 0.080 ppm) were evaluated to get the best estimate of precision at this level (USEPA 1983, 1984).

Performance audits are primarily used to assess the accuracy (or systematic bias) of *individual* ozone monitors. However, when the individual biases of many monitors are combined, and looked at as a group, the variability of average bias can appear random, and therefore be used to assess the *precision of the network*.

For 2003, the most recent year of finalized audit data, ARB staff conducted performance audits for 137 ozone monitors. The average bias of all 137 audits was –2.0%, with a standard deviation of 4.3%. To express standard deviation in units of ppm, we multiply 4.3% by the concentration of interest, in this case, 0.070 ppm. The result is 0.0030 ppm. By convention, this means that ozone measurements are uncertain in the third decimal place, to the degree of plus or minus 0.003 ppm. (This result is consistent with audit data going back to 1999 and preliminary audit data from 2004.)

Since significant figures include all certain digits, *plus the first uncertain digit*, the precision of California's ozone network (as determined above) indicates that ozone measurements should be recorded and reported to the third decimal place ppm.

6.4 Monitor Calibration Methods

Ozone instruments are calibrated by comparing the responses of an ambient monitoring station ozone analyzer to a certified ozone transfer standard. The response to ozone gas is compared at 4 levels and regressed using the "least squares" method. The four levels are approximately 0.400 ppm , 0.300 ppm, 0.200 ppm, and 0.090 ppm. Calibration gas at each of these levels is introduced into the ozone analyzer until a steady and unchanging analyzer response is achieved. Typically, a steady reading of 10 minutes is taken as the calibration

data point. The regression results are not used to correct data; they are used to determine the instrument's linearity and deviation from the true based on the regression slope. An instrument is not adjusted to match the transfer standard unless it is beyond 2% from true (slope of 0.98 to 1.02). We would not expect any difference in accuracy/precision for the 8-hour concentration, since the standard is an average of eight 1-hour concentrations. Precision is not increased or decreased by arithmetic operations.

6.5 Recommendations

Staff recommends that the Board continue to endorse the UV method as the approved method in California for determining compliance with the state Ambient Air Quality Standard for ozone. By reference, therefore, staff recommends all federally approved UV methods be incorporated as California Approved Samplers for ozone. This will result in no change in air monitoring practices, but will align state monitoring requirements with federal requirements. Specifically, we recommend that a new part be added to the California Administrative Code 70100.1, to read, in part:

Ozone Methods. The method for determining compliance with the ozone ambient air quality standard shall be the Federal Equivalent Method for the Determination of Ozone in the Atmosphere (40 CFR, part 53). California Approved Samplers for ozone are set forth in Air Monitoring Quality Assurance Manual Volume IV, Part C: Monitoring Methods for Ozone.

The list of UV methods (USEPA/ORD, 2002) is given at
http://www.epa.gov/ttn/amtic/criteria.html"

The following methods and instruments are *California Approved Samplers for Ozone* for the purposes of determining compliance with the state ambient air quality standard:

Ultraviolet Method for the Determination of Ozone in the Atmosphere, 40 CFR, Chapter 1, Part 50, Appendix D as published in FR 62, 38895, July 18, 1977. The specific instruments approved are:

a. Dasibi Models 1003-AH, 1003-PC, or 1003-RS Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0577-019, as published in FR 42, 28571, June 03, 1977.

b. Dasibi Models 1008-AH, 1008-PC, or 1008-RS Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0383-056, as published in FR 48, 10126, March 10, 1983.

c. DKK-TOA Corp. Model GUX-113E Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0200-134, as published in FR 65, 11308, March 02, 2000.

d. Environics Series 300 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0990-078, as published in FR 55, 38386, September 18, 1990.

e. Environment S.A. Model ozone41M UV Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0895-105, as published in FR 60, 39382, August 02, 1995.

f. Environment S.A. Model ozone42M UV Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0206-148, as published in FR 67, 42557, June 24, 2002.

g. Environment S.A. SANOA Multigas Longpath Monitoring System, USEPA Automated Equivalent Method EQOA-0400-137, as published in FR 65, 26603, May 08, 2000.

h. Horiba Instruments Models APOA-360 and APOA-360-CE Ozone Monitor, USEPA Automated Equivalent Method EQOA-0196-112, as published in FR 61, 11404, March 20, 1996.

i. Monitor Labs/Lear Siegler Model 8810 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0881-053, as published in FR 46, 52224, October 26, 1981.

j. Monitor Labs/Lear Siegler Models ML9810, ML9811, or ML9812, Monitors Labs Model ML9810B, or Wedding & Associates Model 1010 Ozone Analyzers, USEPA Automated Equivalent Method EQOA-0193-091, as published in FR 58, 6964, February 03, 1993.

k. Opsis Model AR 500 and System 300 Open Path Ambient Air Monitoring Systems for Ozone, USEPA Automated Equivalent Method EQOA-0495-103, as published in FR 60, 21518, May 02, 1995.

l. PCI Ozone Corporation Model LC-12 Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0382-055, as published in FR 47, 13572, March 31, 1982.

m. Philips PW9771 03 Analyzer, USEPA Automated Equivalent Method EQOA-0777-023, as published in FR 42, 38931, August 01, 1977; FR 42, 57156, November 01, 1977.

n. Teledyne-Advanced Pollution Instrumentation, Inc. Model 400E Ozone Analyzer, Advanced Pollution Instrumentation, Inc. Model 400/400A Ozone Analyzer, USEPA Automated Equivalent Method EQOA-0992-087, as published in FR 57, 44565, September 28; 1992, FR 63, 31992, June 11, 1998; FR 67, 57811, September 12, 2002.

o. Thermo Electron/Thermo Environmental Instruments Models 49, 49C, USEPA Automated Equivalent Method EQOA-0880-047, as published in FR 45, 57168, August 27, 1980.

6.6 Estimated Costs and Impacts

Because the recommended change reflects the existing practice in air monitoring for ozone, approval of the recommendation will result in no costs or savings to any public agency, or to any private business.

6.7 References

Air Resources Board. September, 1987. Effects of ozone on health, technical support document, pages 5-13.

Code of Federal Regulations. Title 40, Part 50, Appendix D, Measurement principles and calibration procedure for the measurement of ozone in the atmosphere.

Code of Federal Regulation. Title 40, Part 53, Revised requirements for designation of reference and equivalent methods for PM2.5 and ambient air quality surveillance for particulate matter. Pgs 1-28.

McElroy F, Dennis M, Monica N. 1997. Determination of ozone by ultraviolet analysis. A new method for Volume II, Ambient air specific methods, quality assurance handbook for air pollution measurement systems.

USEPA. 1996. Air Quality Criteria for ozone and related photochemical oxidants, EPA/600/P-93/004q-cF, pages 3-90 to 3-102.
http://cfpub.epa.gov/ncea/cfm/ozone.cfm

Title 17, Barclay California Code of Regulation, section 70200.
USEPA/ORD (2002). List of designated reference and equivalent methods. http://www.epa.gov/ttn/amtic/criteria.html

Appendix A (1995). Dasibi model 1003 AH ozone analyzer. Volume II: standard operating procedures for air monitoring, air monitoring quality assurance, California Air Resources Board.
http://www.arb.ca.gov/aaqm/qmosqual/qamanual/qamanual.htm

USEPA, 1984. Special Report, Issues Concerning the Use of Precision and Accuracy Data, EPA-450/4-84-006, pages 2 – 23.
http://www.epa.gov/ttn/amtic/cpreldoc.html

USEPA, 1983. Guideline on the Meaning and Use of Precision and Accuracy Data Required By 40 CFR Part 58, Appendices A and B, EPA-600/4-83-023, pages 6-33. http://www.epa.gov/ttn/amtic/cpreldoc.html

7 Exposure to Ozone

The purpose of this chapter is to provide a characterization of current air quality with respect to ozone. The chapter describes ambient ozone concentrations throughout California, with a focus on the current State 1-hour standard (0.09 ppm) and the proposed State 8-hour standard (0.070 ppm), as well as the federal 1-hour (0.12 ppm) and 8-hour ozone standards (0.08 ppm). The federal one-hour ozone standard is scheduled to be phased out in June 2005. Much of the information relates to current air quality, in particular, ozone data collected during 2001 through 2003. In addition, the chapter provides information about the State and federal area designation processes and data rounding conventions.

Section 7.1 describes the current area designations for the State and federal 1-hour ozone standards, as well as the United States Environmental Protection Agency's (USEPA) recent area designations for the federal 8-hour standard. The area designations indicate which areas of the State attain the health-based standards. Included in the State area designation discussion is information related to the identification of *highly irregular or infrequent events*. Data affected by these types of events are excluded from the State designation process. Section 7.2 gives a characterization of ambient ozone air quality. Information in this section includes discussions of current air quality and the frequency of maximum daily concentrations, as well as information about the ozone season, diurnal patterns of ambient ozone concentration, and historical trend data for each air basin or planning area. Finally, Section 7.3 gives a characterization of exposure to peak 1-hour and 8-hour ozone indicator values throughout the State.

It is important to note that some of the tables and graphs in Sections 7.2 and 7.3 do not include information for the Great Basin Valleys and Northeast Plateau Air Basins because, at the time this Staff Report was prepared, 2001 data for these two areas either were not reliable or not complete. In particular, reliable third quarter 2001 data were not available for the Mammoth Lakes monitoring site in the Great Basin Valleys Air Basin, and data for the Yreka monitoring site in the Northeast Plateau Air Basin were not complete during the 2001 summer months, when high concentrations are expected to occur. Where 2001 data do not impact the air quality statistics, data for these two areas are included (for example, maximum 1-hour and 8-hour concentrations for 2002 and 2003 are included).

7.1 Area Designations for the State and Federal Ozone Standards

7.1.1 Background

The California Health & Safety Code (H&SC) section 39607(e) requires the Air Resources Board (ARB) to establish and periodically review criteria for designating areas as attainment or nonattainment with respect to the State ambient air quality standards. Areas are designated separately for each pollutant for which there is an ambient air quality standard specified in section 70200 of Title 17, California Code of Regulations. The ARB originally adopted State designation criteria in June 1989. The ARB subsequently amended the designation criteria in June 1990, May 1992, December 1992, November 1993, November 1995, September 1998, and January 2004. H&SC section 39608

requires the ARB to use the designation criteria in assessing the designation status of areas in California (ARB 2003).

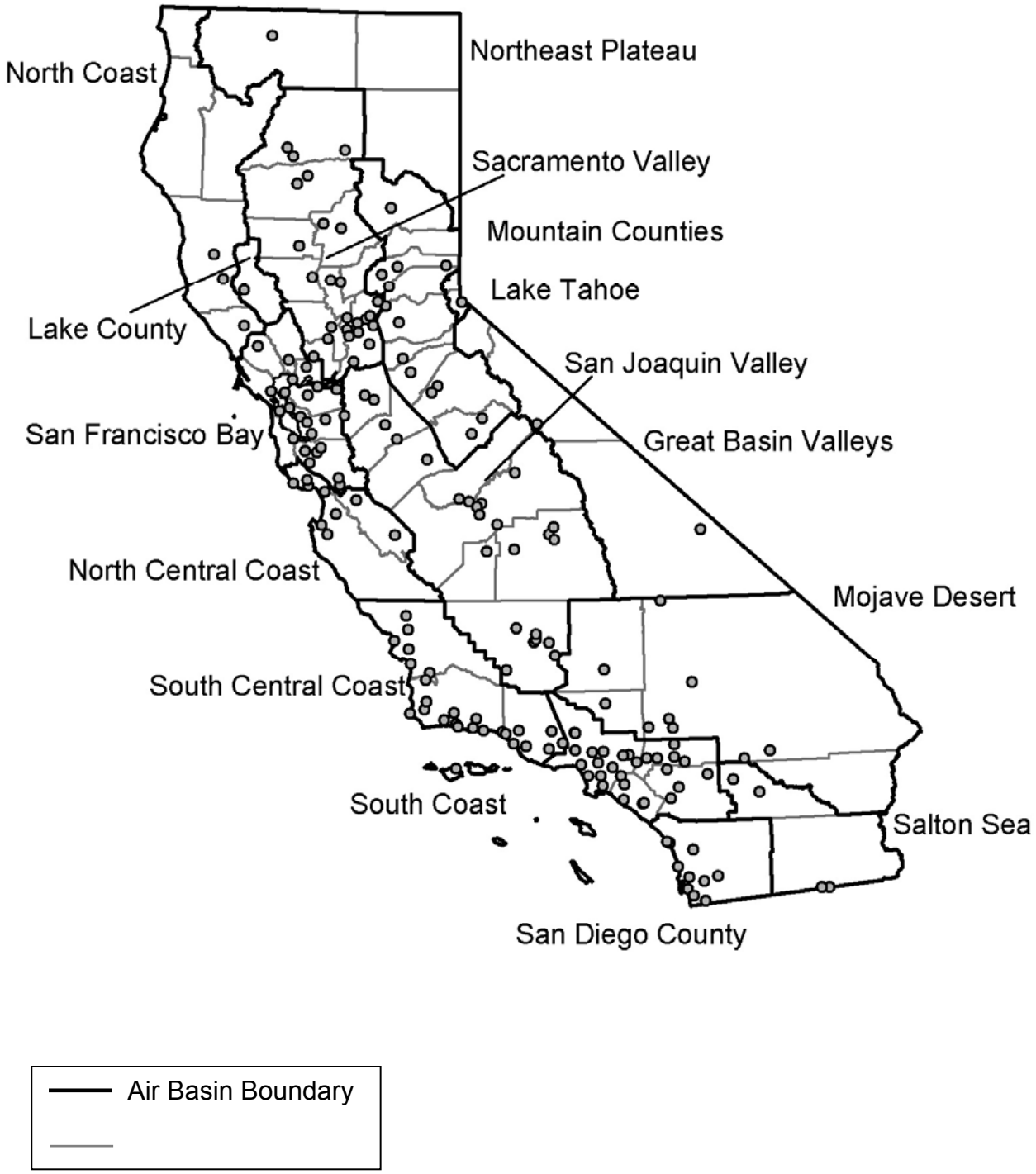
State area designations indicate whether an area meets the health-based State ambient air quality standards. There are three basic designation categories: *nonattainment, attainment,* and *unclassified*. A *nonattainment designation* indicates that the air quality violates a State standard. There is a subcategory of the nonattainment designation called nonattainment-transitional. This designation is given to areas that still violate the State standard, but are making progress and are close to attainment. In contrast to nonattainment, an *attainment designation* indicates that the air quality does not violate the State standard. Finally, an *unclassified designation* indicates that there are insufficient data for determining attainment or nonattainment.

Under State law (H&SC section 40921 and section 40921.5(a) and (c)), areas designated as nonattainment for the State 1-hour ozone standard are also assigned a classification that is commensurate with the severity of their air quality problem and the degree of emission control required to achieve attainment. For example, take two ozone nonattainment areas, with the first area having a measured maximum concentration of 0.10 ppm and the second area having a measured maximum concentration of 0.19 ppm. While both areas are designated as nonattainment, the second area has a more severe ozone problem and will need to implement a more stringent emission control strategy to achieve attainment. Therefore, although these two areas would have the same designation, they would not have the same classification. Under State law (H&SC section 40921 and section 40921.5(a) and (c)), areas are classified as moderate, serious, severe, and extreme with respect to the State 1-hour ozone standard, based on data for calendar years 1989 through 1991.

The USEPA has a similar process for designating and classifying areas with respect to the federal ozone standards. However, the USEPA uses only two designation categories. Similar to the State designations, areas with air quality that violates the federal standard are designated as *nonattainment*. However, areas with air quality that does not violate the standard and areas with insufficient data for determining nonattainment are generally combined in a category called *unclassified/attainment*. Similar to State requirements, the USEPA also classifies ozone nonattainment areas according to the severity of their ozone air quality problem.

Both the ARB and the USEPA designate areas based on recent ambient air quality data. As shown in Figure 7-1, California has an ozone monitoring network with approximately 175 monitors located throughout the State. These monitors are generally operated by the ARB or by local air pollution control or air quality management districts (districts). However, a few sites are operated by other organizations, such as the National Park Service. At each site, a monitor provides continuous hourly averages of ambient ozone concentrations. These hourly measurements can be aggregated into longer-term measurements, such

Figure 7-1 Ozone Monitoring Sites in California (as of December 31, 2002)



as 8-hour averages. Because they are used for regulatory purposes, these measured data must satisfy specific siting and quality assurance procedures established by the ARB and USEPA (additional information about air quality monitoring can be found in Chapter 6). In general, area designations for both the State and federal standards are based on data collected during the previous three years.

7.1.2 Exclusion of Data

The State area designation process has several provisions for excluding high values that are not reasonable to control through the regulatory process. These excluded values are identified as concentrations affected by *highly irregular or infrequent events*. While a concentration identified as a highly irregular or infrequent event "exceeds" the level of the State standard, such an exceedance is not considered a "violation" of the standard. This is important because only a "violation" can trigger a nonattainment designation. As a result, although the State ozone standard is expressed as a concentration that is "not to be exceeded," the designation criteria allow some leeway for excluding exceedances that are not reasonable to control. Under State law, there are three types of highly irregular or infrequent events: extreme concentration events, exceptional events, and unusual concentration events.

An *extreme concentration event* is identified by a statistical procedure (ARB 1993) and is the most frequently used method for excluding values from the State area designation process. This type of event is not necessarily tied to any specific, identifiable event. However, adverse meteorology is one potential cause of an extreme concentration event. Because meteorology is a potential cause of an extreme concentration event and meteorology varies from year-to-year, an area may have several values excluded during years with adverse meteorology and no values excluded during years with more normal meteorology.

In identifying extreme concentration events, a statistical procedure is used to calculate a site-specific and pollutant-specific value representing the concentration that is expected to be exceeded once per year, on average, based on the distribution of data for the site. The site-specific, statistically derived value, commonly referred to as the peak indicator value or the Expected Peak Day Concentration (EPDC), is rounded to the precision of the State standard before being used. The measured or averaged (for example, 8-hour averages) pollutant concentrations are also rounded before being compared with the rounded EPDC. Any rounded concentration that is higher than the rounded EPDC value is identified as an extreme concentration eventand is excluded from the State designation process. Furthermore, these extreme concentrations are not considered violations of the State standard.

In contrast to an extreme concentration event, an *exceptional event* is a specific, identifiable event that causes an exceedance of a standard, but is considered unreasonable to control through the regulatory process. Exceptional events may be identified for both State and federal designation purposes, and are evaluated on a case-by-case basis. An example of an ozone exceptional event is stratospheric ozone intrusion.

Federal guidelines (which are also used in the State designation process) define stratospheric ozone intrusion as occurring when a parcel of air originating in the stratosphere (average height 20 kilometers or about 12.4 miles) is entrained directly to the surface of the earth. Stratospheric ozone intrusions are typically associated with strong frontal passages or severe thunderstorms, and such conditions occur primarily during springtime (USEPA 1986).

Generally, the district identifies questionable data and gathers relevant information to document the cause and effect relationship. For stratospheric ozone intrusion, these data may include the intensity and location of low pressure zones, the relative amount of carbon monoxide present in the air, the relative humidity of the air, and in rare cases, the presence of trace elements characteristic of stratospheric air. The district then submits their request to the ARB for evaluation and potential identification. As stated in the area designation criteria, the ARB will evaluate potential exceptional events only if they have the potential to affect an area's designation status.

Finally, an *unusual concentration event* is an anomalous exceedance of a State standard that cannot be identified as an extreme concentration event or an exceptional event. Unusual concentration events can be identified only for areas designated as attainment or unclassified at the time the exceedance occurs. Furthermore, this type of event is usually identified in areas with limited monitoring data, where we do not have a long-term record for determining what is "characteristic" for the area. In identifying such events, the ARB's Executive Officer must make specific findings based on relevant information. An area may retain its attainment or unclassified designation by excluding an exceedance affected by an unusual concentration event for up to three consecutive years. However, if an exceedance occurs during the fourth year, the area is redesignated as nonattainment, unless the exceedance can be excluded as an extreme concentration event or an exceptional event.

For both State and federal area designations, the size of an area designated for ozone is generally an air basin. However, for the State standards, the ARB may designate a smaller area if it finds (based on air quality data, meteorology, topography, or the distribution of population and emissions) there are areas within an air basin with distinctly different air quality that can be attributed to sources and conditions that do not affect the entire air basin. In this case, the ARB may designate an area smaller than an air basin, using political boundary lines to the extent possible. The smaller designated area must include those sources whose emissions contribute to a violation of the standard. Furthermore, contiguous areas within an air basin that have the same designation, are designated as a single area.

7.1.3 State Area Designations

The State ozone standard is 0.09 ppm for one hour, not to be exceeded. The State area designations are based on a site-by-site comparison of the 1-hour State ozone standard with the maximum measured 1-hour concentration (that is not excluded as a highly irregular or infrequent event) during a three-year period . As described previously, highly irregular or infrequent exceedances may be

excluded as extreme concentration events, exceptional events, or unusual concentration events.

Figure 7-2 shows the area designations for the State 1-hour ozone standard. In addition to San Luis Obispo County and the North Coast Air Basin, which were designated as attainment in January 2004, the Lake County, Lake Tahoe, and Northeast Plateau Air Basins are also designated as attainment (ARB 2003). In contrast, however, most of the rest of the State, including all of the major urban areas, have ozone concentrations that violate the State standard. These areas are designated as nonattainment.

The method used for determining area designation values is generally consistent across all pollutants. First, if there is a valid EPDC, the EPDC is rounded to the given number of decimal places for the applicable State standard (for example, 2 decimal places for the State 1-hour ozone standard). Next, all measured values for the three-year period used in area designations are rounded to the given number of decimal places. All values that are higher than the valid rounded EPDC are excluded as extreme concentration events and therefore, not considered in the area designation process. The value used to designate an area (the designation value) is the highest rounded value for the previous three-year period that is less than or equal to the rounded EPDC. However, if this value is identified as affected by an exceptional event or unusual concentration event, it is excluded from the area designation process and the next highest value becomes the designation value.

7.1.4 Data Rounding Conventions

As noted above, before ozone measurements are used in designating areas for the State standard, they are rounded to the precision of the standard. In addition, the Expected Peak Day Concentration or EPDC is also rounded to the precision of the State standard before it is used to identify and exclude measurements affected by extreme concentration events.

All raw air quality data are stored in the Board's Aerometric Data Analysis and Management (ADAM) database, as they are reported. However, the reported values and the stored values can and do differ very slightly, because ADAM stores numbers in a floating-point format. For example, a number reported as 0.123 might actually be stored as 0.1229999998 or as 0.12300000001. Nonetheless, great care is taken to ensure that these "slight" differences have no impact on calculated values used for area designations.

The precision or given number of decimal places varies for each State standard and depends on how the level of the standard is specified. For example, the current 1-hour ozone standard is 0.09 ppm, which is given to 2 decimal places. In contrast, a proposed 8-hour standard of 0.070 ppm is given to 3 decimal places. Individual measurements and statistics are generally rounded up or down (to the precision of the standard) using the digit just beyond the given number of decimal places and according to standard rounding conventions. Values that are below 5 round down, while those that are equal to or greater than 5 round up. For example, the State 1-hour ozone standard is given to 2 decimal places. Therefore, a measured value of 0.123 ppm rounds to 0.12 ppm because 0.003 is less than 0.005. In contrast, a measured value of 0.127 ppm rounds to 0.13 ppm

because 0.007 is greater than 0.005. Similarly, a measured value of 0.125 ppm rounds to 0.13 ppm because 0.005 exactly equals 0.005.

7.1.5 Federal Area Designations

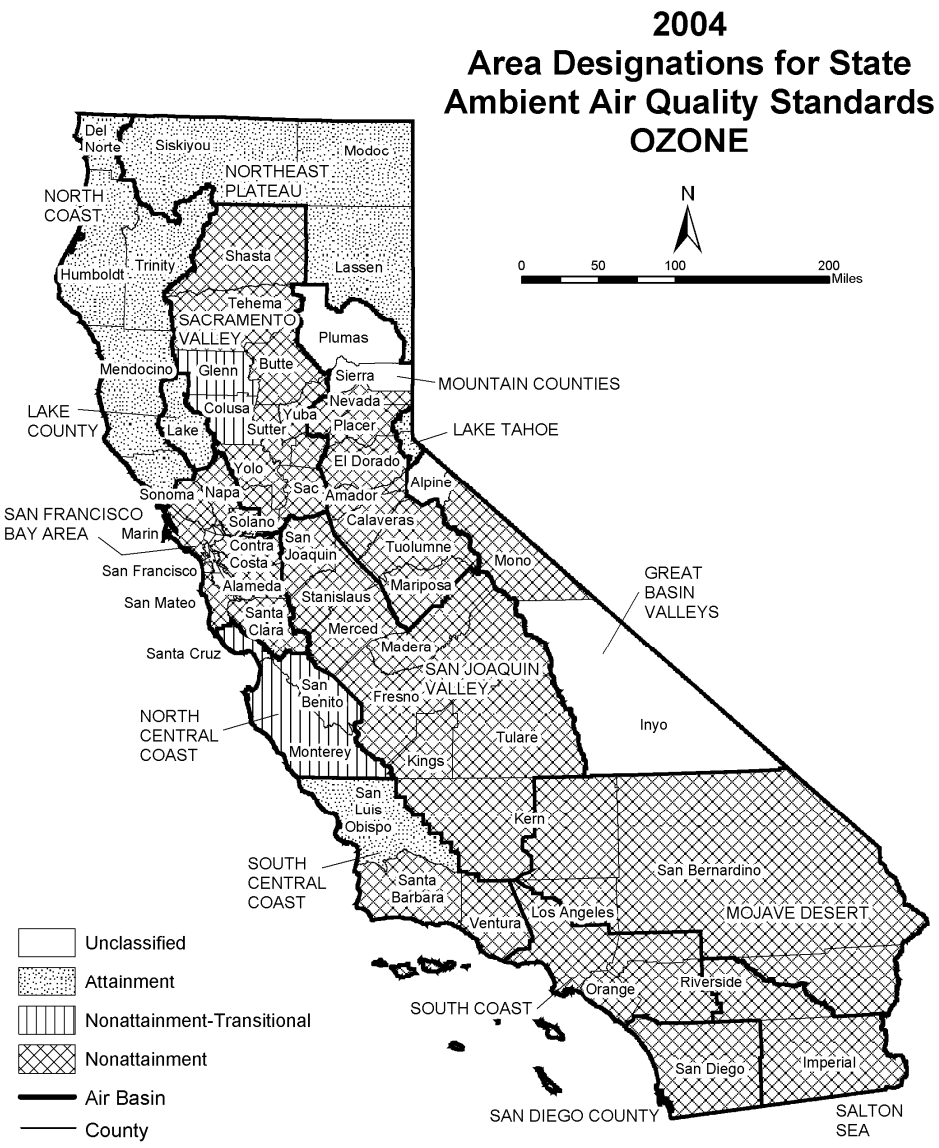
There are two federal standards for ozone: a 1-hour standard and an 8-hour standard. The federal 1-hour ozone standard is 0.12 ppm. For an area to be designated as attainment for the federal 1-hour standard, there may not be more than three violations of the standard at any site in the area during a three-year period. Compliance with the federal 1-hour standard is generally based on comparing the federal standard of 0.12 ppm with the 4th high 1-hour ozone concentration measured at each site in the area during a three-year period. If the 4th high concentration at any site in the area violates the standard, the area is designated as nonattainment. As mentioned earlier, areas that either do not violate the standard or do not have sufficient data to determine compliance with the standard are combined together in a designation category called unclassified/attainment.

Figure 7-3 shows the current area designations for the federal 1-hour ozone standard. Similar to the State designations, Figure 7-3 shows that most of the major urban areas in California are designated as nonattainment for the federal 1-hour standard. Over the last several years, ozone air quality in Butte County, northern Sutter County, Yuba County, Santa Barbara County, Ventura County, San Diego County, eastern Kern County, and the San Francisco Bay Area Air Basin has improved, and air quality in these areas now attains the federal 1-hour ozone standard. Recognizing these improvements, USEPA recently redesignated San Diego County and Santa Barbara County as attainment for the federal 1-hour ozone standard. However, the other four areas have not yet been officially redesignated, and they are shown as nonattainment areas in Figure 7-3.

Although some areas of California no longer violate the federal 1-hour standard, a number of these same areas do violate the new federal 8-hour ozone standard of 0.08 ppm, not to be exceeded. Compliance with the 8-hour standard is based on the annual 4th highest concentration at each site, averaged over three years. Although the federal 8-hour ozone standard is relatively new, long-term data are available for assessing attainment and trends because the 8-hour concentrations are based on an average of 1-hour ozone observations, which have been monitored for many years.

As with the federal 1-hour standard, all monitoring sites within an area must be in compliance with the 8-hour standard for the area to be designated as attainment. The ARB developed recommendations for the federal 8-hour designations and transmitted them to USEPA during early 2004. Figure 7-4 shows the final designations as made by USEPA on April 15, 2004. The final designations are based on air quality data collected during 2001 through 2003. As shown in Figure 7-4, the 8-hour nonattainment areas mirror those for the federal 1-hour standard, with the addition of much of the Mountain Counties Air Basin area. The remainder of the State is included in the unclassified/attainment category.

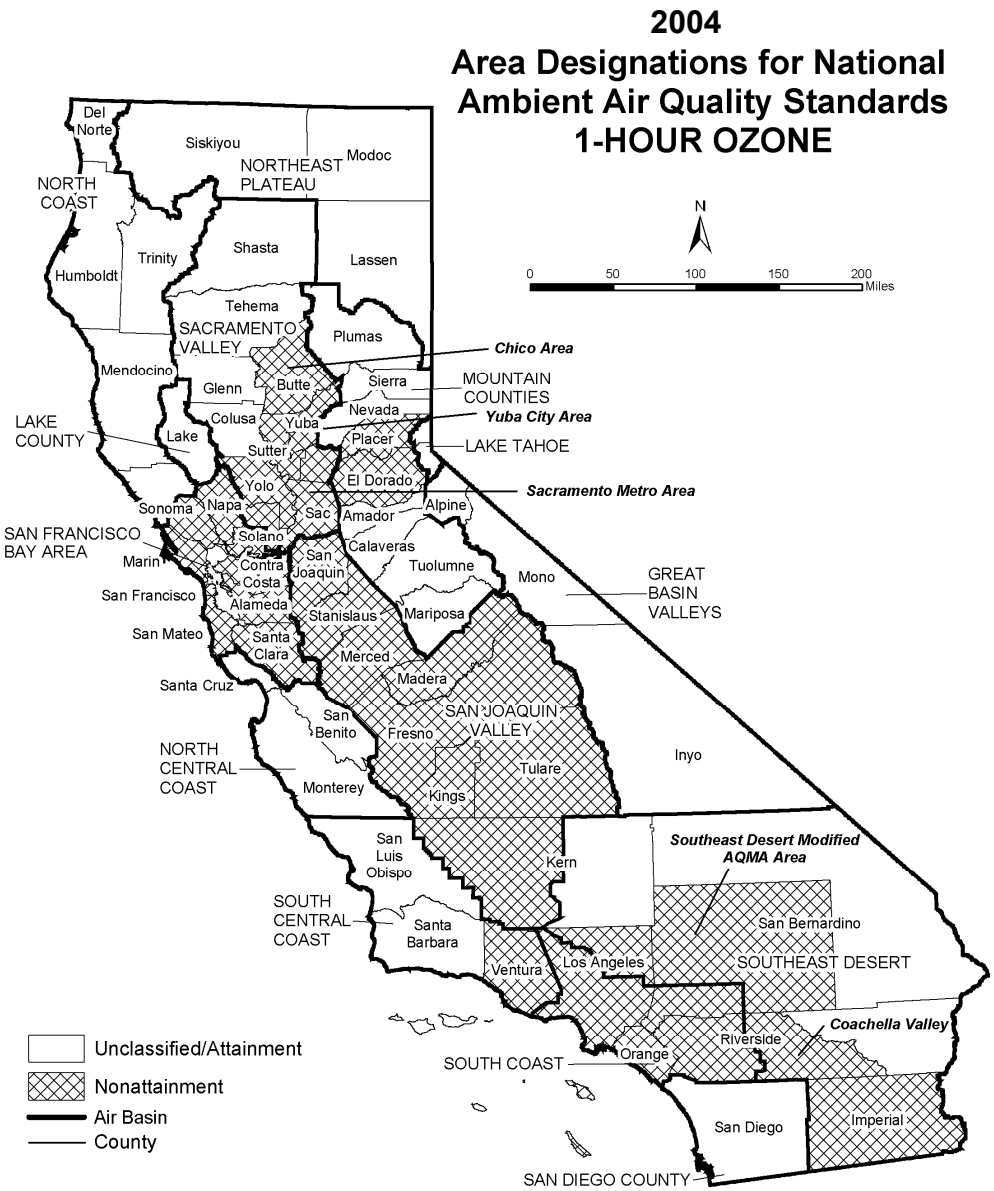
Figure 7-2 2004 Area Designations for the State 1-Hour Ozone Standard



Source Date:
October 2003
Emission Inventory Branch, PTSD

October 18, 2004

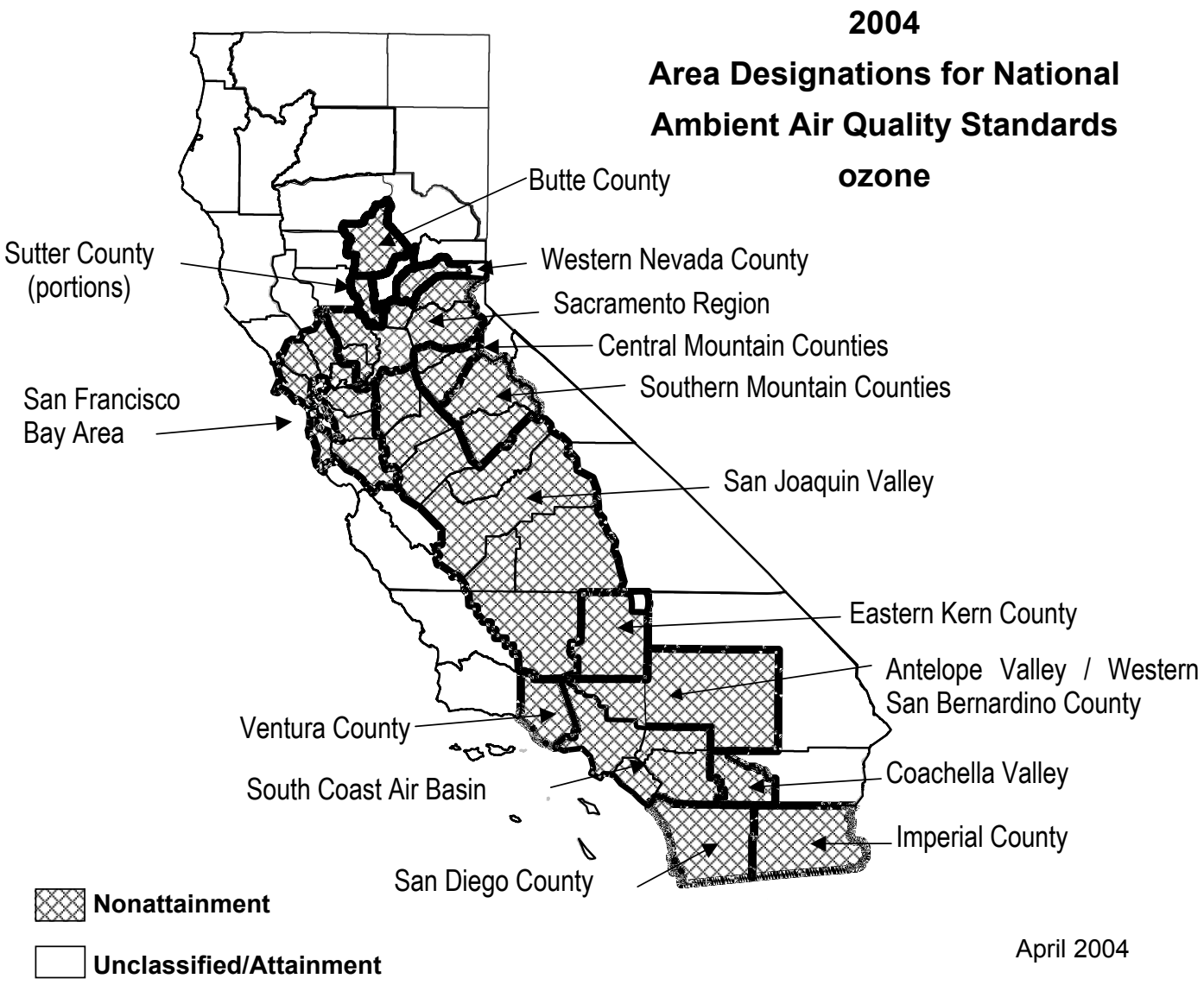
Figure 7-3 2004 Area Designations for the Federal 1-Hour Ozone Standard



Source Date:
October 2003
Emission Inventory Branch, PTSD

October 19, 2004

Figure 7-4 2004 Area Designations for the Federal 8-Hour Ozone Standard



7.2 Characterization of Ambient Ozone Air Quality

7.2.1 Overview

This section discusses air quality with reference to ambient ozone in each of California's air basins and/or planning areas, based on measured and statistically derived values. The information includes summary information about the magnitude and frequency of monitored concentrations for both the 1-hour and 8-hour averaging times. In addition, there is information about the seasonal and diurnal variations and a characterization of ozone concentrations in each area. These discussions include current ozone statistics, as well as historical trends.

Ozone is monitored continuously at approximately 175 sites in California. The data for each monitoring site are reported as 1-hour average concentrations. These 1-hour data can be aggregated into 8-hour average ozone concentrations, and can be summarized as daily, seasonal, or annual maximum 1-hour and 8-hour concentrations. In addition, these data are used in determining the number of days during which measured concentrations exceed the State and federal ozone standards.

For purposes of evaluating long-term ozone air quality trends and population exposures, the maximum concentration usually is not the best measure, because maximum concentrations can be highly influenced by year-to-year variations in meteorology. In contrast to the maximum values, two calculated statistics that provide more stable measures of long-term trends are the *peak indicator value*, or EPDC, and the *moving 3-year mean*. The peak indicator represents the maximum concentration expected to be exceeded once per year, on average. This indicator is based on a statistical calculation using three years of ambient monitoring data and is calculated for each monitoring site in an area. The highest peak indicator value among all sites in an area is generally used when evaluating area-wide air quality. A moving 3-year mean of the annual maximum measured concentrations also tends to be a more stable trend indicator, when compared to the measured maximum concentration. Although the moving 3-year mean is not as robust as the peak indicator, the 3-year mean does tend to dampen some of the year-to-year variation caused by meteorology. This yields data that are more suitable for trend analysis, when compared with data for individual years.

The statistics presented in the following sections represent an air basin, except for the two Sacramento Valley areas and the Mountain Counties area. The Sacramento Valley Air Basin (SVAB) is divided between the Sacramento Metropolitan (Metro) Area and the Upper Sacramento Valley to be consistent with the areas used for federal area designations and air quality planning activities. The Sacramento Metro Area includes the southern urbanized portion of the Sacramento Valley Air Basin (Sacramento, SVAB portion of Solano, and Yolo counties), the southern one-third of Sutter County, the SVAB portion of Placer County, and the portions of El Dorado and Placer counties that lie within the Mountain Counties Air Basin. The remaining portion of the Sacramento Valley Air Basin is included in the Upper Sacramento Valley. Because the Mountain Counties Air Basin portions of El Dorado and Placer counties are included in the Sacramento Metro Area, sites in these areas are not included in the Mountain Counties summary statistics included in this Report.

Data presented in this subsection are evaluated using the maximum measured concentrations, as well as the peak indicator and the number of days the State and federal standards were exceeded. In most cases, the data used reflect data for record extracted from the ARB ADAM (Aerometric Data Analysis and Measurement) or USEPA AIRS (Aerometric Information Retrieval System) databases.

7.2.2 Ambient 1-Hour Ozone Concentrations

Table 7-1 shows the maximum measured 1-hour ozone concentration, the number of days with measured 1-hour concentrations exceeding the State 1-hour standard of 0.09 ppm, and the number of days with measured 1-hour concentrations exceeding the federal 1-hour standard of 0.12 ppm. The table includes statistics for the years 2001 through 2003 and were extracted from the ARB ADAM database during March 2004. It is important to note that the counts of exceedance days reflect area-wide totals. In other words, each day with an exceedance is counted as one day, regardless of the number of individual sites, with concentrations exceeding the standard.

In contrast to Table 7-1, Table 7-2 lists several calculated ozone statistics for each air basin or planning area. The calculated statistics include the 3-year mean of the number of days over the State standard, the peak 1-hour indicator or EPDC, and the 3-year mean of the number of days over the federal 1-hour standard. In addition, Table 7-2 lists the highest 4th high 1-hour ozone concentration, which is an indicator of compliance with the federal 1-hour standard. This statistic represents the maximum of the 4th highest 1-hour ozone concentration measured at any site in each air basin or planning area during the three-year period. Furthermore, the maximum 4th high reflects the federal 1-hour design value, as long as it meets data completeness requirements.

During 2001 through 2003, neither the State nor federal 1-hour standard was exceeded in the Lake County Air Basin, North Coast Air Basin, or Northeast Plateau Air Basin. Data for four additional areas, Great Basin Valleys Air Basin, Lake Tahoe Air Basin, North Central Coast Air Basin, and the Upper Sacramento Valley show exceedances of the State standard, but not the federal 1-hour standard (as described earlier, representative data for the Northeast Plateau Air Basin and Great Basin Valleys Air Basin are available for 2002 and 2003 only). Both the State and federal 1-hour standards were exceeded during at least two of the three years in all other areas.

The highest number of exceedance days for both the State and federal 1-hour standards occurred in the San Joaquin Valley Air Basin and the South Coast Air Basin. Both areas had more than 115 State standard exceedance days and 31 or more federal standard exceedance days during each of the three years. The Sacramento Metro Area, Mojave Desert Air Basin, and Salton Sea Air Basin all averaged more than 50 State standard exceedance days and averaged 6 or more federal standard exceedance days during 2001 through 2003. The remaining five areas (Mountain Counties Air Basin, San Diego Air Basin, San Francisco Bay Area Air Basin, South Central Coast Air Basin, and the Upper Sacramento Valley) averaged from 12 to 45 State standard exceedance days. The Upper Sacramento Valley area had no exceedances of the federal standard

while the Mountain Counties Air Basin, San Diego Air Basin, San Francisco Bay Area Air Basin, and South Central Coast Air Basin each averaged 1 to 2 federal standard exceedance days for the three-year period.

The range of the measured maximum 1-hour concentrations tends to follow a similar pattern. The South Coast Air Basin showed the highest values, with measured concentrations of 0.169 ppm or higher during all three years. The next highest 1-hour ozone concentrations occurred in the Salton Sea Air Basin and San Joaquin Valley Air Basin, which had concentrations of 0.149 ppm or higher during 2001 through 2003. The Sacramento Metro Area, Mojave Desert Air Basin, and San Francisco Bay Area Air Basin had maximum measured concentrations ranging from 0.128 ppm to 0.163 ppm during each of the three years. Maximum 1-hour concentrations in the Mountain Counties Air Basin, San Diego Air Basin, and South Central Coast Air Basin had maximum 1-hour concentrations of 0.120 ppm or higher during each year. Three areas (North Central Coast Air Basin, Upper Sacramento Valley, and Lake Tahoe Air Basin) had maximum 1-hour concentrations above 0.100 ppm during at least two of the three years. The remaining areas (Great Basin Valleys Air Basin, Lake County Air Basin, North Coast Air Basin, and Northeast Plateau Air Basin) had maximum 1-hour ozone concentrations of 0.100 ppm or less during each of the years.

The values for the peak 1-hour indicator and the highest 4th high 1-hour ozone concentration, shown in Table 7-2, reflect similar patterns. The highest values are found in the South Coast and San Joaquin Valley air basins, followed by the Sacramento Metro Area, Salton Sea Air Basin, and Mojave Desert Air Basin. The highest 4th high 1-hour ozone concentration, used as an indicator of compliance with the federal 1-hour standard, is above the level of the standard in each of these five areas.

Table 7-1 Measured Ozone Statistics for 2001-2003 for California Air Basins or Planning Areas

Basin	Year	Maximum 1-Hour Concentration	Days Exceeding State 1-Hour Std	Days Exceeding Federal 1-Hour Std
Great Basin Valleys	2001	Representative data not available		
	2002	0.100	8	0
	2003	0.089	0	0
Lake County	2001	0.070	0	0
	2002	0.090	0	0
	2003	0.070	0	0
Lake Tahoe	2001	0.095	1	0
	2002	0.102	1	0
	2003	0.112	3	0
Mojave Desert	2001	0.146	72	6
	2002	0.157	75	16
	2003	0.163	93	13
Mountain Counties[1]	2001	0.120	29	0
	2002	0.132	41	2
	2003	0.135	45	2
North Central Coast	2001	0.108	3	0
	2002	0.115	8	0
	2003	0.111	3	0
North Coast	2001	0.090	0	0
	2002	0.092	0	0
	2003	0.090	0	0
Northeast Plateau	2001	Representative data not available		
	2002	0.087	0	0
	2003	0.089	0	0
Sacramento Metro Area[2]	2001	0.148	52	3
	2002	0.156	59	10
	2003	0.145	53	6

San Diego	2001	0.141	29	2
	2002	0.121	15	0
	2003	0.125	23	1
San Francisco Bay Area	2001	0.134	15	1
	2002	0.160	16	2
	2003	0.128	19	1
San Joaquin Valley	2001	0.149	123	32
	2002	0.164	127	31
	2003	0.156	137	37
Salton Sea	2001	0.167	81	15
	2002	0.156	68	5
	2003	0.187	69	9
South Central Coast	2001	0.129	34	2
	2002	0.132	24	1
	2003	0.130	45	2
South Coast	2001	0.190	121	36
	2002	0.169	116	45
	2003	0.194	125	64
Upper Sacramento Valley	2001	0.104	12	0
	2002	0.117	17	0
	2003	0.117	19	0

Data Source: ADAM – 03/08/04 and 03/16/04

[1] Mountain Counties Air Basin excludes Cool-Highway 193, Placerville-Gold Nugget Way, and Colfax sites.

[2] Sacramento Metro Area includes the following sites: Cool-Highway 193, Placerville-Gold Nugget Way, and Colfax from Mountain Counties Air Basin and Auburn-Dewitt-C Avenue, Davis-UCD Campus, Elk Grove-Bruceville Road, Folsom-City Corporation Yard, Folsom-Natoma Street, North Highlands-Blackfoot Way, Pleasant Grove-4 miles SW, Rocklin-Rocklin Road, Rocklin-Sierra College, Roseville-N Sunrise Blvd, Sacramento-3801 Airport Road, Sacramento-Del Paso Manor, Sacramento-T Street, Sloughhouse, Vacaville-Elmira Road, Woodland-Gibson Road, and Woodland-Sutter Street from Sacramento Valley Air Basin.

Notes: Days exceeding State and federal 1-hour standards are distinct areawide days, meaning the exceedance day is counted only once, even if multiple sites experienced an exceedance on the same day. The State ozone standard is exceeded when the concentration is equal to or greater than 0.095 ppm. The federal 1-hour ozone standard is exceeded when the concentration is equal to or greater than 0.125 ppm.

Table 7-2 Calculated Ozone Statistics for 2001-2003 for California Air Basins or Planning Areas

Basin	3-Year Mean # Days over State 1-Hr Std	Peak 1-Hour Indicator	3-Year Mean # Days over Federal 1-Hr Std	Highest 4th High 1-Hr Ozone Conc
Great Basin Valleys	Representative data not available			
Lake County	0	0.082	0	0.080
Lake Tahoe	2	0.103	0	0.094
Mojave Desert	80	0.138	12	0.138
Mountain Counties	38	0.117	1	0.117
North Central Coast	5	0.105	0	0.106
North Coast	0	0.082	0	0.083
Northeast Plateau	Representative data not available			
Sacramento Metro Area	55	0.146	6	0.143
Salton Sea	73	0.135	10	0.142
San Diego	22	0.117	1	0.118
San Francisco Bay Area	17	0.130	1	0.123
San Joaquin Valley	129	0.152	33	0.151
South Central Coast	34	0.124	2	0.124
South Coast	121	0.178	48	0.180
Upper Sacramento Valley	16	0.121	0	0.113

Note:
The peak 1-hour indicator and highest 4th high 1-hour ozone concentration reflect data from the high site in the air basin or planning area.

7.2.3 Ambient 8-Hour Ozone Concentrations

The federal ozone standard, promulgated by the USEPA in 1997, is 0.08 ppm for 8 hours. As discussed earlier, ozone is measured continuously, and running 8-hour averages are computed from hourly ozone concentrations. Each of the 8-hour averages is assigned to the first hour of the 8-hour period. For example, an 8-hour average calculated from data collected during the 8-hour period starting at 12 p.m. is assigned to 12 p.m. With complete data, there are twenty-four 8-hour average concentrations calculated for each day. The highest of these daily 8-hour averages is identified as the maximum 8-hour concentration for the day (USEPA 1998).

The federal 8-hour ozone standard is not to be exceeded, based on the fourth highest concentration each year, averaged over three years. In other words, take the fourth highest concentration recorded at a site during each of three years, average these three values together, and then compare the average value to the standard. This comparison is made for each site in an area, and if the value for any site exceeds the standard, the area is nonattainment.

Table 7-3 shows both measured and calculated ozone statistics related to an 8-hour averaging time. The statistics include the maximum 8-hour concentration, the peak 8-hour indicator or EPDC value, the fourth highest 8-hour concentration, the 3-year mean of the fourth highest 8-hour concentration, the number of days on which the federal 8-hour standard was exceeded, and the 3-year mean of the number of days on which the federal 8-hour standard was exceeded. These six statistics are given for each air basin or planning area, for each of the years 2001, 2002, and 2003. These statistics were extracted from the ARB ADAM database during March 2004.

As mentioned earlier, the form of the federal 8-hour standard used for determining attainment is the average of the fourth highest daily concentration during each year of a three-year period. However, this analysis focuses on the peak 8-hour indicator, the maximum 8-hour concentrations, and the number of days on which the maximum concentration exceeded the level of the federal standard. These statistics are used to assess ambient concentrations.

As with the 1-hour statistics, the highest 8-hour values are again found in the South Coast Air Basin and San Joaquin Valley Air Basin. Maximum 8-hour concentrations in the South Coast Air Basin ranged from 0.144 ppm to 0.153 ppm during 2001 through 2003, while maximum 8-hour concentrations in the San Joaquin Valley ranged from 0.120 ppm to 0.132 ppm during the same three-year period. Three other areas, the Mojave Desert Air Basin, the Sacramento Metro Area, and the Salton Sea Air Basin also had a maximum 8-hour concentration above 0.120 ppm during at least one of the three years.

With respect to the federal 8-hour ozone standard, Lake County Air Basin and North Coast Air Basin showed no exceedance days during 2001 through 2003. One area, the Lake Tahoe Air Basin, averaged only one exceedance day for the three-year period, while the North Central Coast Air Basin averaged three 8-hour exceedance days. In contrast, the San Joaquin Valley Air Basin showed the highest average number of exceedance days (123), followed by the South Coast Air Basin (99). The Sacramento Metro Area, Mojave Desert Air Basin, Mountain

Table 7-3 Measured and Calculated Ozone Statistics for Ambient 8-Hour Concentrations 2001 through 2003

Basin	Year	Maximum 8-Hour Conc	Peak 8-Hour Indicator	4th High 8-Hour Conc	3-Year Mean of 4th High Conc	# of Days Fed Std Exceeded	3-Year Mean of # of Days Fed Std Exceeded
Great Basin Valleys	2001	Representative data not available					
	2002	0.088	0.090	0.084	0.081	3	
	2003	0.084	0.084	0.080	0.081	0	
Lake County	2001	0.065	0.071	0.060	0.063	0	
	2002	0.077	0.073	0.072	0.064	0	
	2003	0.061	0.074	0.058	0.063	0	0
Lake Tahoe	2001	0.084	0.079	0.076	0.075	0	
	2002	0.079	0.080	0.077	0.075	0	
	2003	0.103	0.093	0.084	0.084	3	1
Mojave Desert	2001	0.117	0.114	0.106	0.102	65	
	2002	0.123	0.116	0.118	0.106	66	
	2003	0.130	0.118	0.119	0.106	74	68
Mountain Counties	2001	0.106	0.103	0.095	0.097	29	
	2002	0.113	0.106	0.099	0.098	47	42
	2003	0.103	0.107	0.101	0.098	49	
North Central Coast	2001	0.088	0.085	0.079	0.079	2	
	2002	0.094	0.088	0.086	0.081	5	
	2003	0.088	0.089	0.081	0.081	2	3
North Coast	2001	0.073	0.078	0.065	0.069	0	
	2002	0.072	0.069	0.067	0.063	0	
	2003	0.080	0.068	0.062	0.062	0	0
Northeast Plateau	2001	Representative data not available					
	2002	0.075	0.071	0.066	0.055	0	
	2003	0.074	0.073	0.068	0.057	0	
Sacramento Metro Area	2001	0.109	0.113	0.105	0.104	41	
	2002	0.137	0.119	0.111	0.106	47	
	2003	0.122	0.122	0.106	0.107	43	44

Salton Sea	2001	0.113	0.110	0.111	0.100	54	
	2002	0.124	0.118	0.109	0.105	55	
	2003	0.110	0.119	0.105	0.108	47	52
San Diego	2001	0.116	0.101	0.098	0.094	17	
	2002	0.100	0.103	0.096	0.095	13	
	2003	0.103	0.101	0.089	0.093	6	12
San Francisco Bay Area	2001	0.102	0.094	0.094	0.082	7	
	2002	0.106	0.096	0.096	0.082	7	
	2003	0.101	0.098	0.092	0.086	7	7
San Joaquin Valley	2001	0.120	0.119	0.115	0.109	109	
	2002	0.132	0.120	0.125	0.115	125	
	2003	0.127	0.122	0.119	0.115	134	123
South Central Coast	2001	0.113	0.107	0.103	0.101	25	
	2002	0.109	0.106	0.100	0.097	16	
	2003	0.114	0.104	0.100	0.095	35	25
South Coast	2001	0.144	0.144	0.138	0.129	92	
	2002	0.144	0.144	0.138	0.128	96	
	2003	0.153	0.146	0.146	0.131	109	99
Upper Sacramento Valley	2001	0.089	0.102	0.088	0.087	8	
	2002	0.103	0.100	0.099	0.089	16	
	2003	0.099	0.104	0.094	0.089	19	14

Notes: Days exceeding federal 8-hour standard are distinct areawide days, meaning the exceedance day is counted only once, even if multiple sites experienced an exceedance on the same day. The federal 8-hour ozone standard is exceeded when the three-year average of the 4th highest 8-hour concentrations is equal to or greater than 0.085 ppm.

Data Source: ADAM - 3/10/04 and 03/16/04

Counties Air Basin, and Salton Sea Air Basin each averaged between 42 and 68 exceedance days during 2001 through 2003. The remaining four areas averaged between 7 and 25 federal 8-hour exceedance days during the three-year period.

7.2.4 Available 2004 Ozone Data

Although ozone concentrations are monitored continuously at air quality monitoring sites, there is a delay between the time the concentrations are measured and the time they have been quality assured and approved for final use. Because 2003 is the last year for which complete, quality assured data are available, this is the last year used in the trends analyses in this chapter. However, preliminary 2004 data are available for some sites. Table 7-4 lists the available 1-hour and 8-hour ozone statistics for 2004 (note that although the 2004 data for these areas are complete, they are still preliminary and therefore, subject to further review and possible revision). The statistics include the maximum 1-hour concentration, the number of days exceeding the State and the federal 1-hour standards, the maximum 8-hour concentration, and the number of days exceeding the federal 8-hour standard. Statistics are listed for six urban areas in California. These statistics represent the data available in the ARB's AQMIS 2 (Air Quality and Meteorological Information System) database on January 28, 2005.

For all six areas, the values for 2004 are comparable or lower than those for 2003. This is because meteorological conditions during 2003 in many areas of the State were adverse – meaning the meteorological conditions were conducive to ozone formation. Therefore, the ozone concentrations in many areas during 2003 were higher than normal. Overall, during 2004, all six areas had values above one or more of the State and federal standards. Again, the highest values occurred in the South Coast and San Joaquin Valley Air Basins.

Table 7-4 Preliminary 2004 measured Ozone Statistics for Six Urban Areas

Area	Maximum 1-Hour Conc	Days Exceeding State 1-Hour Standard	Days Exceeding Federal 1-Hour Standard	Maximum 8-Hour Conc	Days Exceeding Federal 8-Hour Standard
Sacramento Metro Area	0.12	27	0	0.10	19
San Diego Air Basin	0.13	9	1	0.10	7
San Francisco Bay Area	0.11	7	0	0.08	0
San Joaquin Valley	0.16	103	9	0.13	109
South Coast	0.16	111	27	0.13	87
Ventura County	0.12	22	0	0.10	17

Note: Days exceeding State and federal standards are distinct, areawide days. data are complete, but still preliminary and therefore, subject to further review.

Data Source: AQMIS – 1/28/05

7.2.5 Ozone Season

Ozone is not directly emitted as a pollutant, but is formed in the atmosphere when precursor emissions, namely VOCs and oxides of nitrogen, react in the presence of sunlight. Because of the reaction time involved, the highest ozone concentrations often occur far downwind of the precursor emissions. As a result, ozone is a regional pollutant that often impacts large areas.

Meteorology and terrain play major roles in ozone formation. Generally, low wind speeds or stagnant air, coupled with warm temperatures and cloudless skies, provide the optimum conditions for ozone formation. Therefore, the highest ozone concentrations tend to occur during the summer season. In most urban areas, the ozone season for the current standards runs from May through September. However, the data show that the San Joaquin Valley Air Basin and South Coast Air Basin can record high ozone levels throughout the year. In general, the length or duration of the ozone season reflects the stringency of the standard. In other words, a more stringent standard will have a longer ozone season.

Figures 7.5 and 7.6 and Tables 7.5 and 7.6 show the total number of days each month with exceedances of the State 1-hour and federal 8-hour ozone standards, respectively, during 1990 through 2003. The graphs and tables include information for each of California's largest air basins or planning areas. Although the highest ozone concentrations for both standards tend to occur during the summertime, meteorological conditions can vary from year-to-year and can be a significant factor in the year-to-year variation in ambient ozone. In general, inland areas (such as the San Joaquin Valley, Sacramento Valley, and inland portions of the South Coast Air Basin) tend to experience hotter temperatures and more stagnant conditions than coastal areas. This combination of meteorological conditions can lead to multi-day episodes of elevated ozone concentrations.

To highlight the similarity in the distributions for both the 1-hour and 8-hour standards, Figures 7.5 and 7.6 both are plotted with the same x-axis scale. The trend lines are almost identical for both standards. However, in all areas, the total number of exceedance days for the federal 8-hour standard is the same or lower than the total number of exceedance days for the State 1-hour standard.

Figure 7-4 Seasonal Distribution of the Number of Days Exceeding the State 1-Hour Ozone Standard

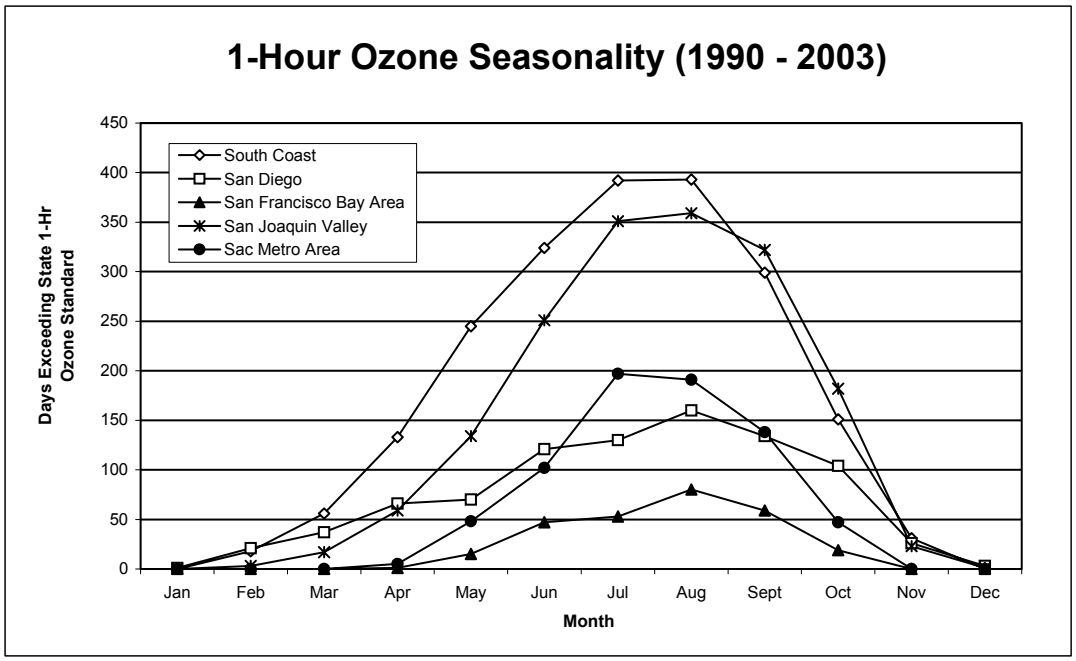


Table 7-5 Total Number of Days with Exceedances of the State 1-Hour Ozone Standard During 1990 to 2003

MONTH	AIR BASIN				
	Sacramento Metropolitan Area	San Diego	San Francisco Bay Area	San Joaquin Valley	South Coast
January	0	1	0	0	0
February	0	21	0	3	18
March	0	37	0	17	56
April	5	66	1	59	133
May	48	70	15	134	245
Jun	102	121	46	251	324
July	196	130	53	351	392
August	191	160	80	359	393
September	138	134	59	322	299
October	47	104	19	182	151
November	0	26	0	23	31
December	0	3	0	1	0

Note: The seasonality is represented by the monthly count of exceedance days that were measured at one or more monitoring sites in an air basin or planning area for the years1990 to 2003.
Data Source: ADAM - 3/18/04

Figure 7-5 Seasonal Distribution of the Number of Days Exceeding the Federal 8-Hour Ozone Standard

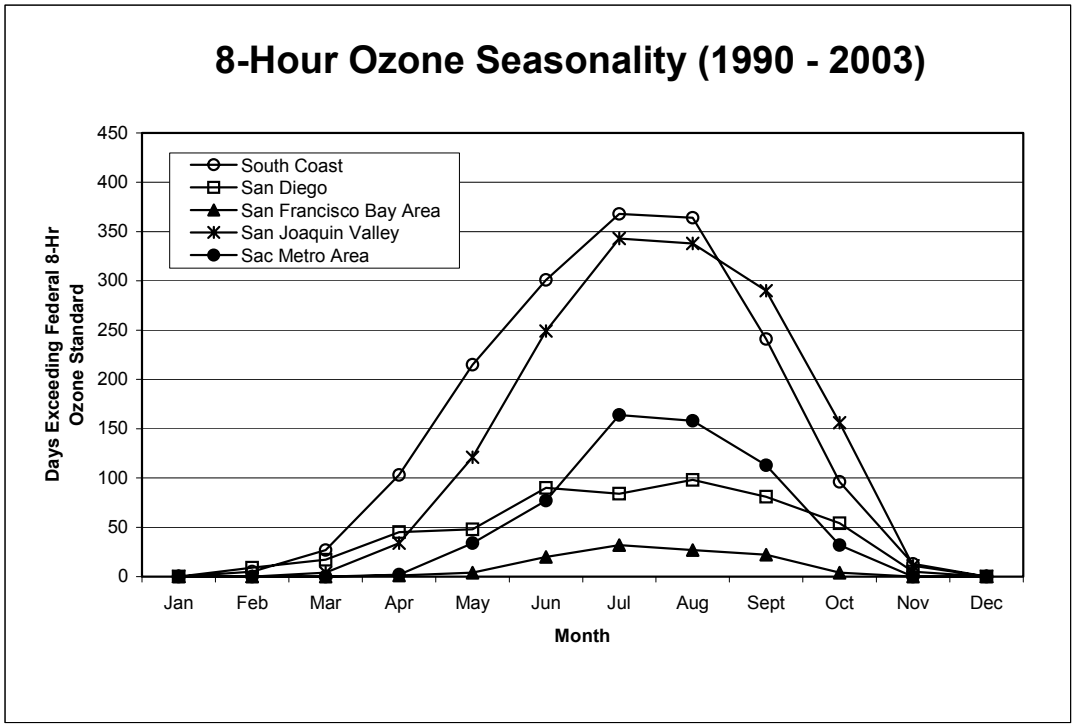


Table 7-6 Total Number of Days with Exceedances of the Federal 8-Hour Ozone Standard During 1990 to 2003

MONTH	AIR BASIN				
	Sacramento Metropolitan Area	San Diego	San Francisco Bay Area	San Joaquin Valley	South Coast
January	0	0	0	0	0
February	0	9	0	0	5
March	0	17	0	4	27
April	2	45	1	34	103
May	34	48	4	121	215
June	77	90	20	249	301
July	164	84	32	343	368
August	158	98	27	338	364
September	113	81	22	290	241
October	32	54	4	156	96
November	0	5	0	11	13
December	0	0	0	0	0

Note: The seasonality is represented by the monthly count of exceedance days that were measured at one or more monitoring sites in an air basin or planning area for the years 1990 to 2003.

Data Source: ADAM - 3/19/04

7.2.6 Frequency of Measured 1-Hour and 8-Hour Ozone Concentrations

The following graphs and tables present frequency distributions of both 1-hour and 8-hour ozone concentrations for each of the years 2001, 2002, and 2003. The frequency distributions represent a summary of the maximum daily ozone concentrations measured at all the sites within each air basin or planning area, as well as for all sites within the State. Maximum ozone concentration levels are aggregated into bins or concentration ranges of 0.01 ppm in size (for example, daily maximum concentrations in the range of 0.00 ppm to 0.01 ppm, 0.01 ppm to 0.02 ppm, 0.02 ppm to 0.03 ppm, etc.). The original input data were taken from the ARB air quality CD (ARB 2004).

It is interesting to note that in many cases, there are a higher number (frequency) of 8-hour observations than 1-hour observations. For example, during 2003, there were a total of 60,138 1-hour observations for California, compared with 60,145 8-hour observations. The higher number of 8-hour observations is a function of how the 8-hour average is calculated. Because the 8-hour average can span hours in two separate days, it is possible to have a valid 8-hour value, but not a valid 1-hour value. As a result, the total number of 8-hour observations can be higher.

Figures 7.7 through 7.12 show frequency information on a statewide basis. In contrast, Tables 7.7 through 7.12 present information for each area of California, as well as for the State as a whole. Each figure and table provides information for an individual year: 2001, 2002, or 2003. The tabular information includes the frequency of the various ranges of maximum daily 1-hour and 8-hour concentrations measured in each area, expressed both as a count and as a percentage of the total. In addition, the tables show the cumulative frequency (again, expressed as a count and as a percentage), from the lowest range to the highest range. The frequency graphs and tables provide information on the frequency of high concentrations for each area, as well as the most frequent, or predominant concentrations levels. This information provides insight about the impact of setting the standards at various levels.

As shown in Figures 7.7 through 7.9, the majority of maximum daily 1-hour ozone concentrations during 2001 to 2003 were below the level of both the State and federal 1-hour standards. Statewide, an average of 44.6 percent of the daily maximums were in the range of 0.03 ppm to 0.05 ppm. During all three years, an average of 97.1 percent of the daily maximum concentrations were at or below 0.10 ppm, the level of the State 1-hour standard. As shown in Figures 7.10 through 7.12, an average of 45.8 percent of the daily maximum 8-hour concentrations were in the range of 0.03 ppm to 0.05 ppm, during the three-year period. An average of 95.2 percent of the maximum concentrations were at or below 0.08 ppm, the level of the federal 8-hour standard.

When looking at the data for individual air basins or planning areas, the results are similar. We do, however, see some variation in the ranges of concentrations represented. For example, the maximum concentrations represented in the South Coast Air Basin range from 0.00 ppm to 0.20 ppm for the 1-hour concentrations and 0.00 ppm to 0.16 ppm for the 8-hour concentrations. In contrast, the concentrations represented in the North Coast Air Basin range from

0.00 ppm to 0.10 ppm for the 1-hour values and 0.00 ppm to 0.08 ppm for the 8-hour values. However, all individual air basins and planning areas show relatively large percentages of concentrations in the 0.03 ppm to 0.05 ppm range, and all areas had at least 37 percent of their maximum 1-hour and 8-hour concentrations at or below 0.05 ppm during 2001 through 2003. Furthermore, more than 50 percent of the 1-hour and 8-hour maximums were at or below 0.06 ppm for all areas during all three years.

Figure 7-6

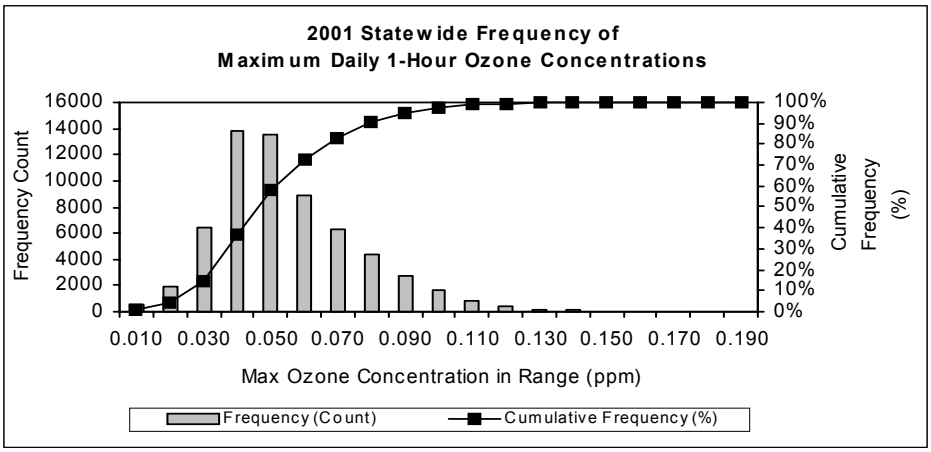


Figure 7-7

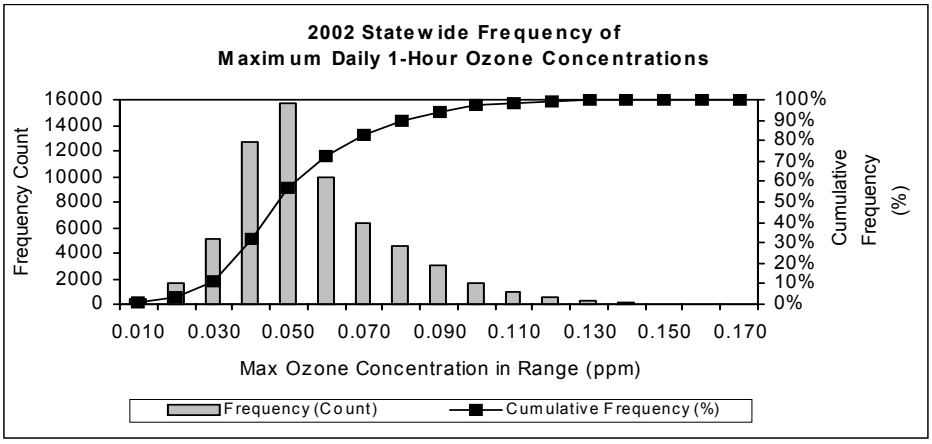


Figure 7-8

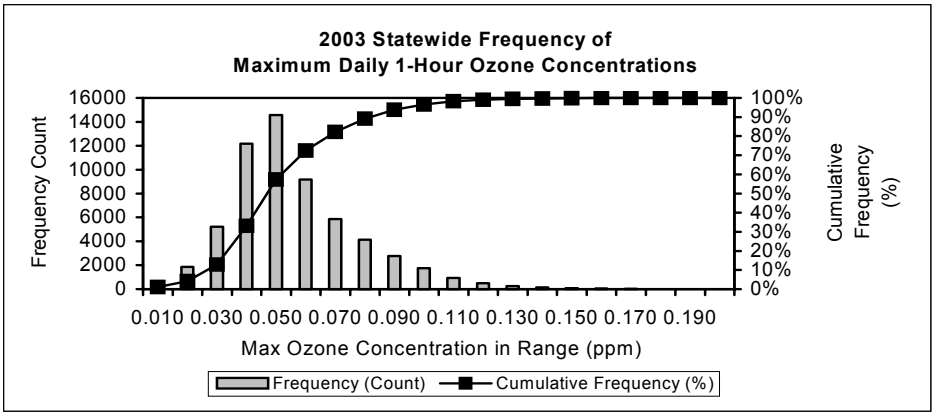


Figure 7-9

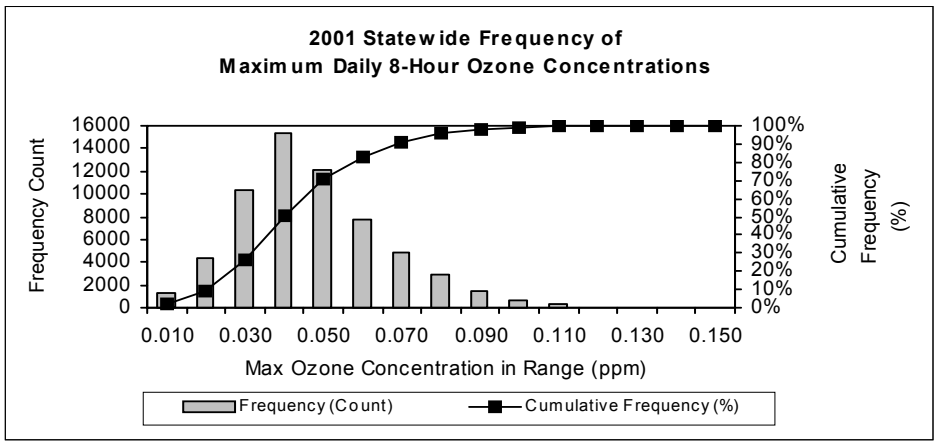


Figure 7-10

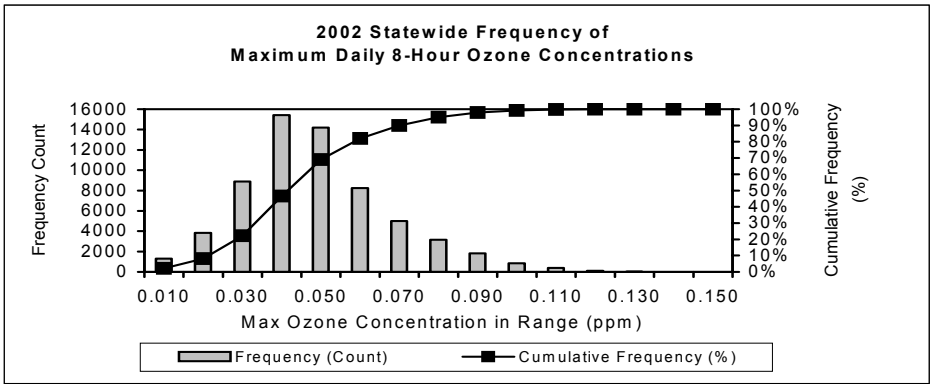


Figure 7-11

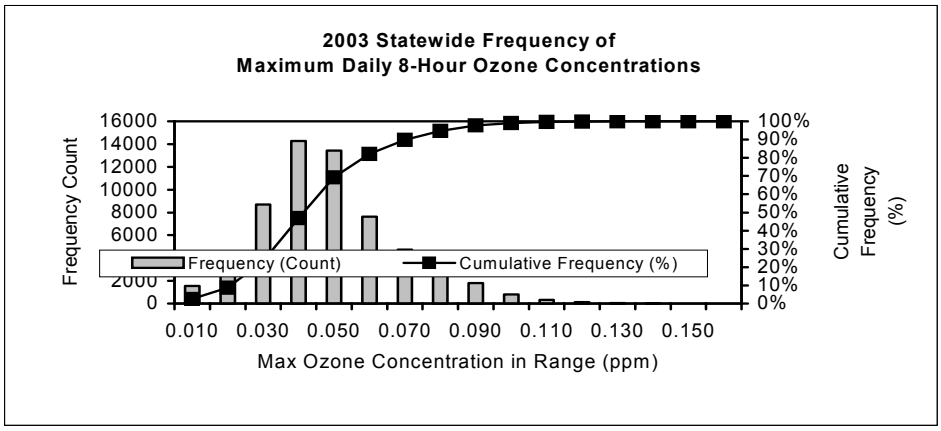


Table 7-7 Frequency of 1-Hour Ozone Concentrations Measured During 2001

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	Representative data not available					
Lake County	0.01	0.02	6	1.6%	6	1.6%
	0.02	0.03	56	15.3%	62	17.0%
	0.03	0.04	138	37.8%	200	54.8%
	0.04	0.05	111	30.4%	311	85.2%
	0.05	0.06	39	10.7%	350	95.9%
	0.06	0.07	15	4.1%	365	100.0%
Lake Tahoe	0.02	0.03	4	0.6%	4	0.6%
	0.03	0.04	69	9.8%	73	10.3%
	0.04	0.05	220	31.2%	293	41.5%
	0.05	0.06	215	30.5%	508	72.0%
	0.06	0.07	124	17.6%	632	89.5%
	0.07	0.08	55	7.8%	687	97.3%
	0.08	0.09	18	2.5%	705	99.9%
	0.09	0.10	1	0.1%	706	100.0%
Mojave Desert	0.00	0.01	3	0.1%	3	0.1%
	0.01	0.02	21	0.7%	24	0.7%
	0.02	0.03	110	3.4%	134	4.2%
	0.03	0.04	585	18.2%	719	22.3%
	0.04	0.05	630	19.6%	1349	41.9%
	0.05	0.06	526	16.3%	1875	58.3%
	0.06	0.07	471	14.6%	2346	72.9%
	0.07	0.08	384	11.9%	2730	84.8%
	0.08	0.09	245	7.6%	2975	92.4%
	0.09	0.10	129	4.0%	3104	96.5%
	0.10	0.11	73	2.3%	3177	98.7%
	0.11	0.12	28	0.9%	3205	99.6%
	0.12	0.13	10	0.3%	3215	99.9%
	0.13	0.14	1	0.0%	3216	99.9%
	0.14	0.15	2	0.1%	3218	100.0%

Table 7-7 (continued)

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	6	0.2%	6	0.2%
	0.01	0.02	19	0.6%	25	0.8%
	0.02	0.03	181	5.9%	206	6.7%
	0.03	0.04	458	15.0%	664	21.7%
	0.04	0.05	657	21.5%	1321	43.2%
	0.05	0.06	561	18.3%	1882	61.5%
	0.06	0.07	517	16.9%	2399	78.4%
	0.07	0.08	396	12.9%	2795	91.3%
	0.08	0.09	188	6.1%	2983	97.5%
	0.09	0.10	47	1.5%	3030	99.0%
	0.10	0.11	24	0.8%	3054	99.8%
	0.11	0.12	7	0.2%	3061	100.0%
North Central Coast	0.00	0.01	3	0.1%	3	0.1%
	0.01	0.02	36	1.0%	39	1.1%
	0.02	0.03	507	14.0%	546	15.1%
	0.03	0.04	1525	42.2%	2071	57.2%
	0.04	0.05	988	27.3%	3059	84.5%
	0.05	0.06	337	9.3%	3396	93.9%
	0.06	0.07	130	3.6%	3526	97.5%
	0.07	0.08	63	1.7%	3589	99.2%
	0.08	0.09	22	0.6%	3611	99.8%
	0.09	0.10	6	0.2%	3617	100.0%
	0.10	0.11	1	0.0%	3618	100.0%
North Coast	0.00	0.01	3	0.3%	3	0.3%
	0.01	0.02	74	6.9%	77	7.2%
	0.02	0.03	266	24.7%	343	31.9%
	0.03	0.04	413	38.4%	756	70.3%
	0.04	0.05	230	21.4%	986	91.7%
	0.05	0.06	65	6.0%	1051	97.8%
	0.06	0.07	20	1.9%	1071	99.6%
	0.07	0.08	3	0.3%	1074	99.9%
	0.08	0.09	1	0.1%	1075	100.0%
Northeast Plateau	Representative data not available					

Table 7-7 (continued)

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Sacra-mento Metro Area	0.00	0.01	55	1.0%	55	1.0%
	0.01	0.02	150	2.8%	205	3.8%
	0.02	0.03	533	9.8%	738	13.6%
	0.03	0.04	1103	20.3%	1841	33.9%
	0.04	0.05	1021	18.8%	2862	52.7%
	0.05	0.06	887	16.3%	3749	69.1%
	0.06	0.07	663	12.2%	4412	81.3%
	0.07	0.08	439	8.1%	4851	89.4%
	0.08	0.09	292	5.4%	5143	94.8%
	0.09	0.10	160	2.9%	5303	97.7%
	0.10	0.11	85	1.6%	5388	99.3%
	0.11	0.12	29	0.5%	5417	99.8%
	0.12	0.13	6	0.1%	5423	99.9%
	0.13	0.14	2	0.0%	5425	100.0%
	0.14	0.15	2	0.0%	5427	100.0%
Salton Sea	0.00	0.01	16	0.7%	16	0.7%
	0.01	0.02	22	1.0%	38	1.7%
	0.02	0.03	89	3.9%	127	5.5%
	0.03	0.04	335	14.6%	462	20.1%
	0.04	0.05	460	20.0%	922	40.1%
	0.05	0.06	467	20.3%	1389	60.4%
	0.06	0.07	355	15.4%	1744	75.9%
	0.07	0.08	228	9.9%	1972	85.8%
	0.08	0.09	138	6.0%	2110	91.8%
	0.09	0.10	98	4.3%	2208	96.0%
	0.10	0.11	47	2.0%	2255	98.1%
	0.11	0.12	18	0.8%	2273	98.9%
	0.12	0.13	15	0.7%	2288	99.5%
	0.13	0.14	7	0.3%	2295	99.8%
	0.14	0.15	3	0.1%	2298	100.0%
	0.16	0.17	1	0.0%	2299	100.0%

Table 7-7 (continued)

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0.00	0.01	4	0.1%	4	0.1%
	0.01	0.02	54	1.5%	58	1.6%
	0.02	0.03	221	6.0%	279	7.6%
	0.03	0.04	767	20.9%	1046	28.5%
	0.04	0.05	1161	31.6%	2207	60.1%
	0.05	0.06	739	20.1%	2946	80.2%
	0.06	0.07	385	10.5%	3331	90.7%
	0.07	0.08	196	5.3%	3527	96.1%
	0.08	0.09	79	2.2%	3606	98.2%
	0.09	0.10	40	1.1%	3646	99.3%
	0.10	0.11	16	0.4%	3662	99.7%
	0.11	0.12	6	0.2%	3668	99.9%
	0.12	0.13	1	0.0%	3669	99.9%
	0.13	0.14	2	0.1%	3671	100.0%
	0.14	0.15	1	0.0%	3672	100.0%
San Francisco Bay Area	0.00	0.01	90	1.2%	90	1.2%
	0.01	0.02	522	7.2%	612	8.5%
	0.02	0.03	1829	25.3%	2441	33.8%
	0.03	0.04	2327	32.2%	4768	66.1%
	0.04	0.05	1398	19.4%	6166	85.4%
	0.05	0.06	517	7.2%	6683	92.6%
	0.06	0.07	231	3.2%	6914	95.8%
	0.07	0.08	154	2.1%	7068	97.9%
	0.08	0.09	85	1.2%	7153	99.1%
	0.09	0.10	38	0.5%	7191	99.6%
	0.10	0.11	15	0.2%	7206	99.8%
	0.11	0.12	9	0.1%	7215	100.0%
	0.12	0.13	2	0.0%	7217	100.0%
	0.13	0.14	1	0.0%	7218	100.0%
San Joaquin Valley	0.00	0.01	35	0.4%	35	0.4%
	0.01	0.02	219	2.6%	254	3.0%
	0.02	0.03	557	6.5%	811	9.5%
	0.03	0.04	1189	14.0%	2000	23.5%
	0.04	0.05	1243	14.6%	3243	38.1%
	0.05	0.06	1080	12.7%	4323	50.8%
	0.06	0.07	1108	13.0%	5431	63.8%
	0.07	0.08	1035	12.2%	6466	76.0%
	0.08	0.09	861	10.1%	7327	86.1%
	0.09	0.10	610	7.2%	7937	93.3%
	0.10	0.11	337	4.0%	8274	97.3%
	0.11	0.12	146	1.7%	8420	99.0%
	0.12	0.13	56	0.7%	8476	99.6%
	0.13	0.14	26	0.3%	8502	100.0%
	0.14	0.15	4	0.0%	8506	100.0%

Table 7-7 (continued)

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Central Coast	0.00	0.01	14	0.2%	14	0.2%
	0.01	0.02	47	0.5%	61	0.7%
	0.02	0.03	527	5.8%	588	6.5%
	0.03	0.04	2442	26.9%	3030	33.4%
	0.04	0.05	2914	32.1%	5944	65.5%
	0.05	0.06	1585	17.5%	7529	83.0%
	0.06	0.07	789	8.7%	8318	91.7%
	0.07	0.08	429	4.7%	8747	96.4%
	0.08	0.09	190	2.1%	8937	98.5%
	0.09	0.10	83	0.9%	9020	99.4%
	0.10	0.11	31	0.3%	9051	99.8%
	0.11	0.12	16	0.2%	9067	100.0%
	0.12	0.13	4	0.0%	9071	100.0%
South Coast	0.00	0.01	197	2.0%	197	2.0%
	0.01	0.02	543	5.5%	740	7.5%
	0.02	0.03	1130	11.4%	1870	18.9%
	0.03	0.04	1825	18.5%	3695	37.4%
	0.04	0.05	1779	18.0%	5474	55.5%
	0.05	0.06	1298	13.2%	6772	68.6%
	0.06	0.07	957	9.7%	7729	78.3%
	0.07	0.08	659	6.7%	8388	85.0%
	0.08	0.09	518	5.2%	8906	90.2%
	0.09	0.10	371	3.8%	9277	94.0%
	0.10	0.11	212	2.1%	9489	96.1%
	0.11	0.12	149	1.5%	9638	97.6%
	0.12	0.13	98	1.0%	9736	98.6%
	0.13	0.14	63	0.6%	9799	99.3%
	0.14	0.15	32	0.3%	9831	99.6%
	0.15	0.16	25	0.3%	9856	99.9%
	0.16	0.17	8	0.1%	9864	99.9%
	0.17	0.18	2	0.0%	9866	100.0%
	0.18	0.19	4	0.0%	9870	100.0%

Table 7-7 (continued)

Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Upper Sacra-mento Valley	0.00	0.01	54	1.6%	54	1.6%
	0.01	0.02	179	5.2%	233	6.8%
	0.02	0.03	365	10.6%	598	17.4%
	0.03	0.04	597	17.3%	1195	34.7%
	0.04	0.05	689	20.0%	1884	54.7%
	0.05	0.06	610	17.7%	2494	72.4%
	0.06	0.07	478	13.9%	2972	86.3%
	0.07	0.08	304	8.8%	3276	95.1%
	0.08	0.09	135	3.9%	3411	99.0%
	0.09	0.10	29	0.8%	3440	99.9%
	0.10	0.11	4	0.1%	3444	100.0%
California	0.00	0.01	480	0.8%	480	0.8%
	0.01	0.02	1892	3.1%	2372	3.9%
	0.02	0.03	6375	10.4%	8747	14.2%
	0.03	0.04	13773	22.4%	22520	36.6%
	0.04	0.05	13501	21.9%	36021	58.5%
	0.05	0.06	8926	14.5%	44947	73.0%
	0.06	0.07	6243	10.1%	51190	83.2%
	0.07	0.08	4345	7.1%	55535	90.2%
	0.08	0.09	2772	4.5%	58307	94.7%
	0.09	0.10	1612	2.6%	59919	97.4%
	0.10	0.11	845	1.4%	60764	98.7%
	0.11	0.12	408	0.7%	61172	99.4%
	0.12	0.13	192	0.3%	61364	99.7%
	0.13	0.14	102	0.2%	61466	99.9%
	0.14	0.15	44	0.1%	61510	99.9%
	0.15	0.16	25	0.0%	61535	100.0%
	0.16	0.17	9	0.0%	61544	100.0%
	0.17	0.18	2	0.0%	61546	100.0%
	0.18	0.19	4	0.0%	61550	100.0%

Table 7-8 Frequency of 1-Hour Ozone Concentrations Measured During 2002

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	0.02	0.03	7	1.4%	7	1.4%
	0.03	0.04	59	12.0%	66	13.4%
	0.04	0.05	154	31.3%	220	44.7%
	0.05	0.06	101	20.5%	321	65.2%
	0.06	0.07	90	18.3%	411	83.5%
	0.07	0.08	43	8.7%	454	92.3%
	0.08	0.09	21	4.3%	475	96.5%
	0.09	0.10	17	3.5%	492	100.0%
Lake County	0.01	0.02	8	2.2%	8	2.2%
	0.02	0.03	45	12.5%	53	14.7%
	0.03	0.04	133	36.8%	186	51.5%
	0.04	0.05	79	21.9%	265	73.4%
	0.05	0.06	53	14.7%	318	88.1%
	0.06	0.07	33	9.1%	351	97.2%
	0.07	0.08	9	2.5%	360	99.7%
	0.08	0.09	1	0.3%	361	100.0%
Lake Tahoe	0.02	0.03	8	1.1%	8	1.1%
	0.03	0.04	72	10.0%	80	11.1%
	0.04	0.05	245	34.0%	325	45.1%
	0.05	0.06	219	30.4%	544	75.5%
	0.06	0.07	111	15.4%	655	90.8%
	0.07	0.08	50	6.9%	705	97.8%
	0.08	0.09	15	2.1%	720	99.9%
	0.10	0.11	1	0.1%	721	100.0%
Mojave Desert	0.00	0.01	2	0.1%	2	0.1%
	0.01	0.02	12	0.4%	14	0.4%
	0.02	0.03	84	2.6%	98	3.0%
	0.03	0.04	396	12.1%	494	15.2%
	0.04	0.05	774	23.7%	1268	38.9%
	0.05	0.06	533	16.3%	1801	55.2%
	0.06	0.07	438	13.4%	2239	68.7%
	0.07	0.08	405	12.4%	2644	81.1%
	0.08	0.09	294	9.0%	2938	90.1%
	0.09	0.10	162	5.0%	3100	95.1%
	0.10	0.11	86	2.6%	3186	97.7%
	0.11	0.12	39	1.2%	3225	98.9%
	0.12	0.13	26	0.8%	3251	99.7%
	0.13	0.14	5	0.2%	3256	99.9%
	0.14	0.15	3	0.1%	3259	100.0%
	0.15	0.16	1	0.0%	3260	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	7	0.2%	7	0.2%
	0.01	0.02	38	1.2%	45	1.4%
	0.02	0.03	136	4.3%	181	5.8%
	0.03	0.04	392	12.5%	573	18.2%
	0.04	0.05	596	19.0%	1169	37.2%
	0.05	0.06	524	16.7%	1693	53.9%
	0.06	0.07	491	15.6%	2184	69.5%
	0.07	0.08	504	16.0%	2688	85.5%
	0.08	0.09	290	9.2%	2978	94.8%
	0.09	0.10	120	3.8%	3098	98.6%
	0.10	0.11	35	1.1%	3133	99.7%
	0.11	0.12	7	0.2%	3140	99.9%
	0.12	0.13	1	0.0%	3141	99.9%
	0.13	0.14	2	0.1%	3143	100.0%
North Central Coast	0.00	0.01	4	0.1%	4	0.1%
	0.01	0.02	48	1.3%	52	1.4%
	0.02	0.03	412	11.3%	464	12.7%
	0.03	0.04	1377	37.8%	1841	50.5%
	0.04	0.05	1181	32.4%	3022	83.0%
	0.05	0.06	349	9.6%	3371	92.5%
	0.06	0.07	160	4.4%	3531	96.9%
	0.07	0.08	68	1.9%	3599	98.8%
	0.08	0.09	29	0.8%	3628	99.6%
	0.09	0.10	11	0.3%	3639	99.9%
	0.10	0.11	3	0.1%	3642	100.0%
	0.11	0.12	1	0.0%	3643	100.0%
North Coast	0.00	0.01	15	1.4%	15	1.4%
	0.01	0.02	46	4.2%	61	5.6%
	0.02	0.03	203	18.5%	264	24.1%
	0.03	0.04	444	40.5%	708	64.7%
	0.04	0.05	245	22.4%	953	87.0%
	0.05	0.06	95	8.7%	1048	95.7%
	0.06	0.07	35	3.2%	1083	98.9%
	0.07	0.08	7	0.6%	1090	99.5%
	0.08	0.09	2	0.2%	1092	99.7%
	0.09	0.10	3	0.3%	1095	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Northeast Plateau	0.00	0.01	6	1.7%	6	1.7%
	0.01	0.02	25	7.2%	31	8.9%
	0.02	0.03	56	16.1%	87	25.0%
	0.03	0.04	70	20.1%	157	45.1%
	0.04	0.05	73	21.0%	230	66.1%
	0.05	0.06	64	18.4%	294	84.5%
	0.06	0.07	40	11.5%	334	96.0%
	0.07	0.08	13	3.7%	347	99.7%
	0.08	0.09	1	0.3%	348	100.0%
Sacra-mento Metro Area	0.00	0.01	53	1.0%	53	1.0%
	0.01	0.02	152	2.9%	205	3.9%
	0.02	0.03	403	7.8%	608	11.7%
	0.03	0.04	931	17.9%	1539	29.6%
	0.04	0.05	1004	19.3%	2543	49.0%
	0.05	0.06	833	16.0%	3376	65.0%
	0.06	0.07	645	12.4%	4021	77.4%
	0.07	0.08	488	9.4%	4509	86.8%
	0.08	0.09	341	6.6%	4850	93.4%
	0.09	0.10	163	3.1%	5013	96.6%
	0.10	0.11	104	2.0%	5117	98.6%
	0.11	0.12	46	0.9%	5163	99.4%
	0.12	0.13	15	0.3%	5178	99.7%
	0.13	0.14	9	0.2%	5187	99.9%
	0.14	0.15	4	0.1%	5191	100.0%
	0.15	0.16	1	0.0%	5192	100.0%
Salton Sea	0.01	0.02	15	0.5%	15	0.5%
	0.02	0.03	130	4.5%	145	5.0%
	0.03	0.04	420	14.6%	565	19.7%
	0.04	0.05	634	22.1%	1199	41.7%
	0.05	0.06	573	20.0%	1772	61.7%
	0.06	0.07	464	16.2%	2236	77.9%
	0.07	0.08	296	10.3%	2532	88.2%
	0.08	0.09	178	6.2%	2710	94.4%
	0.09	0.10	80	2.8%	2790	97.1%
	0.10	0.11	47	1.6%	2837	98.8%
	0.11	0.12	23	0.8%	2860	99.6%
	0.12	0.13	7	0.2%	2867	99.8%
	0.13	0.14	4	0.1%	2871	100.0%
	0.15	0.16	1	0.0%	2872	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0.00	0.01	2	0.1%	2	0.1%
	0.01	0.02	24	0.7%	26	0.8%
	0.02	0.03	159	4.9%	185	5.7%
	0.03	0.04	557	17.0%	742	22.7%
	0.04	0.05	1108	33.9%	1850	56.5%
	0.05	0.06	813	24.8%	2663	81.4%
	0.06	0.07	361	11.0%	3024	92.4%
	0.07	0.08	145	4.4%	3169	96.8%
	0.08	0.09	64	2.0%	3233	98.8%
	0.09	0.10	29	0.9%	3262	99.7%
	0.10	0.11	5	0.2%	3267	99.8%
	0.11	0.12	5	0.2%	3272	100.0%
	0.12	0.13	1	0.0%	3273	100.0%
San Francisco Bay Area	0.00	0.01	128	1.7%	128	1.7%
	0.01	0.02	447	5.9%	575	7.6%
	0.02	0.03	1323	17.6%	1898	25.2%
	0.03	0.04	2518	33.5%	4416	58.7%
	0.04	0.05	1832	24.4%	6248	83.1%
	0.05	0.06	651	8.7%	6899	91.7%
	0.06	0.07	301	4.0%	7200	95.7%
	0.07	0.08	156	2.1%	7356	97.8%
	0.08	0.09	100	1.3%	7456	99.1%
	0.09	0.10	36	0.5%	7492	99.6%
	0.10	0.11	15	0.2%	7507	99.8%
	0.11	0.12	10	0.1%	7517	99.9%
	0.12	0.13	2	0.0%	7519	100.0%
	0.13	0.14	1	0.0%	7520	100.0%
	0.15	0.16	1	0.0%	7521	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Joaquin Valley	0.00	0.01	83	1.0%	83	1.0%
	0.01	0.02	274	3.2%	357	4.1%
	0.02	0.03	570	6.6%	927	10.7%
	0.03	0.04	1096	12.7%	2023	23.4%
	0.04	0.05	1448	16.8%	3471	40.2%
	0.05	0.06	1074	12.4%	4545	52.6%
	0.06	0.07	907	10.5%	5452	63.1%
	0.07	0.08	1002	11.6%	6454	74.7%
	0.08	0.09	848	9.8%	7302	84.5%
	0.09	0.10	656	7.6%	7958	92.1%
	0.10	0.11	359	4.2%	8317	96.2%
	0.11	0.12	180	2.1%	8497	98.3%
	0.12	0.13	95	1.1%	8592	99.4%
	0.13	0.14	31	0.4%	8623	99.8%
	0.14	0.15	13	0.2%	8636	99.9%
	0.15	0.16	5	0.1%	8641	100.0%
	0.16	0.17	1	0.0%	8642	100.0%
South Central Coast	0.00	0.01	8	0.1%	8	0.1%
	0.01	0.02	53	0.6%	61	0.7%
	0.02	0.03	392	4.3%	453	4.9%
	0.03	0.04	1977	21.4%	2430	26.4%
	0.04	0.05	3308	35.9%	5738	62.3%
	0.05	0.06	2023	21.9%	7761	84.2%
	0.06	0.07	845	9.2%	8606	93.4%
	0.07	0.08	374	4.1%	8980	97.4%
	0.08	0.09	154	1.7%	9134	99.1%
	0.09	0.10	52	0.6%	9186	99.7%
	0.10	0.11	22	0.2%	9208	99.9%
	0.11	0.12	6	0.1%	9214	100.0%
	0.12	0.13	2	0.0%	9216	100.0%
	0.13	0.14	1	0.0%	9217	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Coast	0.00	0.01	79	0.8%	79	0.8%
	0.01	0.02	381	3.9%	460	4.7%
	0.02	0.03	854	8.8%	1314	13.5%
	0.03	0.04	1633	16.8%	2947	30.3%
	0.04	0.05	2215	22.7%	5162	53.0%
	0.05	0.06	1470	15.1%	6632	68.1%
	0.06	0.07	950	9.8%	7582	77.9%
	0.07	0.08	722	7.4%	8304	85.3%
	0.08	0.09	494	5.1%	8798	90.3%
	0.09	0.10	336	3.5%	9134	93.8%
	0.10	0.11	226	2.3%	9360	96.1%
	0.11	0.12	164	1.7%	9524	97.8%
	0.12	0.13	104	1.1%	9628	98.9%
	0.13	0.14	71	0.7%	9699	99.6%
	0.14	0.15	21	0.2%	9720	99.8%
	0.15	0.16	15	0.2%	9735	100.0%
	0.16	0.17	4	0.0%	9739	100.0%
Upper Sacra-mento Valley	0.00	0.01	69	1.9%	69	1.9%
	0.01	0.02	184	5.1%	253	7.0%
	0.02	0.03	284	7.8%	537	14.8%
	0.03	0.04	629	17.3%	1166	32.1%
	0.04	0.05	846	23.3%	2012	55.5%
	0.05	0.06	605	16.7%	2617	72.2%
	0.06	0.07	475	13.1%	3092	85.2%
	0.07	0.08	324	8.9%	3416	94.2%
	0.08	0.09	143	3.9%	3559	98.1%
	0.09	0.10	43	1.2%	3602	99.3%
	0.10	0.11	21	0.6%	3623	99.9%
	0.11	0.12	4	0.1%	3627	100.0%

Table 7-8 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
California	0.00	0.01	456	0.7%	456	0.7%
	0.01	0.02	1707	2.7%	2163	3.4%
	0.02	0.03	5066	8.0%	7229	11.4%
	0.03	0.04	12704	20.1%	19933	31.6%
	0.04	0.05	15742	24.9%	35675	56.5%
	0.05	0.06	9980	15.8%	45655	72.3%
	0.06	0.07	6346	10.0%	52001	82.4%
	0.07	0.08	4606	7.3%	56607	89.6%
	0.08	0.09	2975	4.7%	59582	94.4%
	0.09	0.10	1708	2.7%	61290	97.1%
	0.10	0.11	924	1.5%	62214	98.5%
	0.11	0.12	485	0.8%	62699	99.3%
	0.12	0.13	253	0.4%	62952	99.7%
	0.13	0.14	124	0.2%	63076	99.9%
	0.14	0.15	41	0.1%	63117	100.0%
	0.15	0.16	24	0.0%	63141	100.0%
	0.16	0.17	5	0.0%	63146	100.0%

Table 7-9 Frequency of 1-Hour Ozone Concentrations Measured During 2003

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	0.02	0.03	3	0.8%	3	0.8%
	0.03	0.04	64	17.7%	67	18.6%
	0.04	0.05	76	21.1%	143	39.6%
	0.05	0.06	103	28.5%	246	68.1%
	0.06	0.07	61	16.9%	307	85.0%
	0.07	0.08	42	11.6%	349	96.7%
	0.08	0.09	12	3.3%	361	100.0%
Lake County	0.00	0.01	1	0.3%	1	0.3%
	0.01	0.02	14	3.8%	15	4.1%
	0.02	0.03	53	14.6%	68	18.7%
	0.03	0.04	129	35.4%	197	54.1%
	0.04	0.05	109	29.9%	306	84.1%
	0.05	0.06	43	11.8%	349	95.9%
	0.06	0.07	14	3.8%	363	99.7%
	0.07	0.08	1	0.3%	364	100.0%
Lake Tahoe	0.02	0.03	8	0.6%	8	0.6%
	0.03	0.04	93	7.2%	101	7.8%
	0.04	0.05	406	31.5%	507	39.4%
	0.05	0.06	415	32.2%	922	71.6%
	0.06	0.07	253	19.7%	1175	91.3%
	0.07	0.08	77	6.0%	1252	97.3%
	0.08	0.09	25	1.9%	1277	99.2%
	0.09	0.10	9	0.7%	1286	99.9%
	0.11	0.12	1	0.1%	1287	100.0%
Mojave Desert	0.00	0.01	1	0.0%	1	0.0%
	0.01	0.02	18	0.5%	19	0.5%
	0.02	0.03	138	4.0%	157	4.5%
	0.03	0.04	525	15.1%	682	19.6%
	0.04	0.05	820	23.6%	1502	43.2%
	0.05	0.06	562	16.2%	2064	59.4%
	0.06	0.07	446	12.8%	2510	72.3%
	0.07	0.08	378	10.9%	2888	83.1%
	0.08	0.09	264	7.6%	3152	90.7%
	0.09	0.10	173	5.0%	3325	95.7%
	0.10	0.11	90	2.6%	3415	98.3%
	0.11	0.12	32	0.9%	3447	99.2%
	0.12	0.13	17	0.5%	3464	99.7%
	0.13	0.14	7	0.2%	3471	99.9%
	0.14	0.15	1	0.0%	3472	99.9%
	0.15	0.16	1	0.0%	3473	100.0%
	0.16	0.17	1	0.0%	3474	100.0%

Table 7-9 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	20	0.7%	20	0.7%
	0.01	0.02	78	2.7%	98	3.4%
	0.02	0.03	188	6.5%	286	10.0%
	0.03	0.04	337	11.7%	623	21.7%
	0.04	0.05	670	23.3%	1293	45.0%
	0.05	0.06	470	16.4%	1763	61.4%
	0.06	0.07	393	13.7%	2156	75.0%
	0.07	0.08	365	12.7%	2521	87.7%
	0.08	0.09	209	7.3%	2730	95.0%
	0.09	0.10	89	3.1%	2819	98.1%
	0.10	0.11	38	1.3%	2857	99.4%
	0.11	0.12	14	0.5%	2871	99.9%
	0.12	0.13	1	0.0%	2872	100.0%
	0.13	0.14	1	0.0%	2873	100.0%
North Central Coast	0.00	0.01	2	0.1%	2	0.1%
	0.01	0.02	53	1.5%	55	1.5%
	0.02	0.03	428	11.8%	483	13.3%
	0.03	0.04	1309	36.0%	1792	49.2%
	0.04	0.05	1162	31.9%	2954	81.2%
	0.05	0.06	397	10.9%	3351	92.1%
	0.06	0.07	166	4.6%	3517	96.6%
	0.07	0.08	82	2.3%	3599	98.9%
	0.08	0.09	32	0.9%	3631	99.8%
	0.09	0.10	6	0.2%	3637	99.9%
	0.10	0.11	1	0.0%	3638	100.0%
	0.11	0.12	1	0.0%	3639	100.0%
North Coast	0.00	0.01	72	6.6%	72	6.6%
	0.01	0.02	85	7.8%	157	14.4%
	0.02	0.03	207	18.9%	364	33.3%
	0.03	0.04	351	32.1%	715	65.4%
	0.04	0.05	265	24.2%	980	89.6%
	0.05	0.06	75	6.9%	1055	96.4%
	0.06	0.07	34	3.1%	1089	99.5%
	0.07	0.08	3	0.3%	1092	99.8%
	0.08	0.09	2	0.2%	1094	100.0%

Table 7-9 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Northeast Plateau	0.00	0.01	2	0.8%	2	0.8%
	0.01	0.02	9	3.4%	11	4.2%
	0.02	0.03	21	8.0%	32	12.2%
	0.03	0.04	38	14.5%	70	26.7%
	0.04	0.05	66	25.2%	136	51.9%
	0.05	0.06	67	25.6%	203	77.5%
	0.06	0.07	45	17.2%	248	94.7%
	0.07	0.08	12	4.6%	260	99.2%
	0.08	0.09	2	0.8%	262	100.0%
Sacra-mento Metro Area	0.00	0.01	91	2.1%	91	2.1%
	0.01	0.02	222	5.1%	313	7.3%
	0.02	0.03	383	8.9%	696	16.1%
	0.03	0.04	727	16.8%	1423	33.0%
	0.04	0.05	802	18.6%	2225	51.6%
	0.05	0.06	632	14.6%	2857	66.2%
	0.06	0.07	557	12.9%	3414	79.1%
	0.07	0.08	391	9.1%	3805	88.2%
	0.08	0.09	248	5.7%	4053	93.9%
	0.09	0.10	154	3.6%	4207	97.5%
	0.10	0.11	56	1.3%	4263	98.8%
	0.11	0.12	30	0.7%	4293	99.5%
	0.12	0.13	10	0.2%	4303	99.7%
	0.13	0.14	11	0.3%	4314	100.0%
	0.14	0.15	1	0.0%	4315	100.0%
Salton Sea	0.00	0.01	7	0.2%	7	0.2%
	0.01	0.02	70	2.5%	77	2.7%
	0.02	0.03	261	9.3%	338	12.0%
	0.03	0.04	460	16.4%	798	28.4%
	0.04	0.05	667	23.8%	1465	52.2%
	0.05	0.06	530	18.9%	1995	71.1%
	0.06	0.07	353	12.6%	2348	83.7%
	0.07	0.08	203	7.2%	2551	90.9%
	0.08	0.09	116	4.1%	2667	95.0%
	0.09	0.10	64	2.3%	2731	97.3%
	0.10	0.11	36	1.3%	2767	98.6%
	0.11	0.12	27	1.0%	2794	99.6%
	0.12	0.13	9	0.3%	2803	99.9%
	0.13	0.14	1	0.0%	2804	99.9%
	0.14	0.15	2	0.1%	2806	100.0%

Table 7-9 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0.00	0.01	10	0.3%	10	0.3%
	0.01	0.02	66	2.0%	76	2.3%
	0.02	0.03	276	8.5%	352	10.9%
	0.03	0.04	733	22.6%	1085	33.5%
	0.04	0.05	977	30.1%	2062	63.6%
	0.05	0.06	575	17.7%	2637	81.4%
	0.06	0.07	325	10.0%	2962	91.4%
	0.07	0.08	164	5.1%	3126	96.5%
	0.08	0.09	65	2.0%	3191	98.5%
	0.09	0.10	39	1.2%	3230	99.7%
	0.10	0.11	9	0.3%	3239	99.9%
	0.11	0.12	1	0.0%	3240	100.0%
	0.12	0.13	1	0.0%	3241	100.0%
San Francisco Bay Area	0.00	0.01	157	2.3%	157	2.3%
	0.01	0.02	399	5.8%	556	8.0%
	0.02	0.03	1194	17.2%	1750	25.3%
	0.03	0.04	2047	29.5%	3797	54.8%
	0.04	0.05	1748	25.2%	5545	80.0%
	0.05	0.06	715	10.3%	6260	90.3%
	0.06	0.07	331	4.8%	6591	95.1%
	0.07	0.08	166	2.4%	6757	97.5%
	0.08	0.09	88	1.3%	6845	98.8%
	0.09	0.10	51	0.7%	6896	99.5%
	0.10	0.11	22	0.3%	6918	99.8%
	0.11	0.12	8	0.1%	6926	100.0%
	0.12	0.13	3	0.0%	6929	100.0%
San Joaquin Valley	0.00	0.01	154	1.8%	154	1.8%
	0.01	0.02	384	4.6%	538	6.4%
	0.02	0.03	558	6.6%	1096	13.0%
	0.03	0.04	1116	13.3%	2212	26.3%
	0.04	0.05	1197	14.2%	3409	40.6%
	0.05	0.06	1105	13.1%	4514	53.7%
	0.06	0.07	855	10.2%	5369	63.9%
	0.07	0.08	906	10.8%	6275	74.7%
	0.08	0.09	833	9.9%	7108	84.6%
	0.09	0.10	662	7.9%	7770	92.5%
	0.10	0.11	362	4.3%	8132	96.8%
	0.11	0.12	168	2.0%	8300	98.8%
	0.12	0.13	77	0.9%	8377	99.7%
	0.13	0.14	20	0.2%	8397	99.9%
	0.14	0.15	4	0.0%	8401	100.0%
	0.15	0.16	3	0.0%	8404	100.0%

Table 7-9 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Central Coast	0.00	0.01	3	0.0%	3	0.0%
	0.01	0.02	46	0.5%	49	0.5%
	0.02	0.03	547	6.1%	596	6.7%
	0.03	0.04	2265	25.3%	2861	31.9%
	0.04	0.05	3032	33.9%	5893	65.8%
	0.05	0.06	1578	17.6%	7471	83.4%
	0.06	0.07	691	7.7%	8162	91.1%
	0.07	0.08	423	4.7%	8585	95.9%
	0.08	0.09	214	2.4%	8799	98.3%
	0.09	0.10	107	1.2%	8906	99.5%
	0.10	0.11	37	0.4%	8943	99.9%
	0.11	0.12	10	0.1%	8953	100.0%
	0.12	0.13	2	0.0%	8955	100.0%
South Coast	0.00	0.01	59	0.7%	59	0.7%
	0.01	0.02	238	2.8%	297	3.5%
	0.02	0.03	592	6.9%	889	10.4%
	0.03	0.04	1283	14.9%	2172	25.3%
	0.04	0.05	1781	20.7%	3953	46.0%
	0.05	0.06	1319	15.4%	5272	61.4%
	0.06	0.07	876	10.2%	6148	71.6%
	0.07	0.08	676	7.9%	6824	79.5%
	0.08	0.09	520	6.1%	7344	85.5%
	0.09	0.10	369	4.3%	7713	89.8%
	0.10	0.11	291	3.4%	8004	93.2%
	0.11	0.12	209	2.4%	8213	95.7%
	0.12	0.13	139	1.6%	8352	97.3%
	0.13	0.14	106	1.2%	8458	98.5%
	0.14	0.15	57	0.7%	8515	99.2%
	0.15	0.16	43	0.5%	8558	99.7%
	0.16	0.17	19	0.2%	8577	99.9%
	0.17	0.18	6	0.1%	8583	100.0%
	0.18	0.19	1	0.0%	8584	100.0%
	0.19	0.20	1	0.0%	8585	100.0%

Table 7-9 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Upper Sacra-mento Valley	0.00	0.01	79	2.2%	79	2.2%
	0.01	0.02	161	4.5%	240	6.8%
	0.02	0.03	359	10.1%	599	16.9%
	0.03	0.04	698	19.7%	1297	36.5%
	0.04	0.05	780	22.0%	2077	58.5%
	0.05	0.06	582	16.4%	2659	74.9%
	0.06	0.07	453	12.8%	3112	87.7%
	0.07	0.08	253	7.1%	3365	94.8%
	0.08	0.09	135	3.8%	3500	98.6%
	0.09	0.10	36	1.0%	3536	99.6%
	0.10	0.11	10	0.3%	3546	99.9%
	0.11	0.12	3	0.1%	3549	100.0%
California	0.00	0.01	658	1.1%	658	1.1%
	0.01	0.02	1843	3.1%	2501	4.2%
	0.02	0.03	5216	8.7%	7717	12.8%
	0.03	0.04	12175	20.2%	19892	33.1%
	0.04	0.05	14558	24.2%	34450	57.3%
	0.05	0.06	9168	15.2%	43618	72.5%
	0.06	0.07	5853	9.7%	49471	82.3%
	0.07	0.08	4142	6.9%	53613	89.1%
	0.08	0.09	2765	4.6%	56378	93.7%
	0.09	0.10	1759	2.9%	58137	96.7%
	0.10	0.11	952	1.6%	59089	98.3%
	0.11	0.12	504	0.8%	59593	99.1%
	0.12	0.13	259	0.4%	59852	99.5%
	0.13	0.14	146	0.2%	59998	99.8%
	0.14	0.15	65	0.1%	60063	99.9%
	0.15	0.16	47	0.1%	60110	100.0%
	0.16	0.17	20	0.0%	60130	100.0%
	0.17	0.18	6	0.0%	60136	100.0%
	0.18	0.19	1	0.0%	60137	100.0%
	0.19	0.20	1	0.0%	60138	100.0%

Table 7-10 Frequency of 8-Hour Ozone Concentrations Measured During 2001

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	Representative data not available					
Lake County	0.01	0.02	12	3.3%	12	3.3%
	0.02	0.03	60	16.4%	72	19.7%
	0.03	0.04	145	39.7%	217	59.5%
	0.04	0.05	103	28.2%	320	87.7%
	0.05	0.06	42	11.5%	362	99.2%
	0.06	0.07	3	0.8%	365	100.0%
Lake Tahoe	0.01	0.02	4	0.6%	4	0.6%
	0.02	0.03	18	2.5%	22	3.1%
	0.03	0.04	115	16.3%	137	19.4%
	0.04	0.05	255	36.1%	392	55.5%
	0.05	0.06	197	27.9%	589	83.4%
	0.06	0.07	93	13.2%	682	96.6%
	0.07	0.08	23	3.3%	705	99.9%
	0.08	0.09	1	0.1%	706	100.0%
Mojave Desert	0.00	0.01	12	0.4%	12	0.4%
	0.01	0.02	69	2.1%	81	2.5%
	0.02	0.03	261	8.1%	342	10.6%
	0.03	0.04	717	22.3%	1059	32.9%
	0.04	0.05	582	18.1%	1641	51.0%
	0.05	0.06	606	18.8%	2247	69.8%
	0.06	0.07	466	14.5%	2713	84.3%
	0.07	0.08	303	9.4%	3016	93.7%
	0.08	0.09	139	4.3%	3155	98.0%
	0.09	0.10	50	1.6%	3205	99.5%
	0.10	0.11	13	0.4%	3218	99.9%
	0.11	0.12	2	0.1%	3220	100.0%

Table 7-10 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	8	0.3%	8	0.3%
	0.01	0.02	82	2.7%	90	2.9%
	0.02	0.03	311	10.2%	401	13.1%
	0.03	0.04	506	16.5%	907	29.6%
	0.04	0.05	701	22.9%	1608	52.5%
	0.05	0.06	591	19.3%	2199	71.9%
	0.06	0.07	488	15.9%	2687	87.8%
	0.07	0.08	273	8.9%	2960	96.7%
	0.08	0.09	79	2.6%	3039	99.3%
	0.09	0.10	20	0.7%	3059	100.0%
	0.10	0.11	1	0.0%	3060	100.0%
North Central Coast	0.00	0.01	15	0.4%	15	0.4%
	0.01	0.02	173	4.8%	188	5.2%
	0.02	0.03	1072	29.6%	1260	34.8%
	0.03	0.04	1430	39.5%	2690	74.4%
	0.04	0.05	645	17.8%	3335	92.2%
	0.05	0.06	189	5.2%	3524	97.5%
	0.06	0.07	64	1.8%	3588	99.2%
	0.07	0.08	26	0.7%	3614	99.9%
	0.08	0.09	2	0.1%	3616	100.0%
North Coast	0.00	0.01	15	1.4%	15	1.4%
	0.01	0.02	140	13.0%	155	14.4%
	0.02	0.03	333	31.0%	488	45.4%
	0.03	0.04	409	38.1%	897	83.5%
	0.04	0.05	146	13.6%	1043	97.1%
	0.05	0.06	25	2.3%	1068	99.4%
	0.06	0.07	5	0.5%	1073	99.9%
	0.07	0.08	1	0.1%	1074	100.0%
Northeast Plateau	Representative data not available					

Table 7-10 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Sacra-mento Metro Area	0.00	0.01	107	2.0%	107	2.0%
	0.01	0.02	390	7.2%	497	9.2%
	0.02	0.03	860	15.9%	1357	25.0%
	0.03	0.04	1243	22.9%	2600	48.0%
	0.04	0.05	1082	20.0%	3682	67.9%
	0.05	0.06	778	14.4%	4460	82.3%
	0.06	0.07	481	8.9%	4941	91.2%
	0.07	0.08	262	4.8%	5203	96.0%
	0.08	0.09	146	2.7%	5349	98.7%
	0.09	0.10	57	1.1%	5406	99.7%
	0.10	0.11	14	0.3%	5420	100.0%
Salton Sea	0.00	0.01	25	1.1%	25	1.1%
	0.01	0.02	77	3.3%	102	4.4%
	0.02	0.03	244	10.6%	346	15.0%
	0.03	0.04	502	21.8%	848	36.8%
	0.04	0.05	476	20.7%	1324	57.5%
	0.05	0.06	463	20.1%	1787	77.6%
	0.06	0.07	254	11.0%	2041	88.7%
	0.07	0.08	138	6.0%	2179	94.7%
	0.08	0.09	77	3.3%	2256	98.0%
	0.09	0.10	32	1.4%	2288	99.4%
	0.10	0.11	9	0.4%	2297	99.8%
	0.11	0.12	5	0.2%	2302	100.0%
San Diego	0.00	0.01	36	1.0%	36	1.0%
	0.01	0.02	170	4.6%	206	5.6%
	0.02	0.03	515	14.0%	721	19.6%
	0.03	0.04	1118	30.4%	1839	50.1%
	0.04	0.05	988	26.9%	2827	77.0%
	0.05	0.06	529	14.4%	3356	91.4%
	0.06	0.07	216	5.9%	3572	97.3%
	0.07	0.08	66	1.8%	3638	99.0%
	0.08	0.09	27	0.7%	3665	99.8%
	0.09	0.10	7	0.2%	3672	100.0%
	0.11	0.12	1	0.0%	3673	100.0%

Table 7-10 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Francisco Bay Area	0.00	0.01	272	3.8%	272	3.8%
	0.01	0.02	1218	16.9%	1490	20.6%
	0.02	0.03	2385	33.0%	3875	53.6%
	0.03	0.04	2014	27.9%	5889	81.5%
	0.04	0.05	861	11.9%	6750	93.5%
	0.05	0.06	285	3.9%	7035	97.4%
	0.06	0.07	118	1.6%	7153	99.0%
	0.07	0.08	49	0.7%	7202	99.7%
	0.08	0.09	15	0.2%	7217	99.9%
	0.09	0.10	5	0.1%	7222	100.0%
	0.10	0.11	1	0.0%	7223	100.0%
San Joaquin Valley	0.00	0.01	124	1.5%	124	1.5%
	0.01	0.02	510	6.0%	634	7.5%
	0.02	0.03	974	11.5%	1608	18.9%
	0.03	0.04	1276	15.0%	2884	33.9%
	0.04	0.05	1282	15.1%	4166	49.0%
	0.05	0.06	1141	13.4%	5307	62.4%
	0.06	0.07	1069	12.6%	6376	75.0%
	0.07	0.08	971	11.4%	7347	86.5%
	0.08	0.09	678	8.0%	8025	94.4%
	0.09	0.10	347	4.1%	8372	98.5%
	0.10	0.11	110	1.3%	8482	99.8%
	0.11	0.12	16	0.2%	8498	100.0%
South Central Coast	0.00	0.01	34	0.4%	34	0.4%
	0.01	0.02	190	2.1%	224	2.5%
	0.02	0.03	1207	13.3%	1431	15.8%
	0.03	0.04	3139	34.6%	4570	50.4%
	0.04	0.05	2540	28.0%	7110	78.5%
	0.05	0.06	1156	12.8%	8266	91.2%
	0.06	0.07	520	5.7%	8786	96.9%
	0.07	0.08	188	2.1%	8974	99.0%
	0.08	0.09	67	0.7%	9041	99.8%
	0.09	0.10	16	0.2%	9057	99.9%
	0.10	0.11	4	0.0%	9061	100.0%
	0.11	0.12	2	0.0%	9063	100.0%

Table 7-10 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Coast	0.00	0.01	557	5.6%	557	5.6%
	0.01	0.02	1064	10.8%	1621	16.4%
	0.02	0.03	1659	16.8%	3280	33.2%
	0.03	0.04	1958	19.8%	5238	53.0%
	0.04	0.05	1719	17.4%	6957	70.4%
	0.05	0.06	1136	11.5%	8093	81.9%
	0.06	0.07	715	7.2%	8808	89.1%
	0.07	0.08	437	4.4%	9245	93.6%
	0.08	0.09	266	2.7%	9511	96.3%
	0.09	0.10	173	1.8%	9684	98.0%
	0.10	0.11	108	1.1%	9792	99.1%
	0.11	0.12	46	0.5%	9838	99.6%
	0.12	0.13	25	0.3%	9863	99.8%
	0.13	0.14	15	0.2%	9878	100.0%
	0.14	0.15	3	0.0%	9881	100.0%
Upper Sacra-mento Valley	0.00	0.01	118	3.4%	118	3.4%
	0.01	0.02	309	9.0%	427	12.4%
	0.02	0.03	441	12.8%	868	25.2%
	0.03	0.04	744	21.6%	1612	46.8%
	0.04	0.05	701	20.3%	2313	67.1%
	0.05	0.06	588	17.1%	2901	84.2%
	0.06	0.07	362	10.5%	3263	94.7%
	0.07	0.08	155	4.5%	3418	99.2%
	0.08	0.09	29	0.8%	3447	100.0%
California	0.00	0.01	1323	2.1%	1323	2.1%
	0.01	0.02	4408	7.2%	5731	9.3%
	0.02	0.03	10340	16.8%	16071	26.1%
	0.03	0.04	15316	24.9%	31387	51.0%
	0.04	0.05	12081	19.6%	43468	70.6%
	0.05	0.06	7726	12.6%	51194	83.2%
	0.06	0.07	4854	7.9%	56048	91.1%
	0.07	0.08	2892	4.7%	58940	95.8%
	0.08	0.09	1526	2.5%	60466	98.2%
	0.09	0.10	707	1.1%	61173	99.4%
	0.10	0.11	260	0.4%	61433	99.8%
	0.11	0.12	72	0.1%	61505	99.9%
	0.12	0.13	25	0.0%	61530	100.0%
	0.13	0.14	15	0.0%	61545	100.0%
	0.14	0.15	3	0.0%	61548	100.0%

Table 7-11 Frequency of 8-Hour Ozone Concentrations Measured During 2002.

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	0.02	0.03	17	3.5%	17	3.5%
	0.03	0.04	91	18.5%	108	22.0%
	0.04	0.05	153	31.1%	261	53.0%
	0.05	0.06	113	23.0%	374	76.0%
	0.06	0.07	78	15.9%	452	91.9%
	0.07	0.08	32	6.5%	484	98.4%
	0.08	0.09	8	1.6%	492	100.0%
Lake County	0.01	0.02	13	3.6%	13	3.6%
	0.02	0.03	50	13.9%	63	17.5%
	0.03	0.04	131	36.4%	194	53.9%
	0.04	0.05	90	25.0%	284	78.9%
	0.05	0.06	51	14.2%	335	93.1%
	0.06	0.07	20	5.6%	355	98.6%
	0.07	0.08	5	1.4%	360	100.0%
Lake Tahoe	0.01	0.02	3	0.4%	3	0.4%
	0.02	0.03	31	4.3%	34	4.7%
	0.03	0.04	115	16.0%	149	20.7%
	0.04	0.05	269	37.3%	418	58.0%
	0.05	0.06	201	27.9%	619	85.9%
	0.06	0.07	78	10.8%	697	96.7%
	0.07	0.08	24	3.3%	721	100.0%
Mojave Desert	0.00	0.01	8	0.2%	8	0.2%
	0.01	0.02	72	2.2%	80	2.5%
	0.02	0.03	205	6.3%	285	8.7%
	0.03	0.04	545	16.7%	830	25.5%
	0.04	0.05	737	22.6%	1567	48.1%
	0.05	0.06	539	16.5%	2106	64.6%
	0.06	0.07	456	14.0%	2562	78.6%
	0.07	0.08	375	11.5%	2937	90.1%
	0.08	0.09	197	6.0%	3134	96.2%
	0.09	0.10	83	2.5%	3217	98.7%
	0.10	0.11	28	0.9%	3245	99.6%
	0.11	0.12	12	0.4%	3257	100.0%
	0.12	0.13	1	0.0%	3258	100.0%

Table 7-11 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	19	0.6%	19	0.6%
	0.01	0.02	88	2.8%	107	3.4%
	0.02	0.03	252	8.0%	359	11.4%
	0.03	0.04	468	14.9%	827	26.3%
	0.04	0.05	629	20.0%	1456	46.3%
	0.05	0.06	520	16.5%	1976	62.8%
	0.06	0.07	533	17.0%	2509	79.8%
	0.07	0.08	397	12.6%	2906	92.4%
	0.08	0.09	189	6.0%	3095	98.4%
	0.09	0.10	43	1.4%	3138	99.8%
	0.10	0.11	5	0.2%	3143	100.0%
	0.11	0.12	1	0.0%	3144	100.0%
North Central Coast	0.00	0.01	17	0.5%	17	0.5%
	0.01	0.02	170	4.7%	187	5.1%
	0.02	0.03	862	23.7%	1049	28.8%
	0.03	0.04	1509	41.4%	2558	70.2%
	0.04	0.05	768	21.1%	3326	91.3%
	0.05	0.06	188	5.2%	3514	96.5%
	0.06	0.07	86	2.4%	3600	98.8%
	0.07	0.08	31	0.9%	3631	99.7%
	0.08	0.09	11	0.3%	3642	100.0%
	0.09	0.10	1	0.0%	3643	100.0%
North Coast	0.00	0.01	25	2.3%	25	2.3%
	0.01	0.02	106	9.7%	131	12.0%
	0.02	0.03	300	27.4%	431	39.4%
	0.03	0.04	418	38.2%	849	77.5%
	0.04	0.05	176	16.1%	1025	93.6%
	0.05	0.06	58	5.3%	1083	98.9%
	0.06	0.07	10	0.9%	1093	99.8%
	0.07	0.08	2	0.2%	1095	100.0%
Northeast Plateau	0.00	0.01	20	5.7%	20	5.7%
	0.01	0.02	53	15.2%	73	21.0%
	0.02	0.03	64	18.4%	137	39.4%
	0.03	0.04	64	18.4%	201	57.8%
	0.04	0.05	63	18.1%	264	75.9%
	0.05	0.06	64	18.4%	328	94.3%
	0.06	0.07	19	5.5%	347	99.7%
	0.07	0.08	1	0.3%	348	100.0%

Table 7-11 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Sacra-mento Metro Area	0.00	0.01	147	2.8%	147	2.8%
	0.01	0.02	298	5.7%	445	8.6%
	0.02	0.03	703	13.5%	1148	22.1%
	0.03	0.04	1061	20.4%	2209	42.5%
	0.04	0.05	1042	20.1%	3251	62.6%
	0.05	0.06	788	15.2%	4039	77.8%
	0.06	0.07	533	10.3%	4572	88.1%
	0.07	0.08	331	6.4%	4903	94.4%
	0.08	0.09	169	3.3%	5072	97.7%
	0.09	0.10	78	1.5%	5150	99.2%
	0.10	0.11	30	0.6%	5180	99.8%
	0.11	0.12	10	0.2%	5190	100.0%
	0.12	0.13	1	0.0%	5191	100.0%
	0.13	0.14	1	0.0%	5192	100.0%
Salton Sea	0.00	0.01	3	0.1%	3	0.1%
	0.01	0.02	108	3.8%	111	3.9%
	0.02	0.03	333	11.6%	444	15.5%
	0.03	0.04	614	21.4%	1058	36.9%
	0.04	0.05	646	22.5%	1704	59.4%
	0.05	0.06	572	19.9%	2276	79.3%
	0.06	0.07	314	10.9%	2590	90.2%
	0.07	0.08	155	5.4%	2745	95.6%
	0.08	0.09	78	2.7%	2823	98.3%
	0.09	0.10	29	1.0%	2852	99.3%
	0.10	0.11	17	0.6%	2869	99.9%
	0.11	0.12	1	0.0%	2870	100.0%
	0.12	0.13	1	0.0%	2871	100.0%
San Diego	0.00	0.01	12	0.4%	12	0.4%
	0.01	0.02	139	4.2%	151	4.6%
	0.02	0.03	389	11.9%	540	16.5%
	0.03	0.04	877	26.8%	1417	43.3%
	0.04	0.05	1107	33.8%	2524	77.1%
	0.05	0.06	526	16.1%	3050	93.2%
	0.06	0.07	143	4.4%	3193	97.6%
	0.07	0.08	58	1.8%	3251	99.3%
	0.08	0.09	16	0.5%	3267	99.8%
	0.09	0.10	6	0.2%	3273	100.0%

Table 7-11 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Francisco Bay Area	0.00	0.01	326	4.3%	326	4.3%
	0.01	0.02	906	12.0%	1232	16.4%
	0.02	0.03	2071	27.5%	3303	43.9%
	0.03	0.04	2521	33.5%	5824	77.4%
	0.04	0.05	1173	15.6%	6997	92.9%
	0.05	0.06	320	4.3%	7317	97.2%
	0.06	0.07	125	1.7%	7442	98.9%
	0.07	0.08	54	0.7%	7496	99.6%
	0.08	0.09	22	0.3%	7518	99.9%
	0.09	0.10	9	0.1%	7527	100.0%
	0.10	0.11	1	0.0%	7528	100.0%
San Joaquin Valley	0.00	0.01	217	2.5%	217	2.5%
	0.01	0.02	569	6.6%	786	9.1%
	0.02	0.03	868	10.0%	1654	19.1%
	0.03	0.04	1340	15.5%	2994	34.6%
	0.04	0.05	1292	15.0%	4286	49.6%
	0.05	0.06	1082	12.5%	5368	62.1%
	0.06	0.07	998	11.5%	6366	73.7%
	0.07	0.08	915	10.6%	7281	84.3%
	0.08	0.09	755	8.7%	8036	93.0%
	0.09	0.10	379	4.4%	8415	97.4%
	0.10	0.11	187	2.2%	8602	99.5%
	0.11	0.12	35	0.4%	8637	99.9%
	0.12	0.13	4	0.0%	8641	100.0%
	0.13	0.14	1	0.0%	8642	100.0%
South Central Coast	0.00	0.01	23	0.2%	23	0.2%
	0.01	0.02	178	1.9%	201	2.2%
	0.02	0.03	946	10.3%	1147	12.5%
	0.03	0.04	2845	30.9%	3992	43.4%
	0.04	0.05	3143	34.1%	7135	77.5%
	0.05	0.06	1382	15.0%	8517	92.5%
	0.06	0.07	472	5.1%	8989	97.6%
	0.07	0.08	164	1.8%	9153	99.4%
	0.08	0.09	42	0.5%	9195	99.9%
	0.09	0.10	10	0.1%	9205	100.0%
	0.10	0.11	3	0.0%	9208	100.0%

Table 7-11 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Coast	0.00	0.01	340	3.5%	340	3.5%
	0.01	0.02	855	8.8%	1195	12.3%
	0.02	0.03	1385	14.2%	2580	26.5%
	0.03	0.04	2012	20.6%	4592	47.1%
	0.04	0.05	2049	21.0%	6641	68.1%
	0.05	0.06	1261	12.9%	7902	81.1%
	0.06	0.07	761	7.8%	8663	88.9%
	0.07	0.08	445	4.6%	9108	93.5%
	0.08	0.09	286	2.9%	9394	96.4%
	0.09	0.10	180	1.8%	9574	98.2%
	0.10	0.11	102	1.0%	9676	99.3%
	0.11	0.12	53	0.5%	9729	99.8%
	0.12	0.13	9	0.1%	9738	99.9%
	0.13	0.14	6	0.1%	9744	100.0%
	0.14	0.15	2	0.0%	9746	100.0%
Upper Sacra-mento Valley	0.00	0.01	147	4.1%	147	4.1%
	0.01	0.02	269	7.4%	416	11.5%
	0.02	0.03	394	10.9%	810	22.3%
	0.03	0.04	796	21.9%	1606	44.3%
	0.04	0.05	840	23.2%	2446	67.4%
	0.05	0.06	567	15.6%	3013	83.0%
	0.06	0.07	377	10.4%	3390	93.4%
	0.07	0.08	168	4.6%	3558	98.1%
	0.08	0.09	48	1.3%	3606	99.4%
	0.09	0.10	20	0.6%	3626	99.9%
	0.10	0.11	2	0.1%	3628	100.0%
California	0.00	0.01	1304	2.1%	1304	2.1%
	0.01	0.02	3827	6.1%	5131	8.1%
	0.02	0.03	8870	14.0%	14001	22.2%
	0.03	0.04	15407	24.4%	29408	46.6%
	0.04	0.05	14177	22.5%	43585	69.0%
	0.05	0.06	8232	13.0%	51817	82.1%
	0.06	0.07	5003	7.9%	56820	90.0%
	0.07	0.08	3157	5.0%	59977	95.0%
	0.08	0.09	1821	2.9%	61798	97.9%
	0.09	0.10	838	1.3%	62636	99.2%
	0.10	0.11	375	0.6%	63011	99.8%
	0.11	0.12	112	0.2%	63123	100.0%
	0.12	0.13	16	0.0%	63139	100.0%
	0.13	0.14	8	0.0%	63147	100.0%
	0.14	0.15	2	0.0%	63149	100.0%

Table 7-12 Frequency of 8-Hour Ozone Concentrations Measured During 2003.

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Great Basin Valleys	0.02	0.03	6	1.7%	6	1.7%
	0.03	0.04	74	20.5%	80	22.2%
	0.04	0.05	81	22.4%	161	44.6%
	0.05	0.06	110	30.5%	271	75.1%
	0.06	0.07	60	16.6%	331	91.7%
	0.07	0.08	28	7.8%	359	99.4%
	0.08	0.09	2	0.6%	361	100.0%
Lake County	0.00	0.01	1	0.3%	1	0.3%
	0.01	0.02	19	5.2%	20	5.5%
	0.02	0.03	55	15.1%	75	20.5%
	0.03	0.04	130	35.6%	205	56.2%
	0.04	0.05	116	31.8%	321	87.9%
	0.05	0.06	37	10.1%	358	98.1%
	0.06	0.07	7	1.9%	365	100.0%
Lake Tahoe	0.01	0.02	3	0.2%	3	0.2%
	0.02	0.03	33	2.6%	36	2.8%
	0.03	0.04	179	13.9%	215	16.7%
	0.04	0.05	456	35.4%	671	52.1%
	0.05	0.06	413	32.1%	1084	84.2%
	0.06	0.07	154	12.0%	1238	96.2%
	0.07	0.08	37	2.9%	1275	99.1%
	0.08	0.09	10	0.8%	1285	99.8%
	0.09	0.10	1	0.1%	1286	99.9%
	0.10	0.11	1	0.1%	1287	100.0%
Mojave Desert	0.00	0.01	10	0.3%	10	0.3%
	0.01	0.02	84	2.4%	94	2.7%
	0.02	0.03	317	9.1%	411	11.8%
	0.03	0.04	639	18.4%	1050	30.2%
	0.04	0.05	753	21.7%	1803	51.9%
	0.05	0.06	579	16.7%	2382	68.6%
	0.06	0.07	471	13.6%	2853	82.1%
	0.07	0.08	335	9.6%	3188	91.8%
	0.08	0.09	174	5.0%	3362	96.8%
	0.09	0.10	77	2.2%	3439	99.0%
	0.10	0.11	24	0.7%	3463	99.7%
	0.11	0.12	8	0.2%	3471	99.9%
	0.12	0.13	3	0.1%	3474	100.0%

Table 7-12 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0.00	0.01	55	1.9%	55	1.9%
	0.01	0.02	147	5.1%	202	7.0%
	0.02	0.03	248	8.6%	450	15.6%
	0.03	0.04	409	14.2%	859	29.9%
	0.04	0.05	700	24.3%	1559	54.2%
	0.05	0.06	439	15.3%	1998	69.5%
	0.06	0.07	413	14.4%	2411	83.8%
	0.07	0.08	308	10.7%	2719	94.5%
	0.08	0.09	126	4.4%	2845	98.9%
	0.09	0.10	25	0.9%	2870	99.8%
	0.10	0.11	6	0.2%	2876	100.0%
North Central Coast	0.00	0.01	17	0.5%	17	0.5%
	0.01	0.02	186	5.1%	203	5.6%
	0.02	0.03	823	22.6%	1026	28.2%
	0.03	0.04	1431	39.3%	2457	67.5%
	0.04	0.05	835	23.0%	3292	90.5%
	0.05	0.06	217	6.0%	3509	96.5%
	0.06	0.07	99	2.7%	3608	99.2%
	0.07	0.08	26	0.7%	3634	99.9%
	0.08	0.09	4	0.1%	3638	100.0%
North Coast	0.00	0.01	102	9.3%	102	9.3%
	0.01	0.02	132	12.1%	234	21.4%
	0.02	0.03	262	24.0%	496	45.4%
	0.03	0.04	341	31.2%	837	76.6%
	0.04	0.05	203	18.6%	1040	95.2%
	0.05	0.06	46	4.2%	1086	99.5%
	0.06	0.07	5	0.5%	1091	99.9%
	0.07	0.08	1	0.1%	1092	100.0%
Northeast Plateau	0.00	0.01	8	3.1%	8	3.1%
	0.01	0.02	20	7.6%	28	10.7%
	0.02	0.03	32	12.2%	60	22.9%
	0.03	0.04	45	17.2%	105	40.1%
	0.04	0.05	75	28.6%	180	68.7%
	0.05	0.06	61	23.3%	241	92.0%
	0.06	0.07	18	6.9%	259	98.9%
	0.07	0.08	3	1.1%	262	100.0%

Table 7-12 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Sacra-mento Metro Area	0.00	0.01	190	4.4%	190	4.4%
	0.01	0.02	322	7.5%	512	11.9%
	0.02	0.03	565	13.1%	1077	25.0%
	0.03	0.04	840	19.5%	1917	44.4%
	0.04	0.05	849	19.7%	2766	64.1%
	0.05	0.06	666	15.4%	3432	79.6%
	0.06	0.07	427	9.9%	3859	89.5%
	0.07	0.08	252	5.8%	4111	95.3%
	0.08	0.09	136	3.2%	4247	98.5%
	0.09	0.10	48	1.1%	4295	99.6%
	0.10	0.11	11	0.3%	4306	99.8%
	0.11	0.12	6	0.1%	4312	100.0%
	0.12	0.13	1	0.0%	4313	100.0%
Salton Sea	0.00	0.01	29	1.0%	29	1.0%
	0.01	0.02	210	7.5%	239	8.5%
	0.02	0.03	453	16.1%	692	24.7%
	0.03	0.04	616	21.9%	1308	46.6%
	0.04	0.05	648	23.1%	1956	69.7%
	0.05	0.06	413	14.7%	2369	84.4%
	0.06	0.07	234	8.3%	2603	92.7%
	0.07	0.08	106	3.8%	2709	96.5%
	0.08	0.09	58	2.1%	2767	98.6%
	0.09	0.10	29	1.0%	2796	99.6%
	0.10	0.11	11	0.4%	2807	100.0%
San Diego	0.00	0.01	48	1.5%	48	1.5%
	0.01	0.02	224	6.9%	272	8.3%
	0.02	0.03	453	13.9%	725	22.2%
	0.03	0.04	1019	31.2%	1744	53.4%
	0.04	0.05	844	25.8%	2588	79.3%
	0.05	0.06	445	13.6%	3033	92.9%
	0.06	0.07	153	4.7%	3186	97.6%
	0.07	0.08	52	1.6%	3238	99.2%
	0.08	0.09	24	0.7%	3262	99.9%
	0.09	0.10	1	0.0%	3263	99.9%
	0.10	0.11	2	0.1%	3265	100.0%

Table 7-12 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Francisco Bay Area	0.00	0.01	336	4.8%	336	4.8%
	0.01	0.02	712	10.3%	1048	15.1%
	0.02	0.03	1851	26.7%	2899	41.8%
	0.03	0.04	2086	30.1%	4985	71.9%
	0.04	0.05	1352	19.5%	6337	91.4%
	0.05	0.06	365	5.3%	6702	96.6%
	0.06	0.07	138	2.0%	6840	98.6%
	0.07	0.08	62	0.9%	6902	99.5%
	0.08	0.09	30	0.4%	6932	99.9%
	0.09	0.10	4	0.1%	6936	100.0%
	0.10	0.11	1	0.0%	6937	100.0%
San Joaquin Valley	0.00	0.01	356	4.2%	356	4.2%
	0.01	0.02	594	7.1%	950	11.3%
	0.02	0.03	837	10.0%	1787	21.3%
	0.03	0.04	1207	14.4%	2994	35.7%
	0.04	0.05	1197	14.3%	4191	49.9%
	0.05	0.06	1061	12.6%	5252	62.5%
	0.06	0.07	991	11.8%	6243	74.3%
	0.07	0.08	899	10.7%	7142	85.1%
	0.08	0.09	750	8.9%	7892	94.0%
	0.09	0.10	360	4.3%	8252	98.3%
	0.10	0.11	120	1.4%	8372	99.7%
	0.11	0.12	24	0.3%	8396	100.0%
	0.12	0.13	1	0.0%	8397	100.0%
South Central Coast	0.00	0.01	13	0.1%	13	0.1%
	0.01	0.02	222	2.5%	235	2.6%
	0.02	0.03	1208	13.5%	1443	16.1%
	0.03	0.04	2930	32.7%	4373	48.9%
	0.04	0.05	2688	30.0%	7061	78.9%
	0.05	0.06	1058	11.8%	8119	90.7%
	0.06	0.07	492	5.5%	8611	96.2%
	0.07	0.08	218	2.4%	8829	98.7%
	0.08	0.09	95	1.1%	8924	99.7%
	0.09	0.10	21	0.2%	8945	100.0%
	0.10	0.11	3	0.0%	8948	100.0%
	0.11	0.12	1	0.0%	8949	100.0%

Table 7-12 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Coast	0.00	0.01	222	2.6%	222	2.6%
	0.01	0.02	579	6.7%	801	9.3%
	0.02	0.03	1048	12.2%	1849	21.5%
	0.03	0.04	1565	18.2%	3414	39.7%
	0.04	0.05	1866	21.7%	5280	61.5%
	0.05	0.06	1181	13.7%	6461	75.2%
	0.06	0.07	759	8.8%	7220	84.0%
	0.07	0.08	476	5.5%	7696	89.6%
	0.08	0.09	370	4.3%	8066	93.9%
	0.09	0.10	224	2.6%	8290	96.5%
	0.10	0.11	144	1.7%	8434	98.2%
	0.11	0.12	85	1.0%	8519	99.2%
	0.12	0.13	44	0.5%	8563	99.7%
	0.13	0.14	20	0.2%	8583	99.9%
	0.14	0.15	7	0.1%	8590	100.0%
	0.15	0.16	2	0.0%	8592	100.0%
Upper Sacra-mento Valley	0.00	0.01	155	4.4%	155	4.4%
	0.01	0.02	272	7.7%	427	12.1%
	0.02	0.03	516	14.6%	943	26.7%
	0.03	0.04	755	21.4%	1698	48.1%
	0.04	0.05	780	22.1%	2478	70.2%
	0.05	0.06	552	15.6%	3030	85.8%
	0.06	0.07	312	8.8%	3342	94.7%
	0.07	0.08	145	4.1%	3487	98.8%
	0.08	0.09	32	0.9%	3519	99.7%
	0.09	0.10	11	0.3%	3530	100.0%
California	0.00	0.01	1542	2.6%	1542	2.6%
	0.01	0.02	3726	6.2%	5268	8.8%
	0.02	0.03	8707	14.5%	13975	23.2%
	0.03	0.04	14266	23.7%	28241	47.0%
	0.04	0.05	13443	22.4%	41684	69.3%
	0.05	0.06	7643	12.7%	49327	82.0%
	0.06	0.07	4733	7.9%	54060	89.9%
	0.07	0.08	2948	4.9%	57008	94.8%
	0.08	0.09	1811	3.0%	58819	97.8%
	0.09	0.10	801	1.3%	59620	99.1%
	0.10	0.11	323	0.5%	59943	99.7%
	0.11	0.12	124	0.2%	60067	99.9%
	0.12	0.13	49	0.1%	60116	100.0%
	0.13	0.14	20	0.0%	60136	100.0%
	0.14	0.15	7	0.0%	60143	100.0%
	0.15	0.16	2	0.0%	60145	100.0%

7.2.7 Diurnal Variations

Ozone is formed by the reaction of nitrogen oxides and VOCs in the presence of sunlight (see Chapter 3 for further discussion). Because the intensity of sunlight and the strength of the emissions of nitrogen oxides and VOCs that produce ozone vary throughout the day, the concentration of ozone varies throughout the day, as well. In urban areas, the diurnal variation of 1-hour ozone concentrations traces a wave-like pattern throughout the 24-hour day with the highest concentration occurring near 12-noon and the lowest concentration occurring in the early morning, around 6 a.m., Local Standard Time. The 1-hour ozone peak near the 12-noon hour is usually caused by the photochemistry of local emissions.

In certain areas, the diurnal 1-hour ozone profile has more than one peak. Urban areas that lie downwind of larger urban areas can have an ozone diurnal profile with a double peak, one around 12-noon and the other during the mid- to late-afternoon. The ozone peak near the 12-noon hour reflects local emissions, while the later peak results from ozone transported from an upwind area.

Small towns and rural locations, especially in the foothills and higher elevations of the Sierra Nevada Mountains, that are downwind of large urban areas usually have their maximum daily 1-hour ozone concentration anywhere from early afternoon to late evening depending on the distance downwind of the urban area(s). One location on the eastern side of the Sierra Nevada Mountains has observed a daily maximum 1-hour ozone concentration at midnight or in the very early morning. For these types of locations, the early afternoon to nighttime occurrence of the daily maximum ozone concentration is the result of ozone transported from upwind areas.

Under some circumstances, the maximum daily 1-hour ozone concentration can occur during the late morning hours. These late morning maximums are typically caused either by the carryover of ozone aloft from the previous day or from overnight transport of ozone aloft. Ozone aloft reaches the surface when the early morning low-level inversion disappears, as a result of convective mixing.

In contrast to the 1-hour concentrations, which reflect a single measurement, the 8-hour concentrations reflect an average of eight hourly values. As such, the 8-hour concentrations generally span the period during which the highest 1-hour concentrations occur. Based on air quality data for 2001 through 2003, the most common starting time for 8-hour concentrations that exceed the federal standard is 10 a.m. or 11 a.m. These high 8-hour periods then extend on through the 5 p.m. or 6 p.m. observation. 8-hour concentrations that exceed the level of the federal ozone standard can begin at other hours, but the rate is less than half of those beginning at 10 a.m. to 11 a.m.

7.2.8 Characterization of Ozone by Air Basin or Planning Area

Ozone air quality has improved substantially over the last two decades in most areas of California, despite significant population and economic growth. On a statewide basis (using the site with the highest value for each year), the peak 1-hour indicator decreased 54 percent and the peak 8-hour indicator decreased 47 percent from 1982 to 2003. In contrast, the statewide population increased 45 percent, and the average number of vehicle miles traveled each day statewide

increased 104 percent during the same time period. Even though there has been substantial improvement in ozone air quality, current levels still represent a significant concern in many areas. The following subsections describe the trends in both 1-hour and 8-hour average ozone concentrations for each air basin or planning area of California.

7.2.8.1 Great Basin Valleys Air Basin

The Great Basin Valleys Air Basin (GBVAB) includes all of Alpine, Inyo, and Mono counties, located along the eastern border of California. Much of the area is sparsely populated, and the air basin as a whole, is home to less than 1 percent of California's population. As previously discussed, at the time this Staff Report was prepared, representative data were not available for the Great Basin Valleys Air Basin during 2001. Therefore, air quality statistics using 2001 data are not included in the following discussion.

Similar to many other areas of the State, ozone concentrations in the GBVAB tend to be highest during the summer months. Ozone levels, as indicated by the peak 1-hour indicator, changed little in the GBVAB from 1982 through 2003. The maximum 1-hour concentration shows more variation than the peak indicator, especially during the early 1990s. These variations may have been caused by year-to-year changes in meteorology. Both the peak 1-hour indicator and the maximum 1-hour concentration statistics show similar values during the two end years: 1982 and 2000 for the peak indicator and 1982 and 2003 for the maximum concentration. The 8-hour values show similar trends, however, the 8-hour peak indicator has been a bit more stable than the 1-hour peak indicator. Similar to the 1-hour trend, the 8-hour peak indicator values for the two end years (1982 and 2000) and the maximum 8-hour values for the two end years (1982 and 2003) are nearly identical.

Figures 7.13 and 7.14 both show a slight increase in the maximum 1-hour and 8-hour values for 2002 when compared with 2000 and 2003. During 2002, the maximum 1-hour ozone concentration was 0.10 ppm, which exceeds the State ozone standard. Between 1997 and 2000, there were no exceedances of the State ozone standard. There was also an increase during 2002 in the number of days with concentrations exceeding the State standard (8 days during 2002, compared with 0 days during 1999 and 2003) and the federal 8-hour standard (3 days during 2002, compared with 0 days during 1999 and 2003). These year-to-year variations are probably attributable to variations in meteorology rather than overall changes in emissions.

Figure 7-12

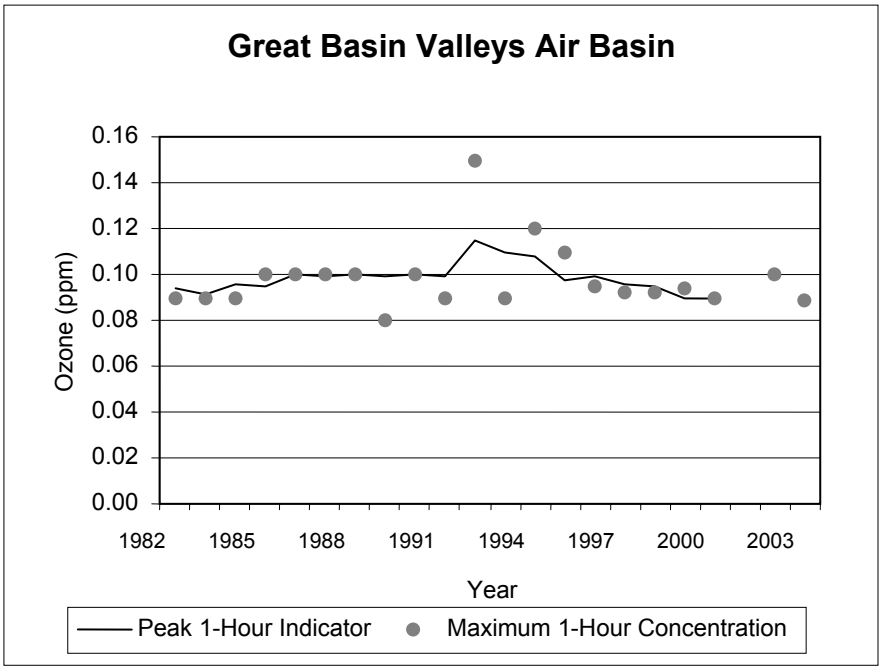
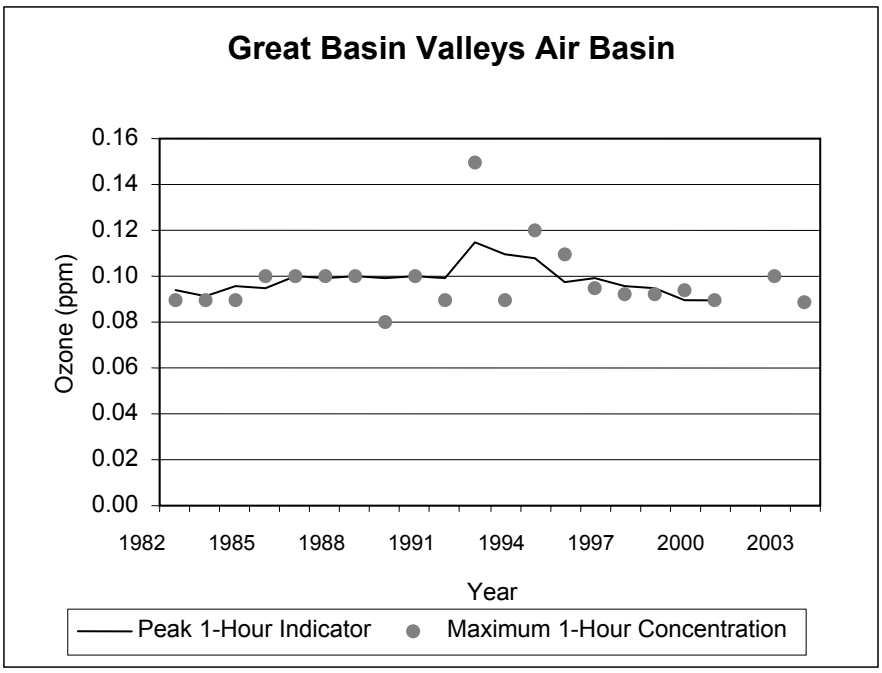


Figure 7-13



7.2.8.2 Lake County Air Basin

The Lake County Air Basin (LCAB) comprises all of Lake County. Less than 1 percent of the State's population lives in the LCAB. This air basin is the only area of California that attains all of the State ambient air quality standards, including the State ozone standard. Air quality in LCAB also does not violate either the 1-hour or 8-hour federal standards.

Ozone levels are relatively low in the LCAB. During 1982 through 2003, the peak 1-hour indicator varied between 0.074 ppm and 0.087 ppm. In contrast, the maximum 1-hour concentration was more variable, ranging between 0.06 ppm and 0.09 ppm. This is not unexpected, since the maximum 1-hour concentration is less stable than the peak indicator and therefore, more affected by year-to-year changes in meteorology. The 8-hour values show a similar trend over the same time period. Again, the maximum 8-hour concentration shows more variation, with concentrations ranging between 0.05 ppm and 0.08 ppm. The 8-hour peak indicator shows less variation, ranging between 0.060 ppm and 0.076 ppm.

Figure 7-14

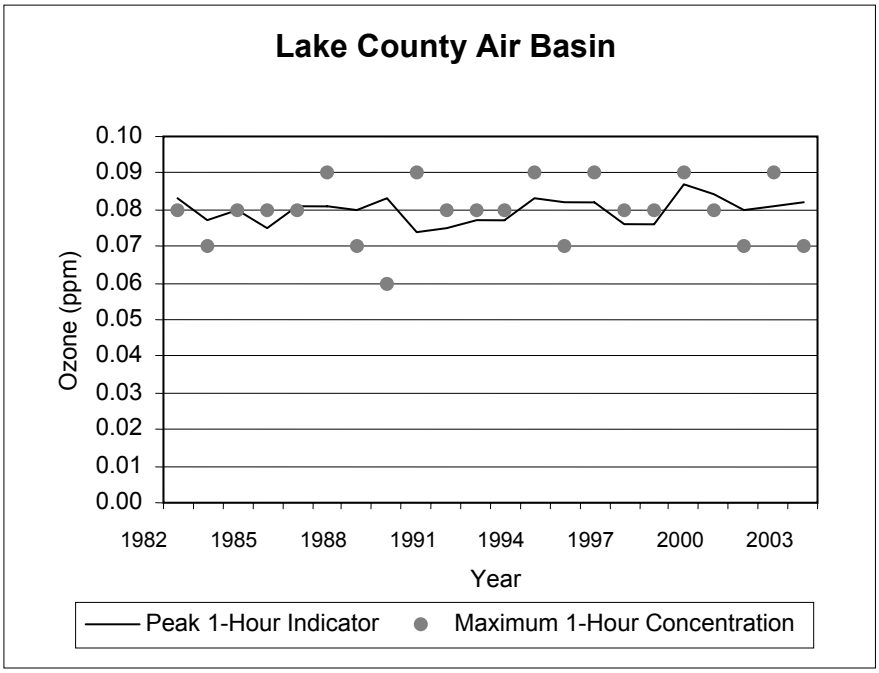
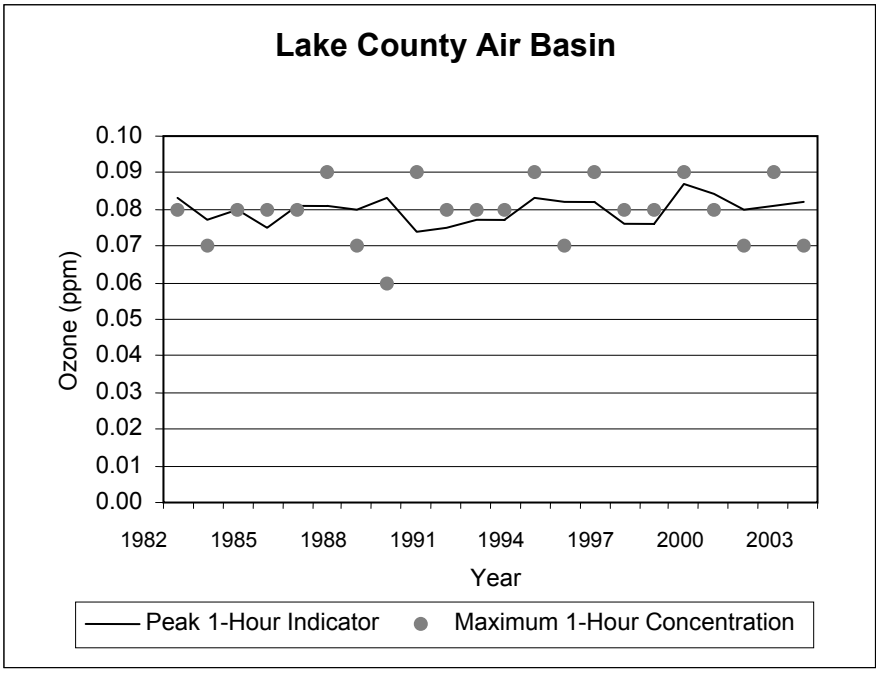


Figure 7-15



7.2.8.3 Lake Tahoe Air Basin

The Lake Tahoe Air Basin (LTAB) consists of the eastern portions of El Dorado and Placer counties. Less than 1 percent of the State's population lives in the LTAB. Ozone concentrations in this air basin tend to be highest during the summer months. However, the highest concentrations in the LTAB are still relatively low, compared to other areas of California.

Similar to the Lake County Air Basin, ozone levels in the LTAB do not show a substantial change during the 1982 through 2003 time period. The peak indicators and the maximum measured concentrations for both the 1-hour and 8-hour averaging times were similar for 1982 and 2001. All of the trend lines show a slight upswing during the last year or two. However, several more years of data are needed to determine if the upswing will continue or if it was caused by variations in meteorology.

The number of days with concentrations above the State standard remains low during the last three years (1, 1, and 3 days during 2001, 2002, and 2003, respectively). The number of days with concentrations above the federal 8-hour standard was similarly low (0, 0, and 3 days during 2001, 2002, and 2003, respectively). At this time, the LTAB remains designated as attainment for the State ozone standard and unclassified/attainment for the federal 1-hour ozone standard. The area is also unclassified/attainment for the federal 8-hour ozone standard.

Figure 7-16

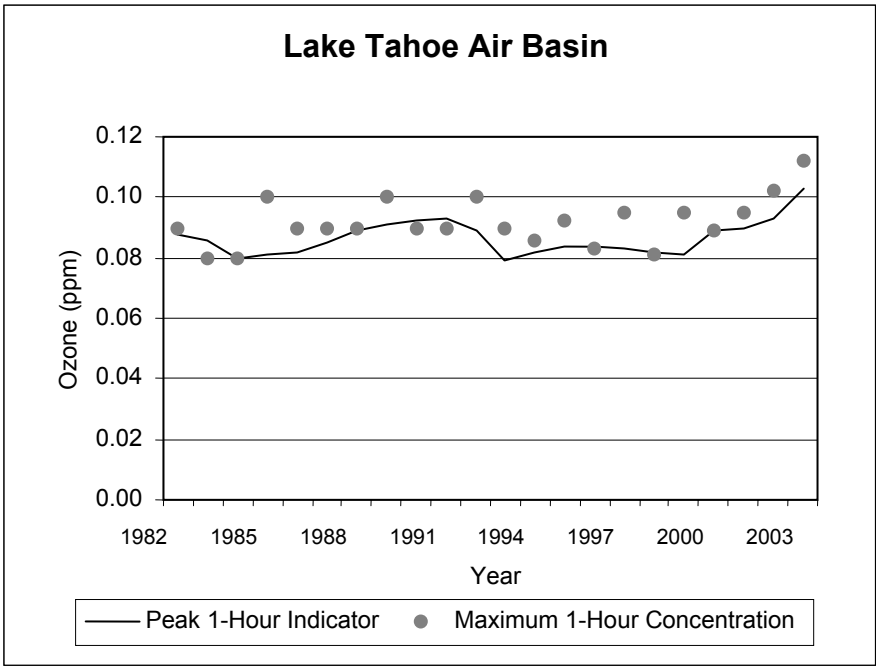
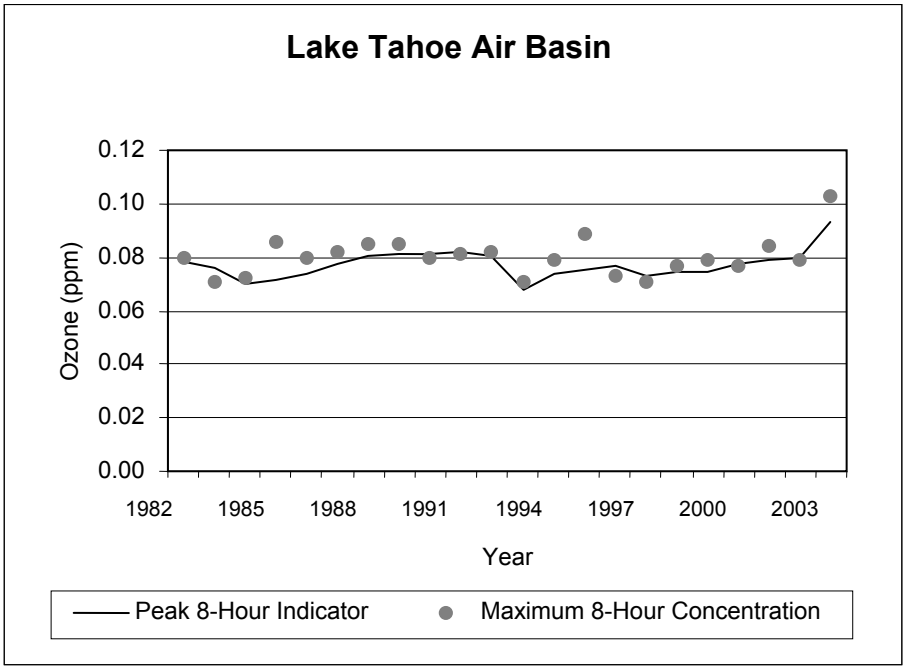


Figure 7-17



7.2.8.4 Mojave Desert Air Basin

The Mojave Desert Air Basin (MDAB) covers a large part of California's high desert. The air basin includes the eastern half of Kern County, the northeastern portion of Los Angeles County, all of San Bernardino County except for the southwestern corner, and the eastern third of Riverside County. Less than 3 percent of California's population resides in the MDAB. Because of the year-round warm temperatures, and therefore, less distinct seasonality, the ozone season in the MDAB can be longer than in other areas of California.

Overall, the peak 1-hour indicator shows a decrease of 37 percent, and the peak 8-hour indicator shows a decrease of 32 percent from 1982 to 2003. During the same time period, the maximum 1-hour concentration declined about 19 percent, and the maximum 8-hour concentration declined about 10 percent. There is a fair amount of year-to-year variation in the values for both averaging times, probably caused by year-to-year variations in meteorology. There has also been a substantial decrease in the number of exceedance days for both the State and federal standards. State standard exceedance days declined from 120 days in 1982 to 93 days in 2003. During this same time period, the number of federal 1-hour exceedance days was 38 in 1982 compared with only 13 in 2003. With respect to the federal 8-hour concentrations, there were 104 exceedance days in 1982 compared with 74 exceedance days in 2003.

Figure 7-18

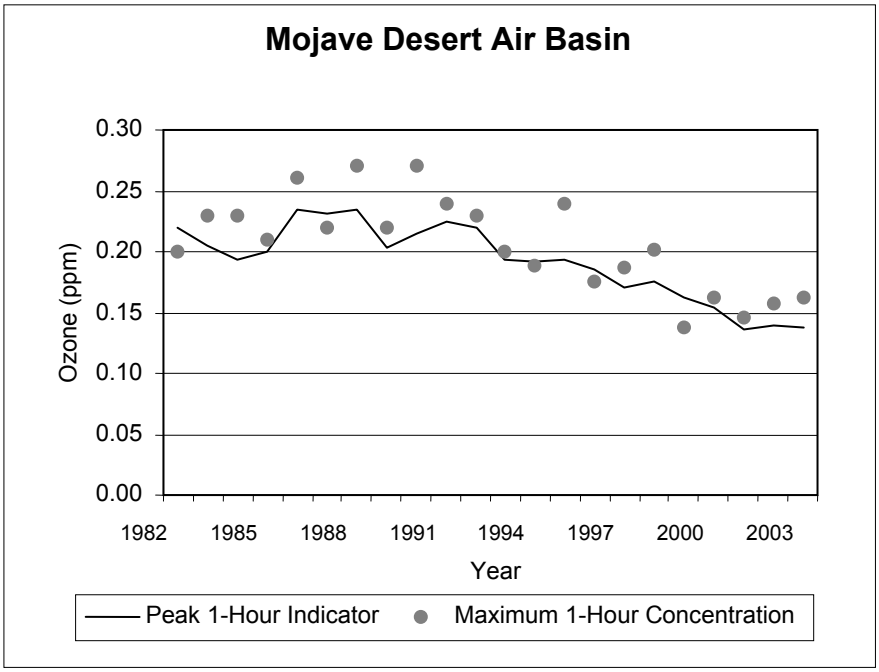
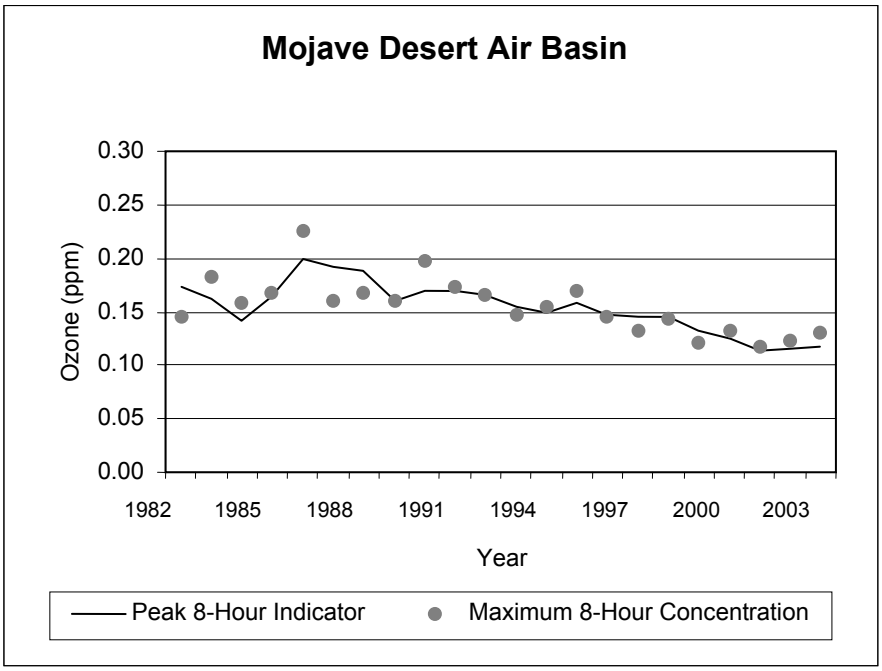


Figure 7-19



7.2.8.5 Mountain Counties Air Basin

For the purposes of this document, the Mountain Counties Air Basin (MCAB) comprises the central and northern portions of the Sierra Nevada mountain region, including Amador, Calaveras, Mariposa, Nevada, Plumas, Sierra, and Tuolumne counties (the MCAB portions of Placer and El Dorado counties are included in the Sacramento Metropolitan Area). The MCAB is thinly populated, its communities separated from one another by the region's complex terrain. A little less than 1 percent of the State's population lives in the MCAB.

Unlike the rest of California, ozone data are available for a representative number of sites in the Mountain Counties Air Basin only since 1987. Between 1987 and 2003, the maximum 1-hour ozone concentrations show a fair amount of year-to-year variation. The trend line for the peak 1-hour indicator is much more stable. This indicator has decreased since 1998, but shows little change during the last three years. During 2001 through 2003, the State ozone standard was exceeded an average of 38 days per year, while the federal 1-hour standard was exceeded an average of only once per year.

Compared with the 1-hour trend lines, the 8-hour trend lines show little change over the 22-year time period. Both the maximum 8-hour concentrations and the peak 8-hour indicator values are relatively stable. While the maximum 8-hour concentrations for 1982 and 2003 show a difference of only 0.008 ppm, there is essentially no difference in the peak 8-hour indicator values for the two end years (0.106 ppm for 1982 versus 0.107 ppm for 2003). During 2001 through 2003, the federal 8-hour ozone standard was exceeded an average of 43 times per year.

Figure 7-20

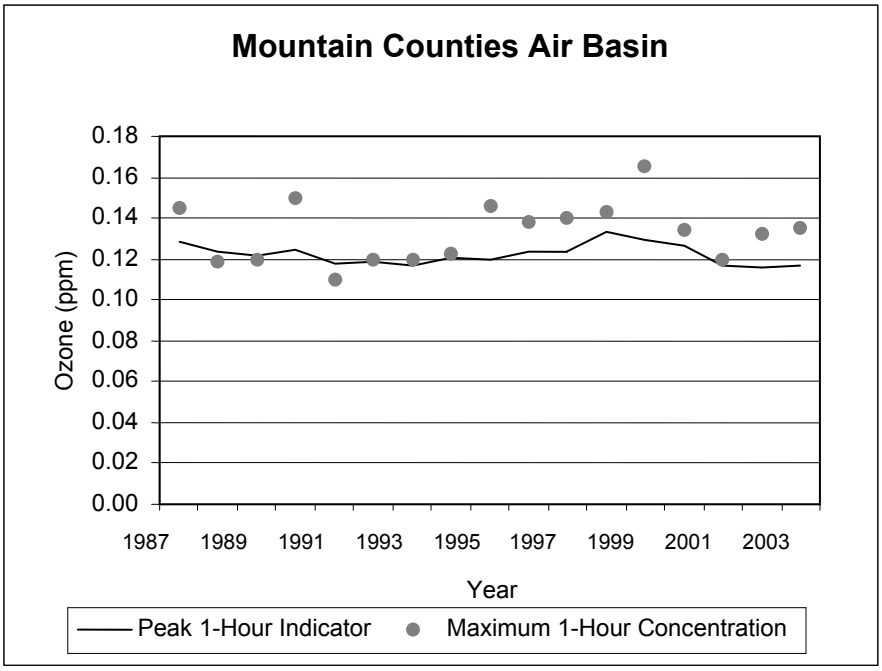
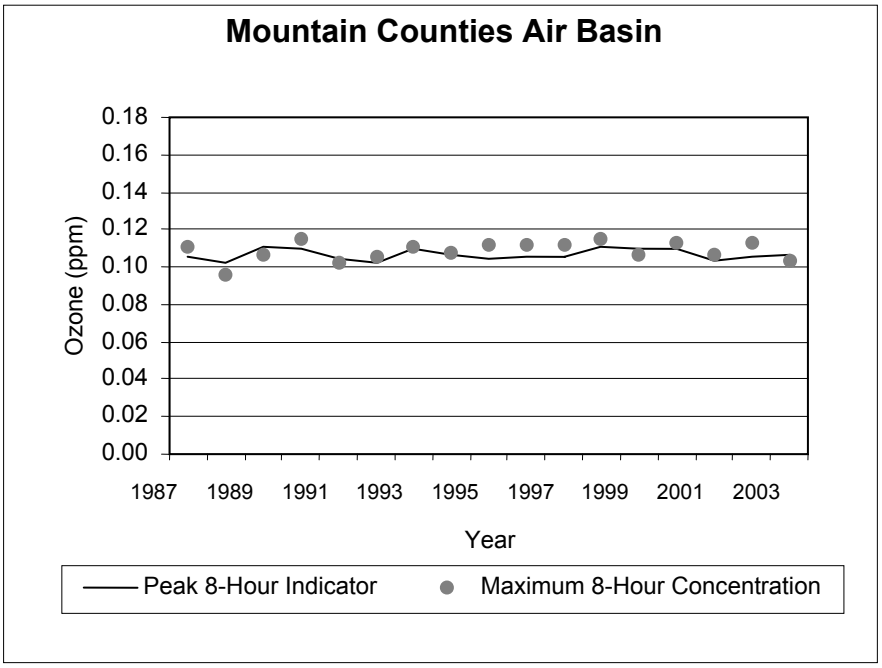


Figure 7-21



7.2.8.6 North Central Coast Air Basin

The North Central Coast Air Basin (NCCAB) is located on the coast of California and includes all of Monterey, San Benito and Santa Cruz counties. About 2 percent of the State's population live in the NCCAB.

The NCCAB enjoys relatively clean air, with only a few exceedances of the State ozone standard each year. The NCCAB trend lines for both the 1-hour values and the 8-hour values are relatively flat from 1982 through 1986, with substantial increases in 1987. During 1987, a new monitoring site was established at the Pinnacles National Monument in San Benito County, and ozone concentrations at this site were higher than those measured at the other existing monitoring sites in the NCCAB. Since 1987, the peak 1-hour indicator declined 28 percent, and the maximum 1-hour concentration declined 24 percent. The 8-hour trend lines show similar reductions, with the peak 8-hour indicator declining 23 percent and the maximum 8-hour concentration declining 21 percent since 1987.

During 2001 through 2003, there was an average of only 4 days with concentrations above the State standard during each year. While ozone concentrations in the NCCAB did not exceed the federal 1-hour standard during this time period, 8-hour concentrations exceeded the federal standard an average of 3 days per year during 2001 through 2003.

Figure 7-22

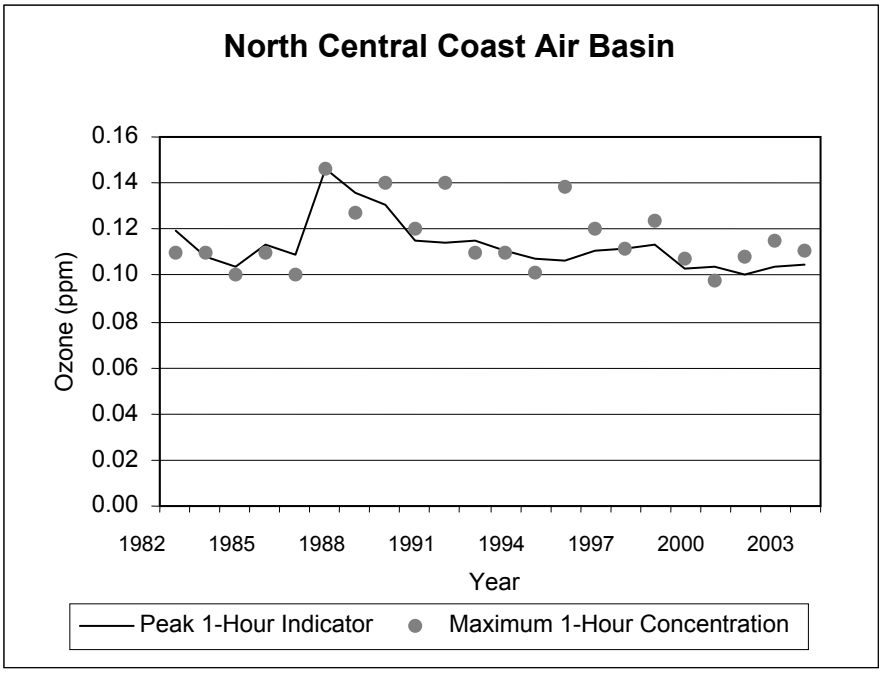
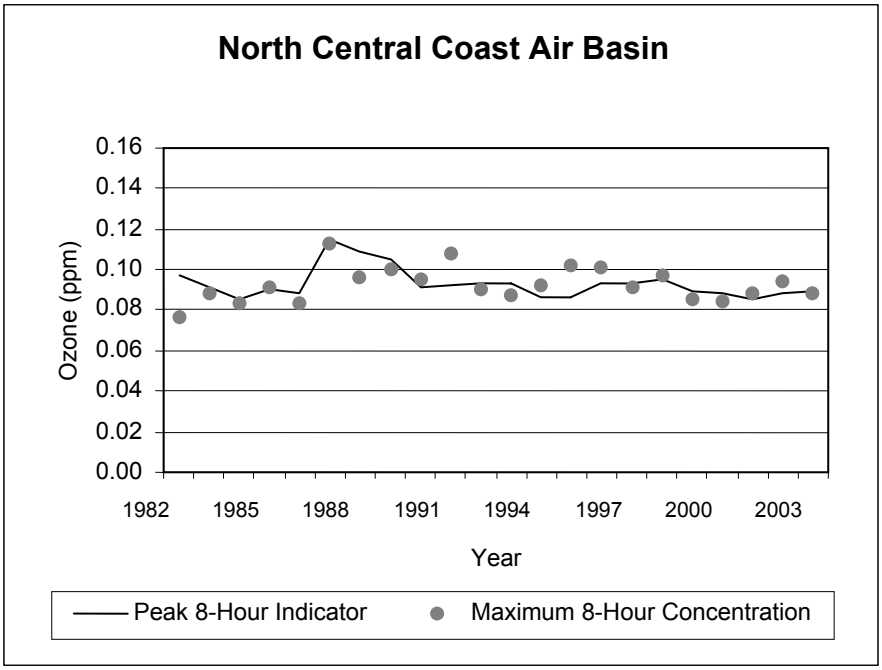


Figure 7-23



7.2.8.7 North Coast Air Basin

The North Coast Air Basin (NCAB) includes all of Del Norte, Humboldt, Mendocino, and Trinity counties, as well as the northern portion of Sonoma County. Slightly less than 1 percent of the State's population lives in the NCAB.

Ozone air quality in the NCAB does not exceed any of the State or federal ozone standards. However, both the 1-hour and the 8-hour ozone statistics for the NCAB show a general increase in 1992, coinciding with the establishment of a new ozone monitoring site at the Healdsburg Municipal Airport in Sonoma County. Since that time, the Healdsburg site has consistently recorded the highest ozone concentrations in the NCAB. The values for both averaging times during 1992 through 2003 show considerable variation, probably caused by year-to-year changes in meteorology. However, the values for all variables show a general overall decline since 1999. Recognizing the progress made in the NCAB, the ARB redesignated the NCAB as attainment for the State ozone standard in January 2004. In addition, the entire NCAB is designated as unclassified/attainment for both the federal 1-hour standard and the federal 8-hour standard.

Figure 7-24

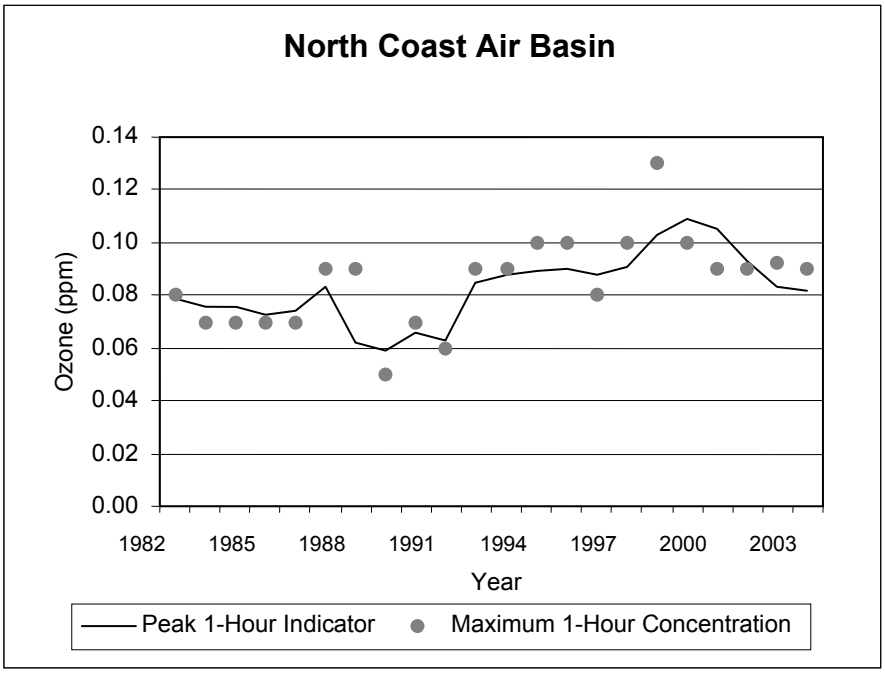
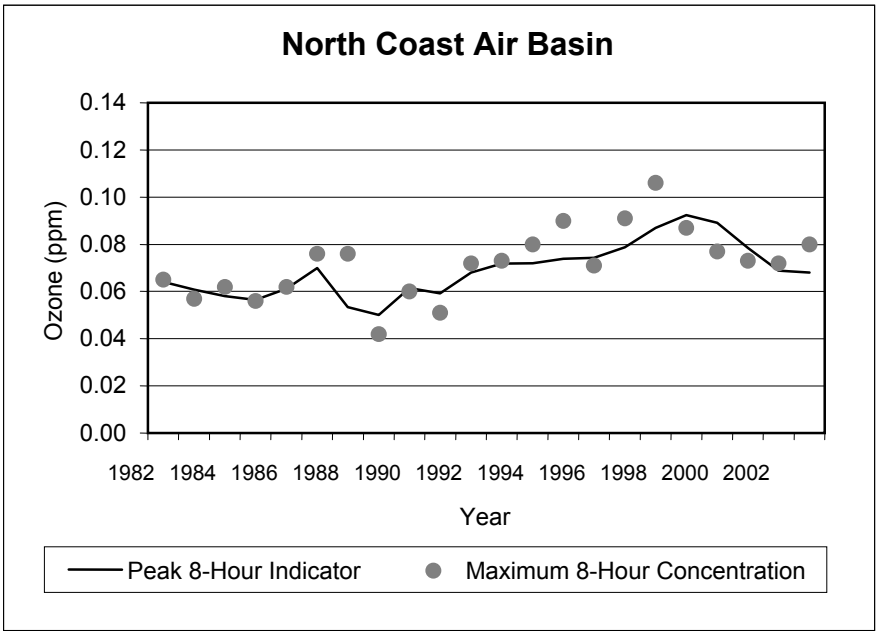


Figure 7-25



7.2.8.8 Northeast Plateau Air Basin

The Northeast Plateau Air Basin (NEPAB) is located in the northeast corner of California and includes Lassen, Modoc, and Siskiyou counties. The area is sparsely populated, housing less than 1 percent of California's population. As previously discussed, representative data are not available for the Northeast Plateau Air Basin during 2001. Therefore, air quality statistics using 2001 data are not included in the following discussion.

Ozone concentrations in this air basin tend to be relatively low, and neither the State nor federal 1-hour ozone standards were exceeded during the past 22 years. Trend lines for both the 1-hour and 8-hour statistics follow the same general pattern, and are relatively flat throughout the entire time period. However, there is some variability, caused by year-to-year variations in meteorology. Maximum concentrations for both averaging times show a slight upswing during the last several years, but the values remain below both the State and federal ozone standards. Additional years of data are needed to determine if an upward trend will continue.

Figure 7-26

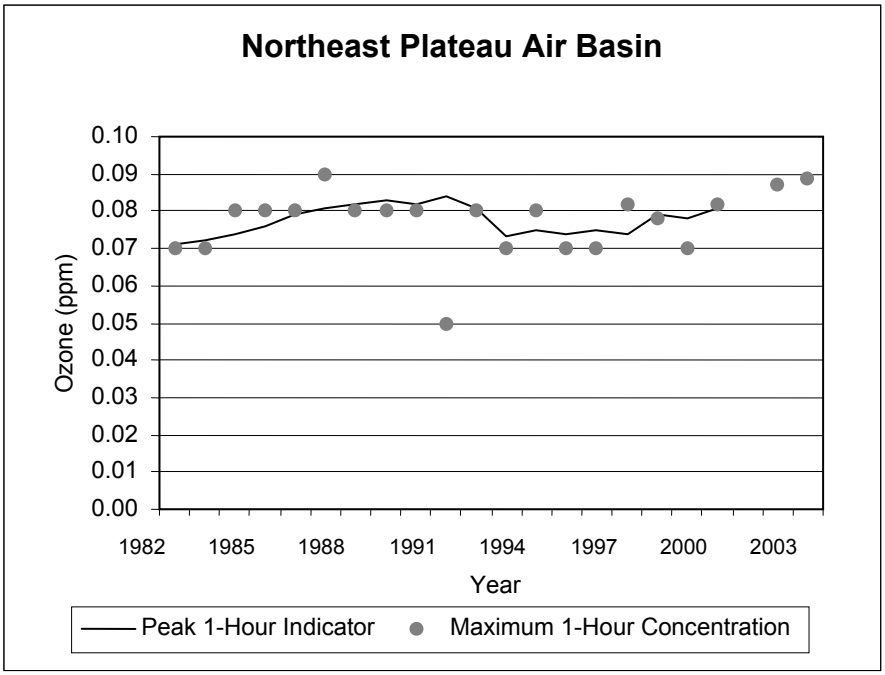
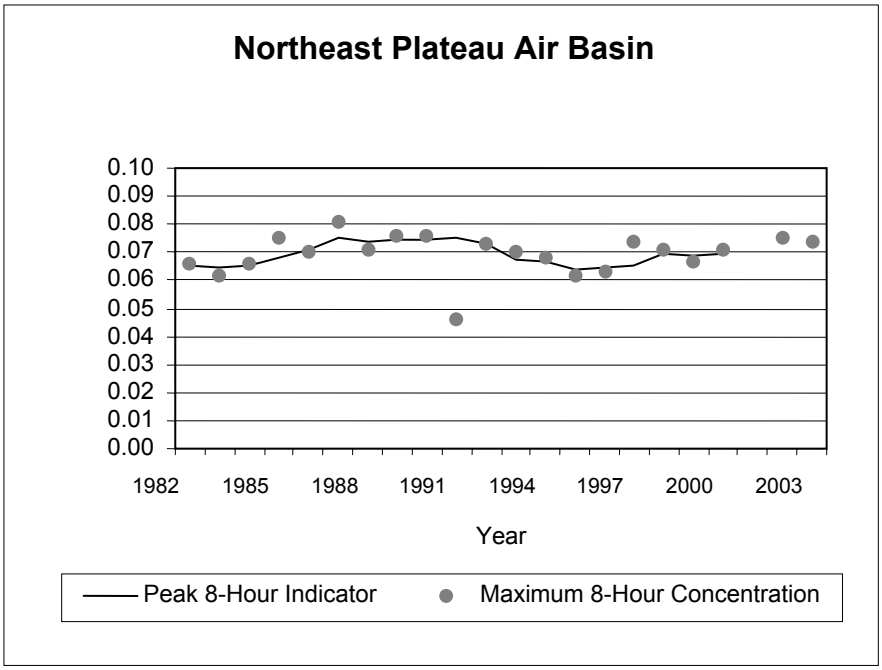


Figure 7-27



7.2.8.9 Sacramento Metro Area

The Sacramento Metro Area includes all of the urbanized southern portion of the Sacramento Valley Air Basin or SVAB (all of Sacramento and Yolo counties, the SVAB portion of Solano County, the southern one-third of Sutter County, and the SVAB portion of Placer County), as well as the portions of El Dorado and Placer counties that are located within the Mountain Counties Air Basin. The Sacramento Metro Area is consistent with the area used for federal 1-hour ozone area designations and air quality planning. In terms of population, the Sacramento Metro Area is home to about 6 percent of California's citizens.

Similar to many other areas of the State, ozone concentrations tend to be highest during the summer months, with the ozone season running from May through October. Peak 1-hour ozone levels in the Sacramento Metro Area have not declined as quickly over the last several years as they have in some other urban areas. The maximum peak 1-hour indicator remained fairly constant during the 1980s. Since then, the peak 1-hour indicator has decreased slightly, and the overall decline during the 22-year period is about 16 percent. The maximum measured 1-hour concentrations have also decreased, but with more year-to-year variation. During 2001 through 2003, the Sacramento Metro Area had an average of 55 State standard exceedance days and 6 federal 1-hour exceedance days per year.

The 8-hour statistics show less variation than the 1-hour statistics and again, little change over the 22-year time period. From 1982 to 2003, the peak 8-hour indicator decreased about 10 percent and the maximum 8-hour concentration decreased only 8 percent. During 2001 through 2003, ozone concentrations in the Sacramento Metro Area exceeded the federal 8-hour standard an average of 44 days per year.

Figure 7-28

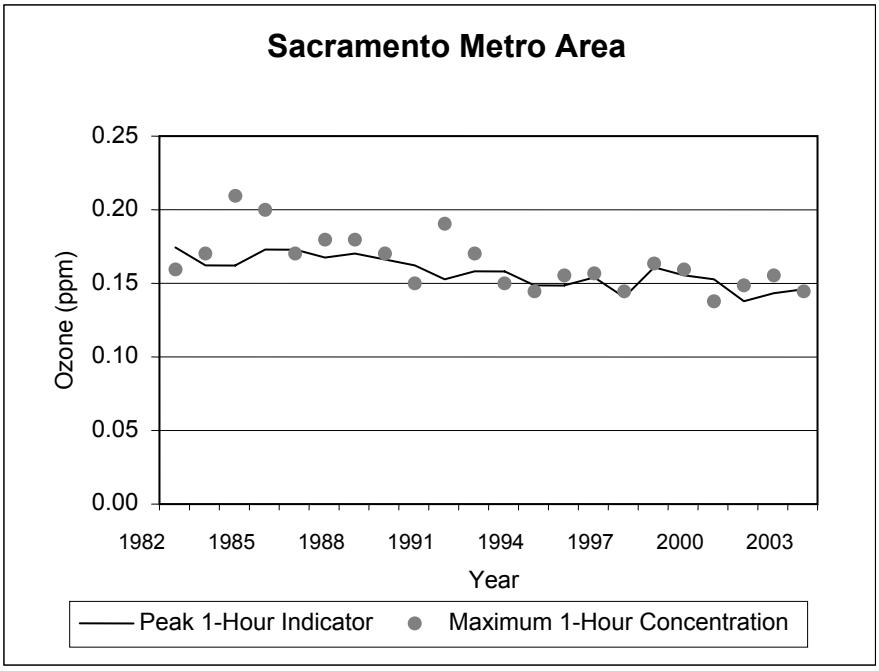
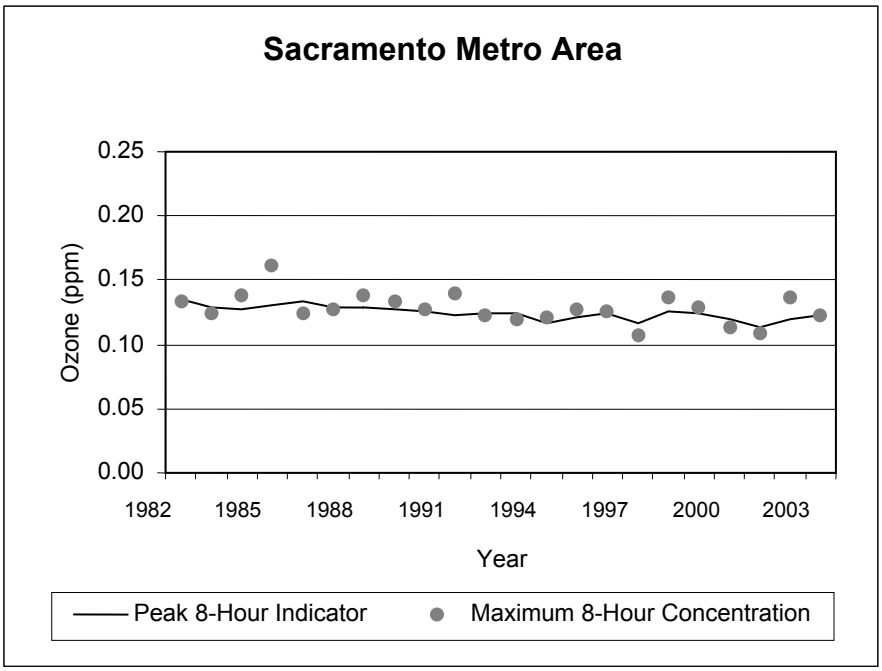


Figure 7-29



7.2.8.10 Salton Sea Air Basin

The Salton Sea Air Basin (SSAB) occupies the southeast corner of California and includes all of Imperial County, as well as the central portion of Riverside County. Less than 2 percent of the State's population lives in this air basin.

Ozone concentrations in the SSAB have exceeded the State standard and both federal ozone standards on multiple occasions. The maximum 1-hour ozone concentrations for 1982 through 2003 show substantial variation between years, with much of this variation probably caused by year-to-year changes in meteorology. However, the trend line for the peak 1-hour indicator is more stable across the 22-year period, showing a steady decline. The overall decline in the peak 1-hour indicator is 35 percent from 1982 to 2003. During each of the years 2001 through 2003, the number of exceedance days for the State ozone standard ranged between 68 and 81, and between 5 and 15 for the federal 1-hour standard.

Although much less variable, the peak 8-hour indicator and maximum 8-hour concentrations also show a steady decline over the entire time period. The peak 8-hour indicator declined 26 percent, while the maximum 8-hour concentration showed a drop of 23 percent. During the most recent three years, 2001 through 2003, ozone concentrations in the Salton Sea Air Basin exceeded the federal 8-hour standard an average of 52 days per year.

Figure 7-30

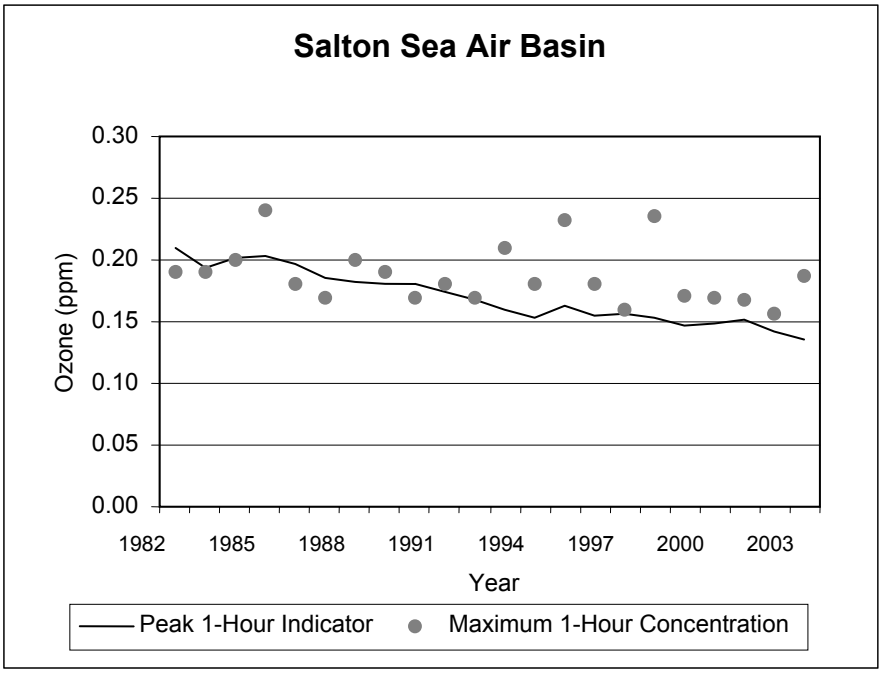
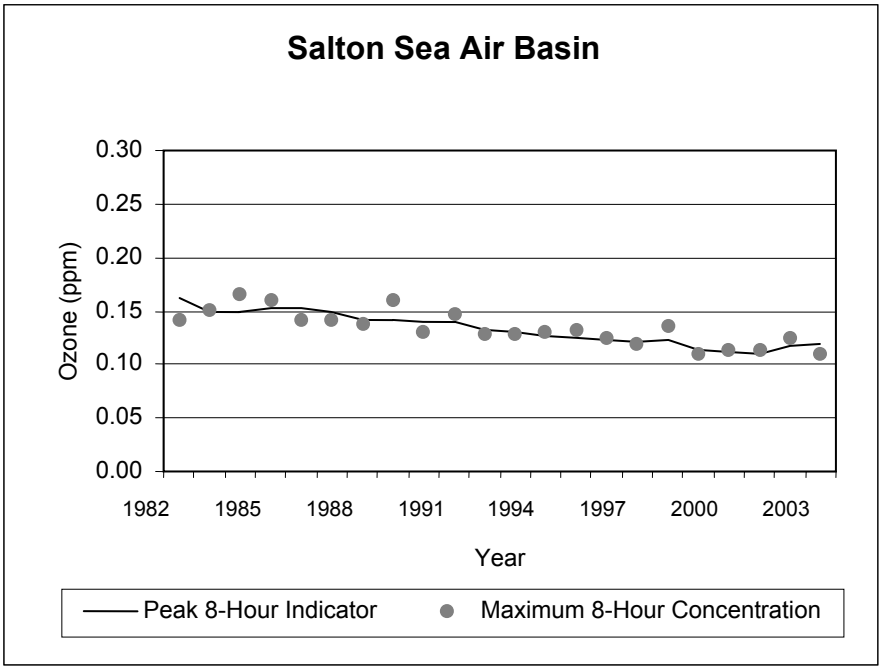


Figure 7-31



7.2.8.11 San Diego Air Basin

The San Diego Air Basin (SDAB) lies in the southwest corner of California and includes all of San Diego County. About 8 percent of California's population live in the SDAB. However, the population and emissions are concentrated mainly in the western portion of the air basin. Because of its southerly location and proximity to the ocean, much of the SDAB has a relatively mild climate, without marked seasonality.

Both the 1-hour and 8-hour ozone statistics for the SDAB indicate a substantial improvement in ozone air quality since 1982. The peak 1-hour indicator shows an overall decline of 42 percent – from 0.203 ppm in 1982 to 0.117 ppm in 2003. The measured maximum 1-hour concentration shows a similar reduction – 46 percent from 1982 to 2003. The peak 8-hour indicator also shows a similar overall decline, 38 percent, from 0.1616 ppm in 1982 to 0.1008 ppm in 2003. The measured maximum 8-hour concentration shows a 46 percent decline from 1982 to 2003.

The number of exceedance days in the SDAB has also dropped substantially over the 22-year period. There were 120 State standard exceedance days and 47 federal 1-hour exceedance days in 1982. These numbers compare with 23 State standard exceedance days and only 1 federal 1-hour exceedance day during 2003. Based on the improvements in this area, the USEPA recently redesignated the San Diego Air Basin as attainment for the federal 1-hour ozone standard. However, although the SDAB now attains the federal 1-hour ozone standard, the federal 8-hour standard continues to pose a more substantial problem, with an average of 12 exceedance days per year during 2001 through 2003.

Figure 7-32

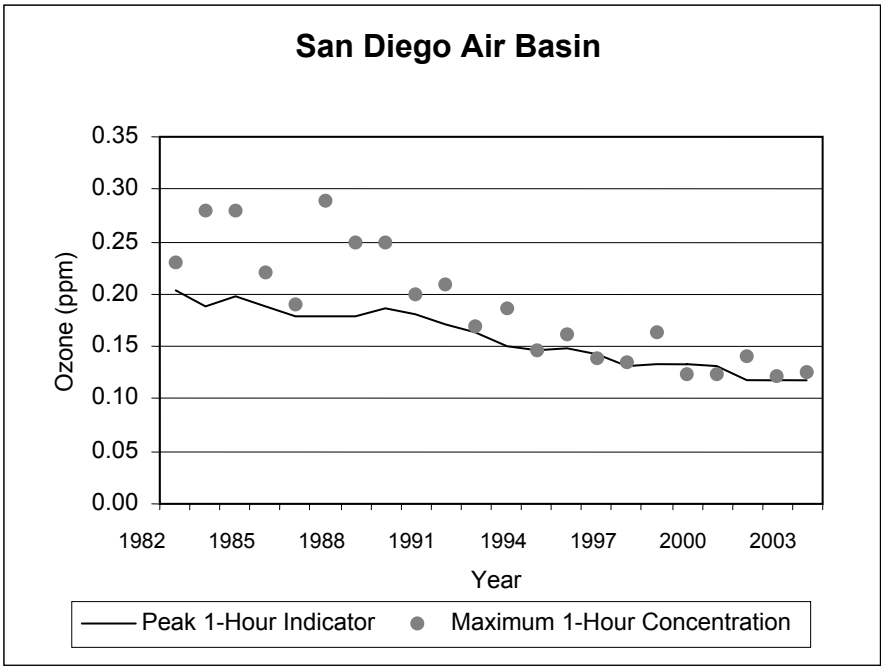
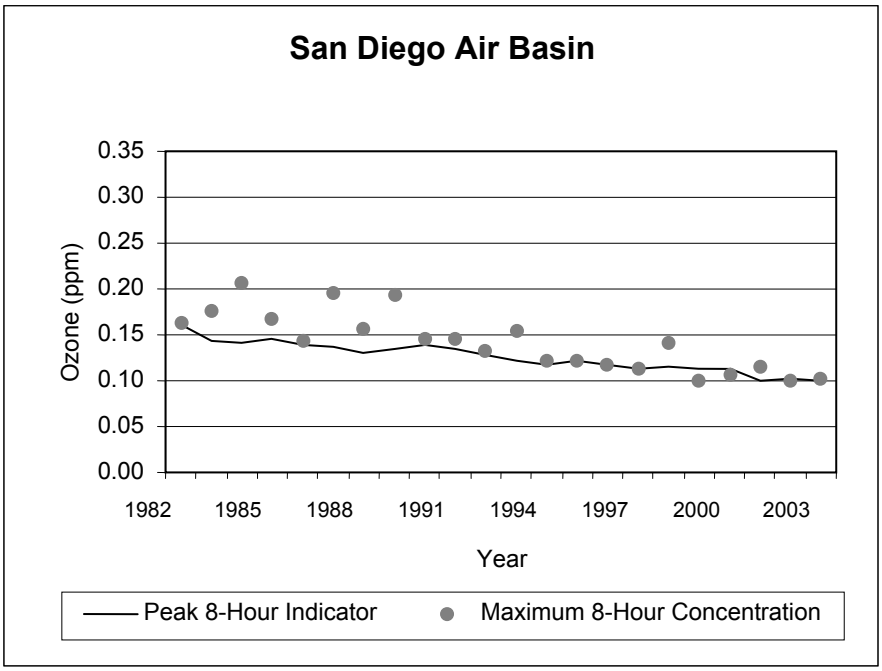


Figure 7-33



7.2.8.12 San Francisco Bay Area Air Basin

The nine-county San Francisco Bay Area Air Basin (SFBAAB) includes all of Alameda, Contra Costa, Marin, Napa, San Francisco, San Mateo, and Santa Clara counties, the southern half of Sonoma County, and the southwestern portion of Solano County. The SFBAAB is California's second largest urban area and is home to about 19 percent of California's citizens. Because of the SFBAAB's more favorable climate, with cooler temperatures and better ventilation, ozone concentrations in the SFBAAB are much lower than they are in the South Coast Air Basin and San Joaquin Valley Air Basin.

Although ozone air quality trends for the SFBAAB do not show a consistent downward trend, there have been overall improvements during the 22-year trend period. Furthermore, much of the year-to-year variation in the trends is probably attributable to variations in meteorology. The peak 1-hour indicator declined about 16 percent from 1982 to 2003, and the maximum 1-hour concentration declined about 15 percent. There is a much greater difference in the percentage changes of the two 8-hour statistics. The peak 8-hour indicator declined about 19 percent from 1982 to 2003, while the maximum 8-hour concentration declined only about 6 percent. However, it is important to keep in mind that changes in meteorology have a much greater influence on maximum concentrations than on peak indicator values. In contrast to previous years, values for 2001 through 2003 are lower and relatively stable compared with values during the prior few years, for both the 1-hour and 8-hour statistics.

The decreases in exceedance days have been more impressive than the decreases in concentrations and peak indicator values. During 1982, there were 36 State and 5 federal 1-hour exceedance days, compared with 19 State and 1 federal 1-hour exceedance days during 2003. This represents a reduction of nearly 50 percent in the number of State exceedance days and 80 percent in the number of federal 1-hour exceedance days. With respect to the federal 8-hour standard, there were 13 exceedance days in 1982 compared with 7 exceedance days during 2003. This represents a 46 percent decrease.

Figure 7-34

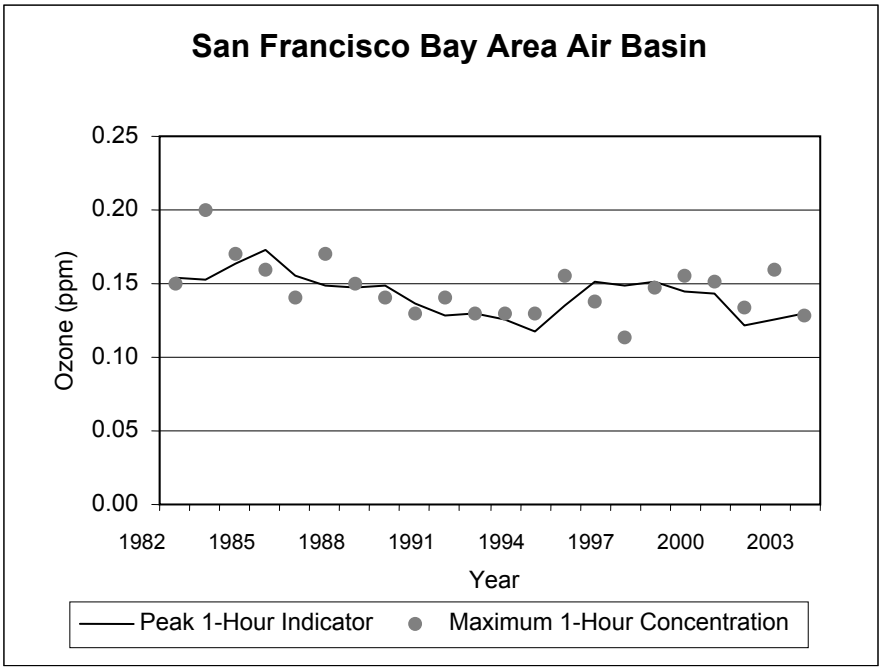
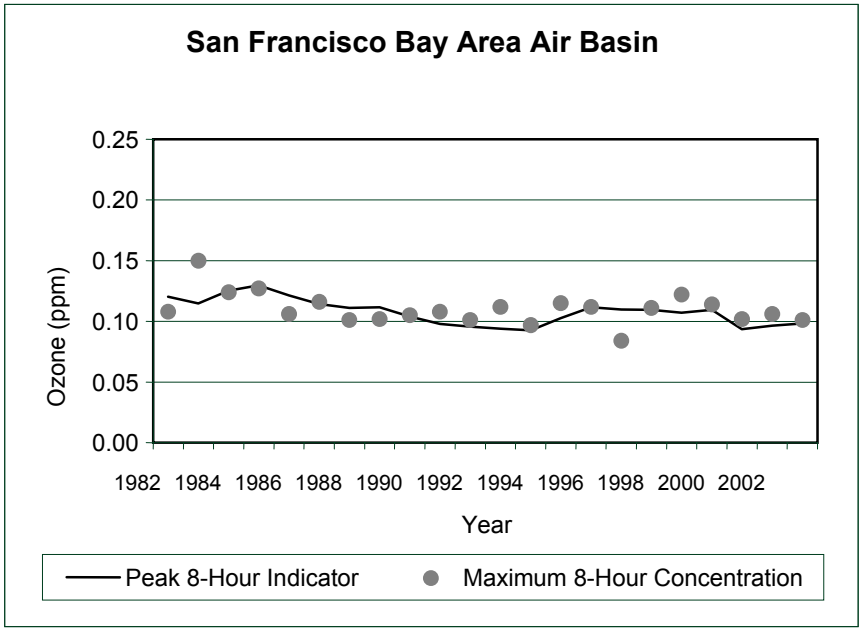


Figure 7-35



7.2.8.13 San Joaquin Valley Air Basin

The San Joaquin Valley Air Basin (SJVAB) occupies the southern two-thirds of California's Central Valley. The eight-county area includes Fresno, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare counties, as well as the western portion of Kern County. Close to 10 percent of the State's population resides in the SJVAB. Similar to other inland areas, the San Joaquin Valley has hot dry summers. Generally, the ozone season extends from April through October. Currently, the SJVAB is designated as nonattainment for both the State and federal 1-hour ozone standards. The SJVAB is also nonattainment for the federal 8-hour standard.

The ozone problem in the SJVAB ranks among the most severe in the State. While the average maximum 1-hour and 8-hour concentrations in the SJVAB during 2001 through 2003 were not vastly different from those in the Sacramento Metro Area, the number of exceedance days was substantially higher. During these three years, the SJVAB had an annual average of 129 State and 33 federal 1-hour exceedance days compared with 55 State and 6 federal 1-hour exceedance days in the Sacramento Metro Area. For the federal 8-hour standard, the SJVAB had an annual average of 123 exceedance days during 2001 through 2003, compared with 44 exceedance days in the Sacramento Metro Area.

In contrast to these comparisons, during 2001 through 2003, the SJVAB had a lower average maximum 1-hour concentration than the South Coast Air Basin (0.156 ppm compared with 0.184 ppm), but a slightly higher 3-year average of State standard exceedance days (129 days compared with 121 days). The same pattern holds true for the 8-hour statistics. During 2001 through 2003, the average maximum 8-hour concentration for the SJVAB was 0.126 ppm compared with 0.147 ppm for the South Coast Air Basin. However, during the same three years, the average number of federal 8-hour exceedances days was 123 for the SJVAB compared with 99 for the South Coast Air Basin.

Despite the severity of the SJVAB ozone problem, the area has made some progress in reducing ambient ozone. From 1982 to 2003, the peak 1-hour indicator decreased 19 percent, and the maximum 1-hour ozone concentration decreased 13 percent. In comparison to these modest declines, the decrease in the number of exceedance days is more substantial. Since the late 1980s, the number of federal 1-hour exceedance days has declined 50 percent. However, the decrease in the number of State exceedance days has not been as great (only 12 percent since the late 1980s).

With respect to the federal 8-hour standard, the pattern is similar, but the declines are smaller. From 1982 to 2003, the peak 8-hour indicator decreased about 12 percent, while the maximum 8-hour concentration decreased only about 5 percent. The decrease in the number of federal 8-hour exceedance days is much smaller than the decrease in federal 1-hour exceedance days. Since the late 1980s, the number of federal 8-hour exceedance days decreased only about 12 percent. This smaller decrease in 8-hour exceedances may stem from the fact that although the peak concentrations have been reduced, there are still a

substantial number of hours with concentrations above the level of the 8-hour standard, and these hours contribute to exceedances of the 8-hour standard.

Although the San Joaquin Valley has seen an overall improvement in ozone air quality, this region remains a challenge. High concentration areas still remain, especially in the major urban areas of Fresno and Bakersfield. The geography and climate pose a significant challenge to air quality progress, as high summer temperatures coupled with poor ventilation in the valley area contribute to high ozone levels.

Figure 7-36

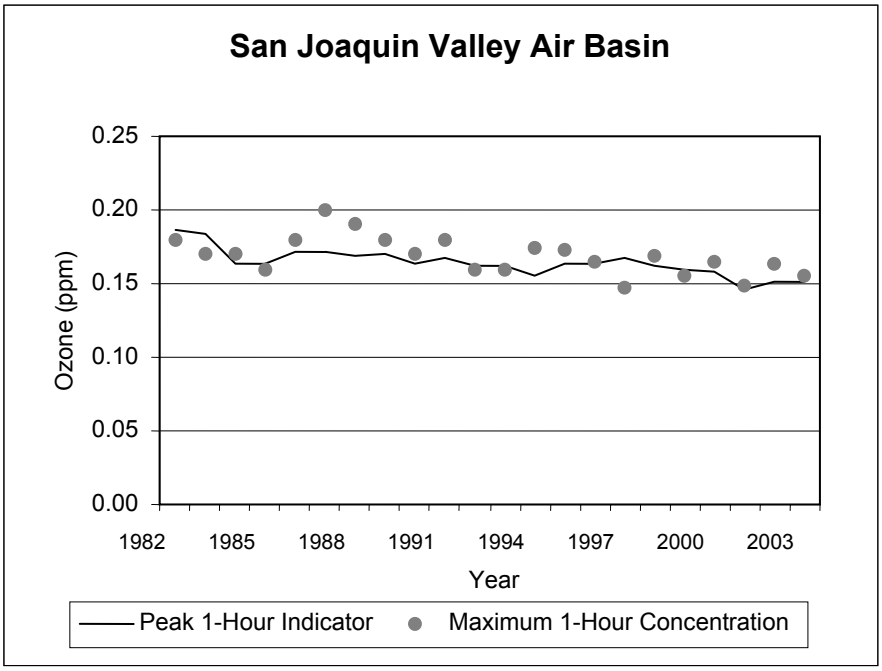
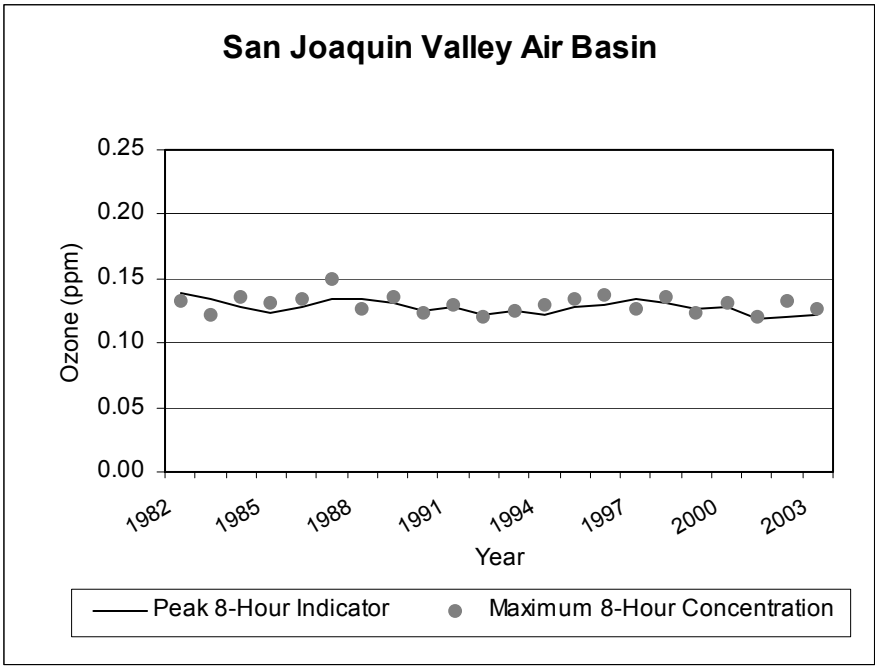


Figure 7-37



7.2.8.14 South Central Coast Air Basin

The South Central Coast Air Basin (SCCAB) includes all of San Luis Obispo, Santa Barbara, and Ventura counties. About 4 percent of the State's total population live in the SCCAB.

During January 2004, the Board designated San Luis Obispo as attainment for the State ozone standard. The remainder of the SCCAB is designated as nonattainment for the State ozone standard. For the federal 1-hour standard, both San Luis Obispo County and Santa Barbara County are designated as unclassified/attainment while Ventura County remains designated as nonattainment. However, Ventura County has made considerable progress and now qualifies as attainment for the federal 1-hour standard.

Ozone statistics for both the 1-hour and 8-hour averaging times show similar trends, with a consistent decline over the 22-year trend period. The peak 1-hour and 8-hour indicators show overall drops of 37 percent and 36 percent, respectively, from 1982 to 2003. The maximum 1-hour and 8-hour concentrations show a substantial amount of variation from one year to the next, probably caused by year-to-year differences in meteorology. However, between 1982 and 2003, the maximum 1-hour concentration shows an overall decline of 43 percent and the maximum 8-hour concentration shows an overall decline of 32 percent.

The number of exceedance days declined by an even larger percentage. The number of State standard exceedance days decreased from 145 in 1982 to 45 in 2003 (an overall decrease of about 69 percent). During this same time period, the number of federal 1-hour exceedance days decreased from 72 to only 2 (an overall reduction of 97 percent). The number of 8-hour exceedance days also shows a substantial decrease, a 72 percent reduction from 1982 to 2003. As mentioned above, the entire SCCAB has now either attained or is close to attaining the federal 1-hour ozone standard. With respect to the federal 8-hour standard, Ventura County is nonattainment, whereas San Luis Obispo and Santa Barbara counties are attainment for the federal 8-hour standard.

Figure 7-38

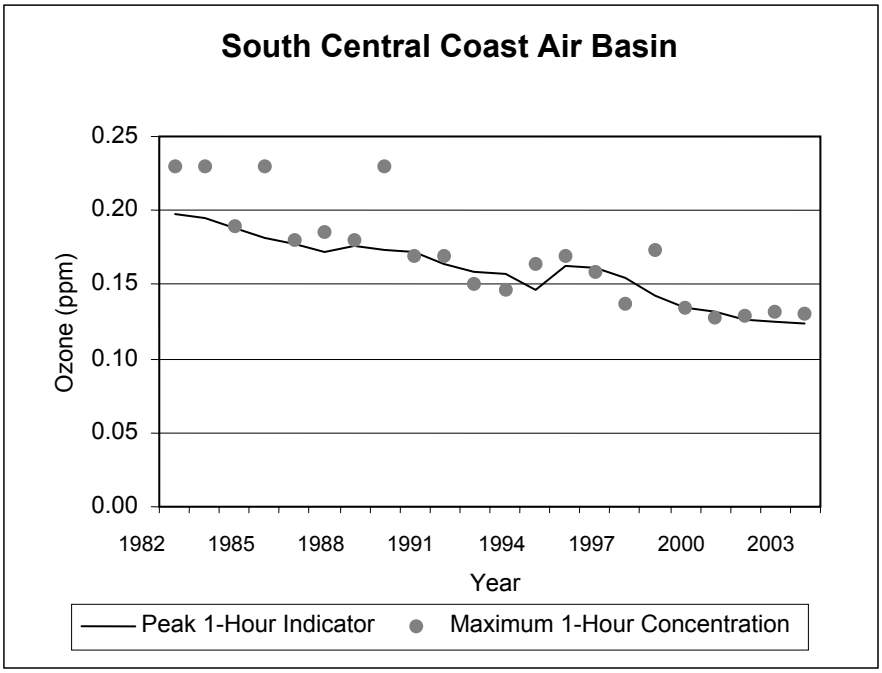
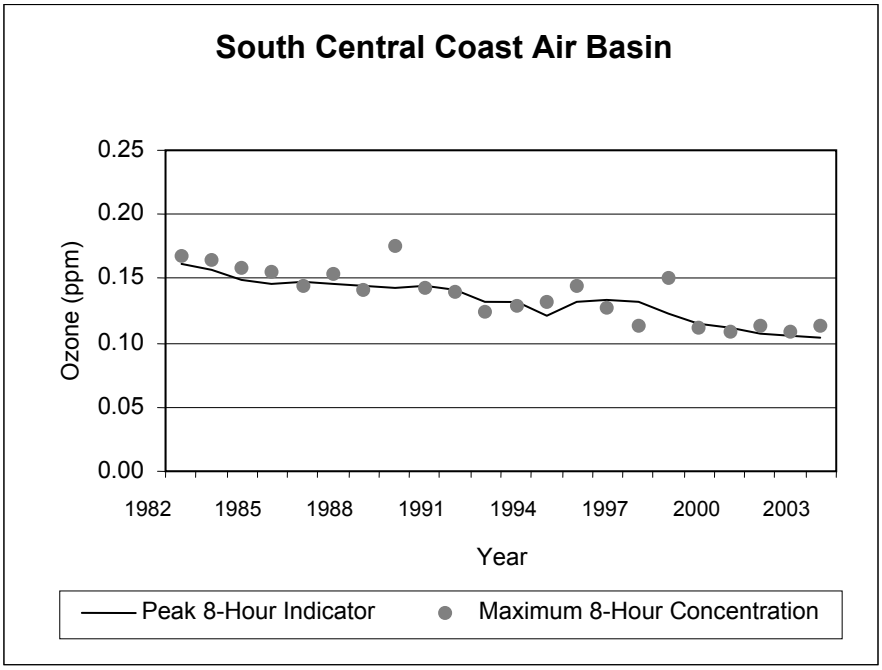


Figure 7-39



7.2.8.15 South Coast Air Basin

The South Coast Air Basin (SoCAB) includes California's largest metropolitan region, and 43 percent of the State's population lives within its boundaries. The area includes the southern two-thirds of Los Angeles County, all of Orange County, and the western urbanized portions of Riverside and San Bernardino counties. The area's warm sunny weather, associated with a persistent high-pressure system, is conducive to the formation of ozone. The ozone problem is further aggravated by surrounding mountains, frequent low inversion heights, and stagnant air conditions. These factors all act together to trap pollutants within the SoCAB.

The ozone problem in the SoCAB is one of the most severe in the State, in terms of maximum concentrations and number of days with exceedances of the standards. However, there has been considerable improvement over the years. The long tradition of emission control programs in the SoCAB has had a continuing impact on the area's ozone air quality. The long-term trends show substantial improvements in ozone air quality, despite substantial increases in population and the number of vehicle miles traveled each day.

Overall, the peak 1-hour indicator declined 54 percent from 1982 to 2003, and the peak 8-hour indicator declined 47 percent. The maximum measured concentrations show similar rates of decline during the same time period, with the maximum 1-hour concentration decreasing 52 percent and the maximum 8-hour concentration decreasing 42 percent. From 1982 to 2003, the number of State standard exceedance days decreased about 37 percent. During this same 22-year period, the number of days with concentrations above the federal 1-hour standard decreased 58 percent and the number of days with concentrations above the federal 8-hour standard decreased 34 percent.

The 2003 ozone season in the South Coast Air Basin was worse than any ozone season during the previous five years. Furthermore, there were more days with concentrations above the ozone standards than during any year since 1998. The region also recorded its first Stage 1 smog alert since 1998. A Stage 1 alert occurs when the 1-hour ozone concentration reaches 0.20 ppm. Because the 2003 Stage 1 smog alert occurred at a special study site (Rim of the World High School), it is not included as part of the data reflected in Figures 7.41 and 7.42. ARB staff analyses indicate that 2003 had more days with meteorological conditions that are conducive to higher ozone than in the last 24 years. Therefore, the higher values were substantially influenced by meteorology rather than overall changes in emissions.

Figure 7-40*

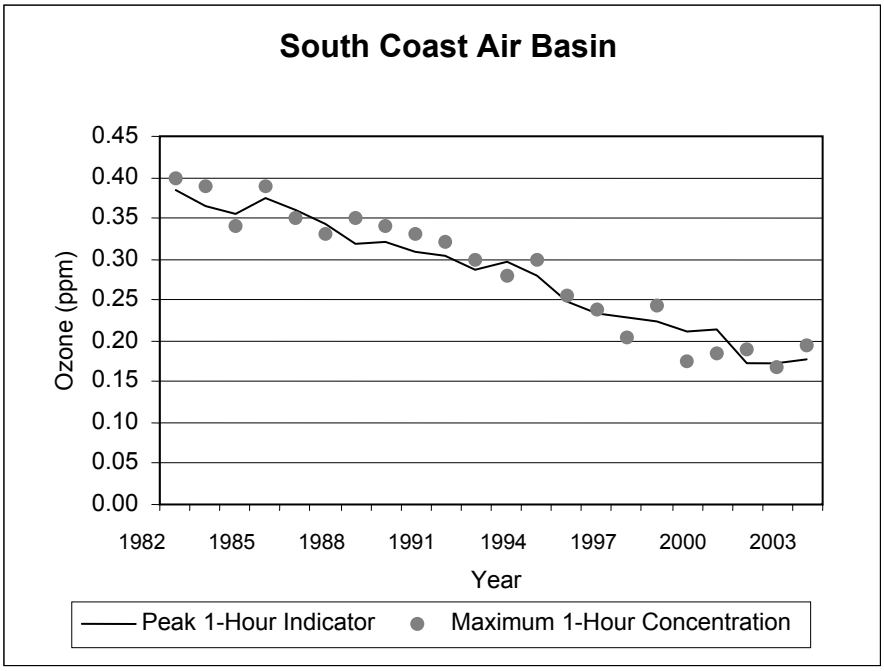
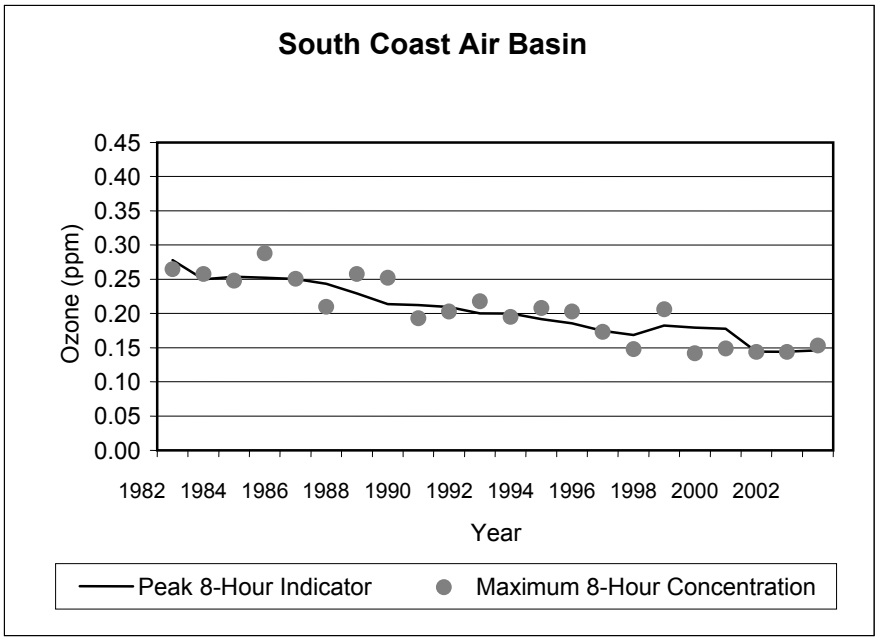


Figure 7-41*



*Values for the South Coast Air Basin do not include data for the Rim of the World High School site.

7.2.8.16 Upper Sacramento Valley Area

The Upper Sacramento Valley (USV) generally comprises the northern two-thirds of the Sacramento Valley Air Basin. The USV includes the northern two-thirds of Sutter County and all of Butte, Colusa, Glenn, Shasta, Tehama, and Yuba counties. Less than 2 percent of the State's population live in the USV.

Although the USV attains the federal 1-hour ozone standard, ambient concentrations continue to exceed the State standard. Furthermore, portions of the USV are nonattainment for the federal 8-hour ozone standard. Overall, the 1-hour and 8-hour ozone statistics for the USV show little change over the 22-year period. During 1982 through 2003, the maximum 1-hour concentrations ranged from 0.10 ppm to 0.14 ppm, with a difference of only 6 percent in the values for 1982 and 2003. Maximum 8-hour concentrations ranged from 0.89 ppm to 0.126 ppm during the same time period, with almost no difference between the end-year values (0.096 ppm in 1982 versus 0.099 ppm in 2003). The peak 1-hour and 8-hour indicator values also show little change across the entire trend period, and the values for the end-years are very close, for both averaging times. During 2001 through 2003, the USV experienced an average of 16 days each year with concentrations exceeding the State 1-hour ozone standard and 14 days each year with concentrations exceeding the federal 8-hour standard. There were no exceedances of the federal 1-hour ozone standard during this same time period.

Figure 7-42

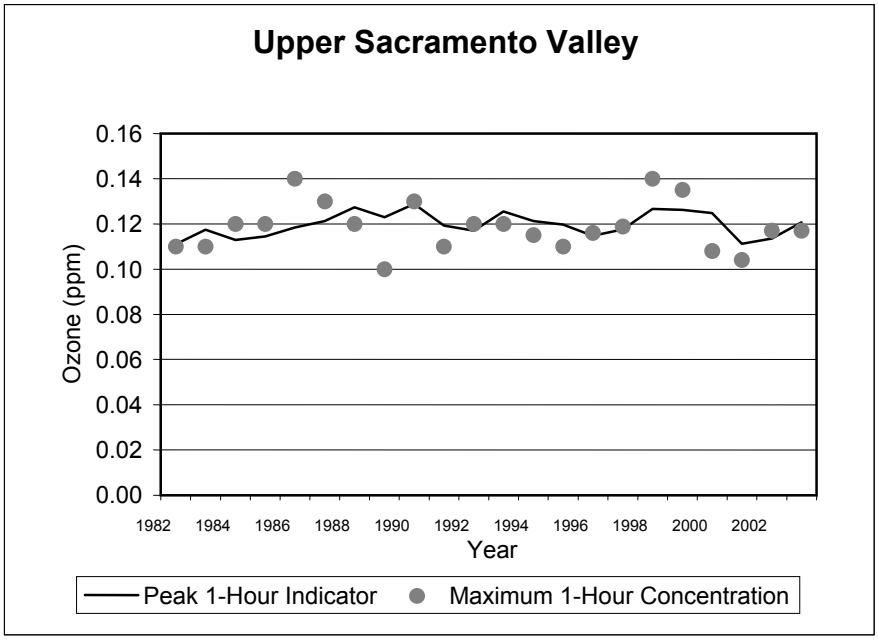
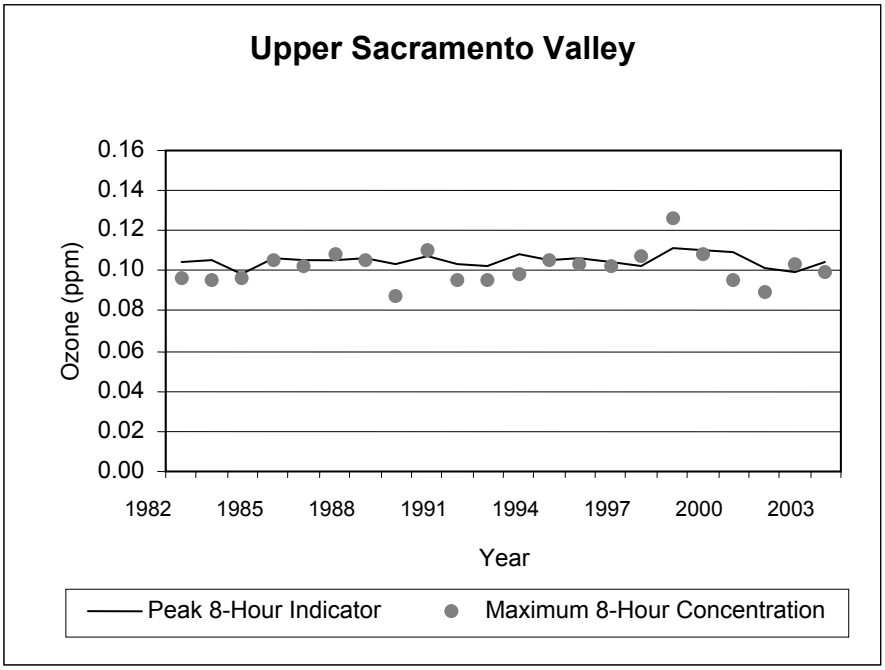


Figure 7-43



7.3 Analysis of Peak Ozone Exposure in California

7.3.1 Introduction

This section provides information on the ranges of peak outdoor 1-hour and 8-hour ozone concentrations to which people in different parts of California are potentially exposed. The term “potentially” is used because we realize that daily activity patterns influence a person’s exposure. For example, being inside a building will decrease a person’s exposure to outdoor ozone concentrations in their vicinity. However, any person who is outdoors during the peak concentration period will be exposed to the peak concentration. Furthermore, the exposures presented here provide an integrated regional perspective rather than an indication of exposure at any individual location.

This exposure analysis is based solely on “outdoor” ozone data, as measured by the statewide network of ambient ozone monitoring sites. The following tables and graphs present information on the population that could be exposed to different peak ozone concentrations within each air basin or planning area in California, as well as for the State as a whole. It is important to note that no exposure information is included for the Great Basin Valleys and Northeast Plateau air basins. As described in Section 7.4.2, below, the exposure indicator is based on three years of data (2001 through 2003). At the time this Staff Report was prepared, the third quarter 2001 ozone data for the Great Basin Valleys were under investigation by the local district and ARB. These data appeared to be low, by a factor of 10, and therefore, could not be used to calculate a reliable peak value. In contrast, the 2001 ozone data for the Northeast Plateau are missing for key summer months, when ozone is expected to be highest. Therefore, these data are not sufficient for calculating a realiable peak value.

7.3.2 Calculation of Peak Outdoor Ozone Exposures

This analysis is based on the Inverse Distance Weighting method from the Geostatistical Analyst 8.2 software. For this discussion, ozone peak indicator values and population counts were associated by census tract and merged to assemble a distribution of exposures across a range of peak ozone concentrations.

Concentrations of many air pollutants, including ozone, may change substantially from place to place. Accordingly, population exposure estimates tend to be more accurate when the population data and air quality data on which they are based are highly resolved, geographically. Population counts by census tract provide a convenient source of highly resolved population data. A typical census tract contains several thousand people. As a result, densely populated areas have many census tracts, while sparsely populated areas have very few.

Air quality data from the statewide network of monitors were also resolved to each census tract. The concentration assigned to a census tract was a weighted-average of the concentrations measured at the monitors. The weight assigned to each monitor was a function of its distance from the centroid or center of mass of the census tract, using an inverse distance weighting function (1/distance to a power). In this way, close monitors are more influential than are distant monitors. Using a weighting factor of 1/distance squared is a common

practice. However, the Geostatistical Analyst 8.2 software was able to modify the power used, in order to improve the estimations. In addition to the weighting factor, a search radius of 50 kilometers was specified. However, each census tract interpolation was based on observations for at least three neighboring sites, even if those sites were beyond the 50 kilometer search radius. Geographical barriers such as mountain ranges that may impede the movement of emissions and pollutants were not considered in the exposure calculations, but this omission had little impact on the results since monitors typically collect data in populated areas on both sides of such barriers.

Ambient ozone data used in this exposure analysis were extracted from the ARB air quality data CD (ARB 2004) and from the ARB ADAM database, and they represent the annual 1-hour and 8-hour peak indicator values for each site during the 2001 through 2003 time period. The peak indicator, sometimes referred to as the Expected Peak Day Concentration or EPDC, is a highly precise estimate of the 99.73 percentile (364/365 percentile) of the 1-hour or 8-hour ozone concentrations measured at a monitoring site. An exponential-tail model is used to calculate the peak indicator, making use of the highest twenty percent of all daily maximum values during a 3-year period (ARB 1993). Because the peak indicator represents the highest concentration expected to occur once per year, on average, the exposure results provide an assessment of the highest expected 1-hour and 8-hour exposures for each monitor.

Distributions for peak exposures were prepared for both a 1-hour and an 8-hour averaging time, using year 2000 census data. On a statewide basis, about 84 percent of California's population is exposed to peak 1-hour values above the State standard (0.09 ppm) and about 67 percent are exposed to peak 8-hour values above the federal 8-hour ozone standard (0.08 ppm).

Complete results of the exposure analyses for both the 1-hour and 8-hour averaging times are given in the following tables and figures. For each averaging time, there are results for each of the air basins or planning areas, as well as for the State as a whole. For each area, the population is allocated to different peak ozone in 0.005 ppm concentration ranges. The cumulative percent of the population exposed to each range of values above the level of the relevant standard is also included.

Table 7-13 and Figures 7-45 through 7-59 show the distribution of exposure estimates based on the peak 1-hour indicator. All areas of the State except Lake County Air Basin show some percentage of their population exposed to peak 1-hour ozone levels above 0.09 ppm. Furthermore, six areas, Mojave Desert, Sacramento Metro Area, Salton Sea, San Joaquin Valley, South Coast, and Upper Sacramento Valley, have 98 to 100 percent of their population exposed to peak 1-hour ozone levels above 0.09 ppm.

The highest 1-hour exposures occur in the Mojave Desert, Salton Sea, San Joaquin Valley, and South Coast areas. In these four areas, 23 percent or more of the population was exposed to peak 1-hour ozone levels above 0.130 ppm. People in the Mojave Desert and South Coast areas were exposed to the highest 1-hour peak ozone values – in the range of 0.155 to 0.160 ppm in the Mojave Desert and 0.170 to 0.175 ppm in the South Coast. In comparison, the

maximum exposure levels were somewhat lower in the San Joaquin Valley (0.150 ppm to 0.155 ppm) and Salton Sea (0.145 ppm to 0.150 ppm). Regardless of how they compare, the peak exposure values for all four of these areas are still well above the level of the current State 1-hour ozone standard.

Table 7-14 and Figures 7.60 through 7.74 show the distribution of exposure estimates based on the peak 8-hour indicator. All areas of the State except the Lake County Air Basin show some exposure to peak 8-hour ozone levels above 0.08 ppm. In contrast, Salton Sea and San Joaquin Valley both show 100 percent of their population exposed to peak 8-hour ozone levels above 0.080 ppm. For the Mojave Desert, Sacramento Metro Area, and Upper Sacramento Valley, more than 98 percent of the population is exposed to peak 8-hour ozone levels above 0.080 ppm. However, the severity of these exposures varies greatly among all these areas.

To assess the level of outdoor exposure, one must look not only at the percent of population exposed, but also the range of concentrations reflected by the percentage value. For example, in the Upper Sacramento Valley, nearly 100 percent of the population is exposed to peak 8-hour ozone levels above 0.080 ppm. However, the highest peak indicator value is between 0.100 ppm and 0.105 ppm. In contrast, only about 86 percent of the population in the South Coast Air Basin is exposed to peak 8-hour ozone levels above 0.080 ppm. However, 34 percent of these exposures occur at levels above 0.105 ppm. Furthermore, the population in the South Coast Air Basin is much larger than the population in the Upper Sacramento Valley. As a result, the peak 8-hour ozone exposures in the South Coast Air Basin have a greater impact on public health compared to exposures in the Upper Sacramento Valley. These factors are very important to consider when interpreting the peak exposure data.

Table 7-13 Summary of Ozone Peak 1-Hour Indicator Population-Weighted Exposure

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
Great Basin Valleys	Representative data not available				
Lake County	0.075	0.08	1451	2.5%	
	0.080	0.085	54346	93.2%	
	0.085	0.090	2512	4.3%	
		LC Total:	**58309**	**Total >0.09**	**0.0%**
Lake Tahoe	0.080	0.085	11253	24.4%	
	0.085	0.090	3310	7.2%	
	0.090	0.095	1364	3.0%	3.0%
	0.095	0.100	3909	8.5%	11.4%
	0.100	0.105	7990	17.3%	28.7%
	0.105	0.110	6072	13.1%	41.9%
	0.110	0.115	4857	10.5%	52.4%
	0.115	0.120	7445	16.1%	68.5%
		LT Total:	**46200**	**Total >0.09**	**68.5%**
Mojave Desert	0.075	0.080	14619	1.8%	
	0.080	0.085	1858	0.2%	
	0.085	0.090	0	0.0%	
	0.090	0.095	2115	0.3%	0.3%
	0.095	0.100	13003	1.6%	1.9%
	0.100	0.105	43493	5.3%	7.2%
	0.105	0.110	49988	6.1%	13.3%
	0.110	0.115	7048	0.9%	14.2%
	0.115	0.120	34908	4.3%	18.4%
	0.120	0.125	3575	0.4%	18.9%
	0.125	0.130	97684	12.0%	30.8%
	0.130	0.135	124785	15.3%	46.1%
	0.135	0.140	266115	32.6%	78.7%
	0.140	0.145	146078	17.9%	96.6%
	0.145	0.150	8483	1.0%	97.6%
	0.150	0.155	2381	0.3%	97.9%
	0.155	0.160	609	0.1%	98.0%
		MD Total:	**816742**	**Total >0.09**	**98.0%**

Table 7-13 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
Mountain Counties	0.085	0.090	26088	9.9%	
	0.090	0.095	7393	2.8%	2.8%
	0.095	0.100	9935	3.8%	6.6%
	0.100	0.105	4993	1.9%	8.5%
	0.105	0.110	54037	20.5%	29.0%
	0.110	0.115	73533	27.9%	56.8%
	0.115	0.120	58102	22.0%	78.9%
	0.120	0.125	24067	9.1%	88.0%
	0.125	0.130	5549	2.1%	90.1%
		MC Total:	**263697**	**Total >0.09**	**90.1%**
North Central Coast	0.070	0.075	6025	0.8%	
	0.075	0.080	209446	29.5%	
	0.080	0.085	201570	28.4%	
	0.085	0.090	179902	25.3%	
	0.090	0.095	42330	6.0%	6.0%
	0.095	0.100	63684	9.0%	14.9%
	0.100	0.105	7641	1.1%	16.0%
		NCC Total:	**710598**	**Total >0.09**	**16.0%**
North Coast	0.065	0.070	24886	8.0%	
	0.070	0.075	22103	7.1%	
	0.075	0.080	48516	15.6%	
	0.080	0.085	57361	18.5%	
	0.085	0.090	47285	15.2%	
	0.090	0.095	96345	31.1%	31.1%
	0.095	0.100	13572	4.4%	35.4%
		NC Total:	**310068**	**Total >0.09**	**35.4%**
Northeast Plateau	Representative data not available				
Sacramento Metro Area	0.085	0.090	2703	0.2%	
	0.090	0.095	0	0.0%	0.0%
	0.095	0.100	734	0.0%	0.0%
	0.100	0.105	30024	1.7%	1.8%
	0.105	0.110	157159	9.0%	10.7%
	0.110	0.115	324179	18.5%	29.2%
	0.115	0.120	358081	20.4%	49.6%
	0.120	0.125	387246	22.1%	71.7%
	0.125	0.130	343817	19.6%	91.3%
	0.130	0.135	145127	8.3%	99.6%
	0.135	0.140	4941	0.3%	99.8%
		SMA	**1754011**	**Total >0.09**	**99.8%**

Table 7-13 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
Salton Sea	0.095	0.100	1139	0.2%	0.2%
	0.100	0.105	13406	2.9%	3.1%
	0.105	0.110	16123	3.5%	6.6%
	0.110	0.115	28677	6.2%	12.7%
	0.115	0.120	120808	25.9%	38.7%
	0.120	0.125	71786	15.4%	54.1%
	0.125	0.130	85422	18.3%	72.4%
	0.130	0.135	81352	17.5%	89.9%
	0.135	0.140	37889	8.1%	98.0%
	0.140	0.145	6448	1.4%	99.4%
	0.145	0.150	2836	0.6%	100.0%
		SS Total:	**465886**	**Total >0.09**	**100.0%**
San Diego	0.075	0.080	10139	0.4%	
	0.080	0.085	60883	2.2%	
	0.085	0.090	287031	10.2%	
	0.090	0.095	618435	22.0%	22.0%
	0.095	0.100	1081023	38.4%	60.4%
	0.100	0.105	579186	20.6%	81.0%
	0.105	0.110	108618	3.9%	84.8%
	0.110	0.115	60378	2.1%	87.0%
	0.115	0.120	7066	0.3%	87.2%
	0.120	0.125	1011	0.0%	87.3%
		SD Total:	**2813770**	**Total >0.09**	**87.3%**
San Francisco Bay Area	0.060	0.065	182456	2.7%	
	0.065	0.070	707493	10.6%	
	0.070	0.075	602682	9.0%	
	0.075	0.080	801287	12.0%	
	0.080	0.085	411973	6.2%	
	0.085	0.090	329285	4.9%	
	0.090	0.095	436796	6.6%	6.6%
	0.095	0.100	500334	7.5%	14.1%
	0.100	0.105	1309795	19.7%	33.7%
	0.105	0.110	1139535	17.1%	50.8%
	0.110	0.115	155743	2.3%	53.2%
	0.115	0.120	19512	0.3%	53.5%
	0.120	0.125	64229	1.0%	54.4%
		SFBA	**6661120**	**Total >0.09**	**54.4%**

Table 7-13 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
San Vallev	0.095	0.100	9814	0.3%	
	0.100	0.105	322715	10.1%	10.1%
	0.105	0.110	165526	5.2%	15.3%
	0.110	0.115	228875	7.2%	22.5%
	0.115	0.120	689818	21.6%	44.1%
	0.120	0.125	658731	20.7%	64.8%
	0.125	0.130	360413	11.3%	76.1%
	0.130	0.135	244276	7.7%	83.7%
	0.135	0.140	362643	11.4%	95.1%
	0.140	0.145	96589	3.0%	98.1%
	0.145	0.150	41823	1.3%	99.4%
	0.150	0.155	8162	0.3%	99.7%
		SJV Total:	**3189385**	**Total >0.09**	**99.7%**
South Coast	0.065	0.070	96532	6.9%	
	0.070	0.075	156513	11.2%	
	0.075	0.080	82498	5.9%	
	0.080	0.085	84029	6.0%	
	0.085	0.090	334320	23.9%	
	0.090	0.095	135552	9.7%	9.7%
	0.095	0.100	118109	8.4%	18.1%
	0.100	0.105	80854	5.8%	23.9%
	0.105	0.110	88803	6.3%	30.3%
	0.110	0.115	64995	4.6%	34.9%
	0.115	0.120	60142	4.3%	39.2%
	0.120	0.125	45428	3.2%	42.5%
	0.125	0.130	26760	1.9%	44.4%
	0.130	0.135	19269	1.4%	45.8%
	0.135	0.140	4692	0.3%	46.1%
		SCC	**1398496**	**Total >0.09**	**46.1%**

Table 7-13 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
South Coast	0.080	0.085	13235	0.1%	
	0.085	0.090	265666	1.8%	
	0.090	0.095	852643	5.8%	5.8%
	0.095	0.100	994109	6.8%	12.7%
	0.100	0.105	1400457	9.6%	22.3%
	0.105	0.110	1536657	10.5%	32.8%
	0.110	0.115	1027585	7.0%	39.8%
	0.115	0.120	1223463	8.4%	48.2%
	0.120	0.125	960987	6.6%	54.8%
	0.125	0.130	672583	4.6%	59.4%
	0.130	0.135	672383	4.6%	64.0%
	0.135	0.140	720309	4.9%	69.0%
	0.140	0.145	665763	4.6%	73.5%
	0.145	0.150	618711	4.2%	77.8%
	0.150	0.155	710721	4.9%	82.6%
	0.155	0.160	1061720	7.3%	89.9%
	0.160	0.165	686129	4.7%	94.6%
	0.165	0.170	499248	3.4%	98.0%
	0.170	0.175	8513	0.1%	98.1%
		SC Total:	**14590882**	**Total**	**98.1%**
Upper Sacramento Valley	0.085	0.090	1450	0.2%	
	0.090	0.095	35840	5.9%	5.9%
	0.095	0.100	261253	43.3%	49.2%
	0.100	0.105	148057	24.5%	73.8%
	0.105	0.110	129396	21.4%	95.2%
	0.110	0.115	20956	3.5%	98.7%
	0.115	0.120	6566	1.1%	99.8%
		USV Total:	**603518**	**Total**	**99.8%**

Table 7- 13 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.09 ppm
California	0.060	0.065	182456	0.5%	
	0.065	0.070	845294	2.5%	
	0.070	0.075	829558	2.5%	
	0.075	0.080	1167956	3.5%	
	0.080	0.085	896508	2.7%	
	0.085	0.090	1481723	4.4%	
	0.090	0.095	2230408	6.6%	6.6%
	0.095	0.100	3071868	9.1%	15.7%
	0.100	0.105	3948611	11.7%	27.4%
	0.105	0.110	3457662	10.2%	37.7%
	0.110	0.115	1996826	5.9%	43.6%
	0.115	0.120	2585911	7.7%	51.2%
	0.120	0.125	2217060	6.6%	57.8%
	0.125	0.130	1592228	4.7%	62.5%
	0.130	0.135	1287192	3.8%	66.3%
	0.135	0.140	1396589	4.1%	70.5%
	0.140	0.145	914878	2.7%	73.2%
	0.145	0.150	671853	2.0%	75.2%
	0.150	0.155	721264	2.1%	77.3%
	0.155	0.160	1062329	3.1%	80.5%
	0.160	0.165	686129	2.0%	82.5%
	0.165	0.170	499248	1.5%	84.0%
	0.170	0.175	8513	0.0%	84.0%
		State	**33752064**	**Total >0.09**	**84.0%**

Figure 7-44

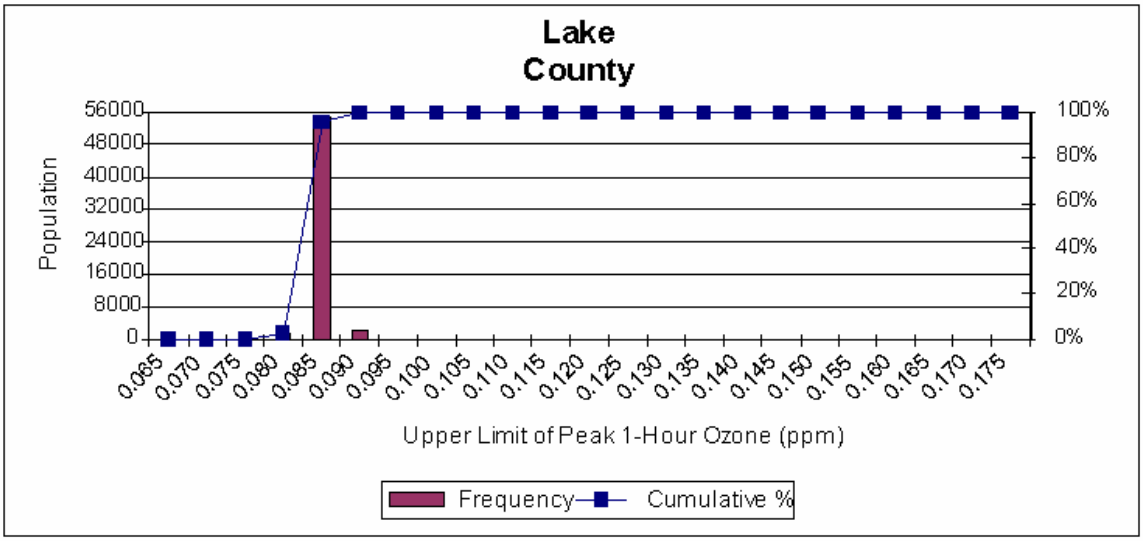


Figure 7-45

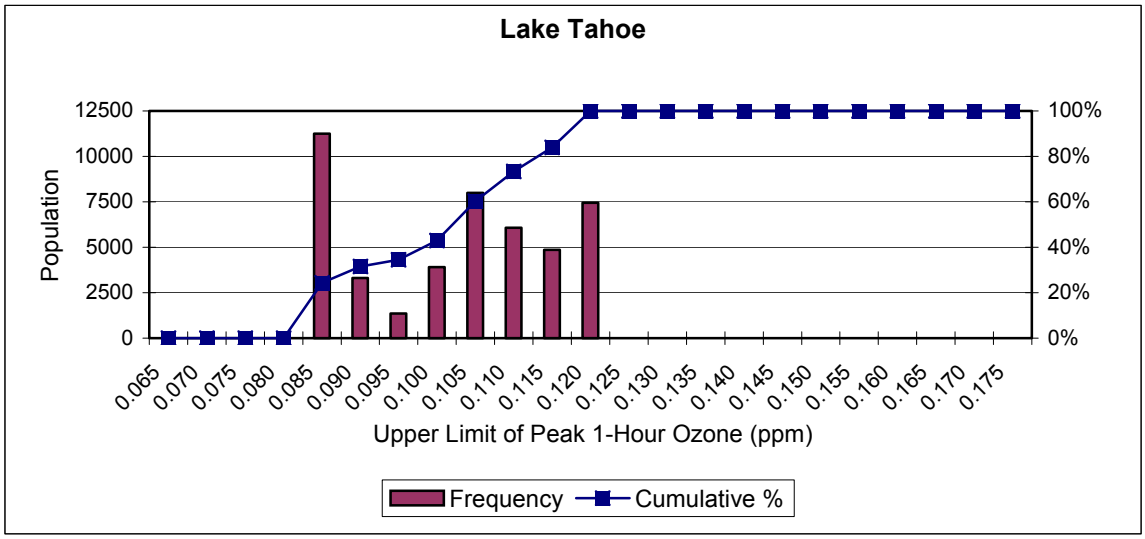


Figure 7-46

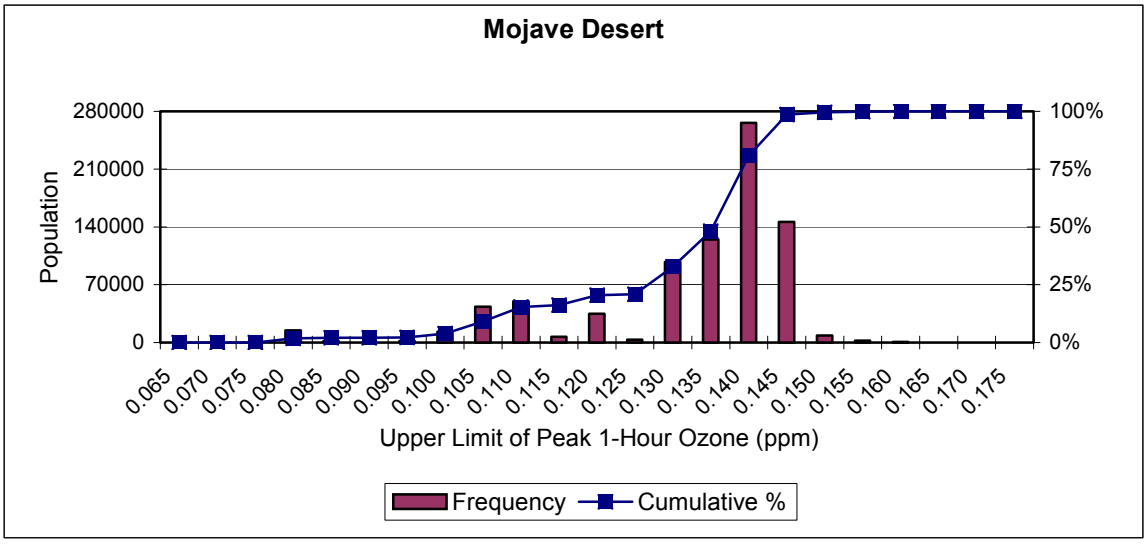


Figure 7-47

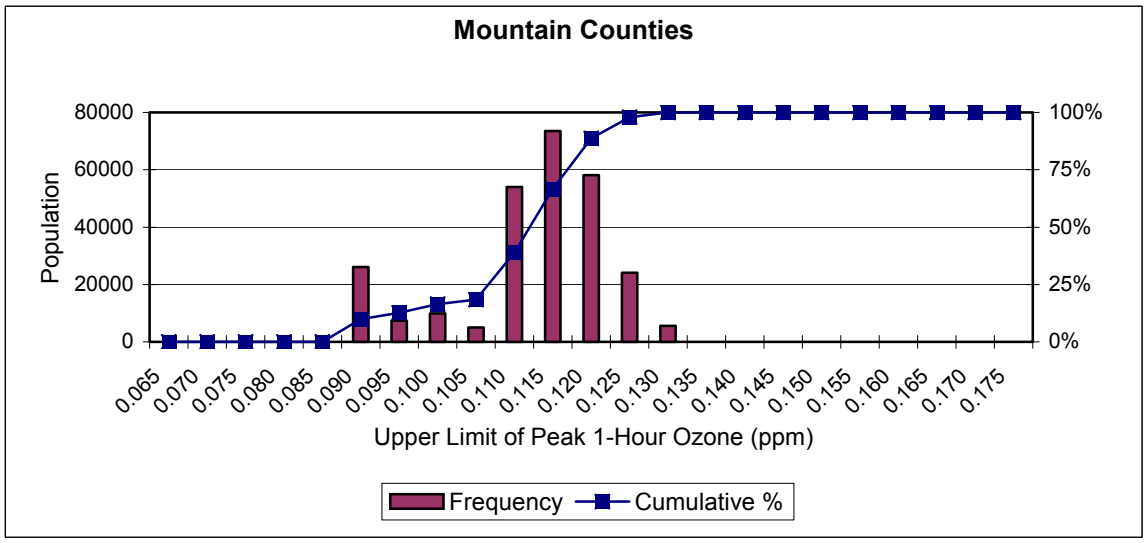


Figure 7-48

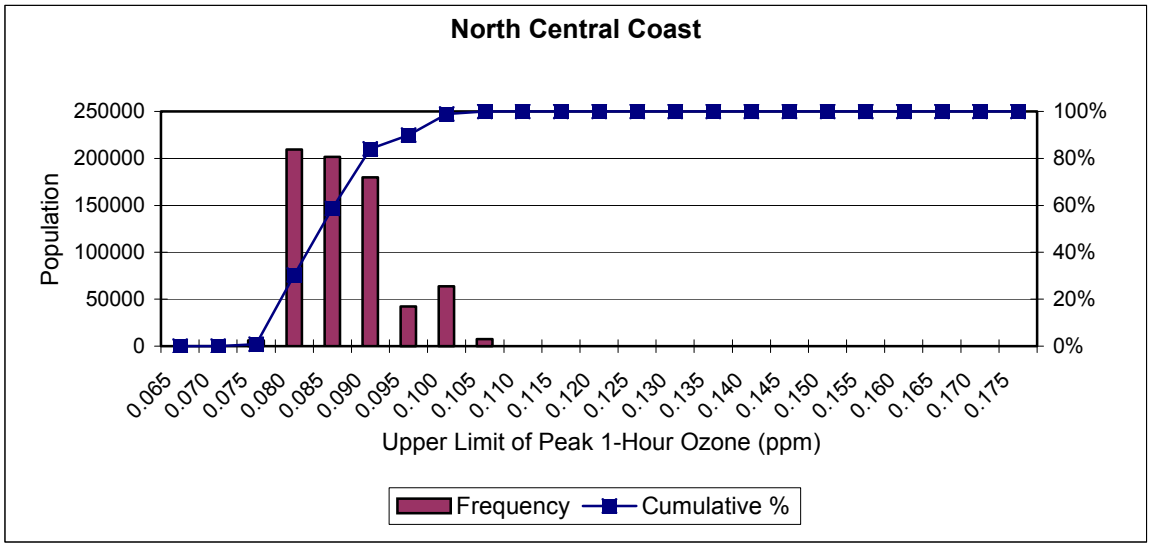


Figure 7-49

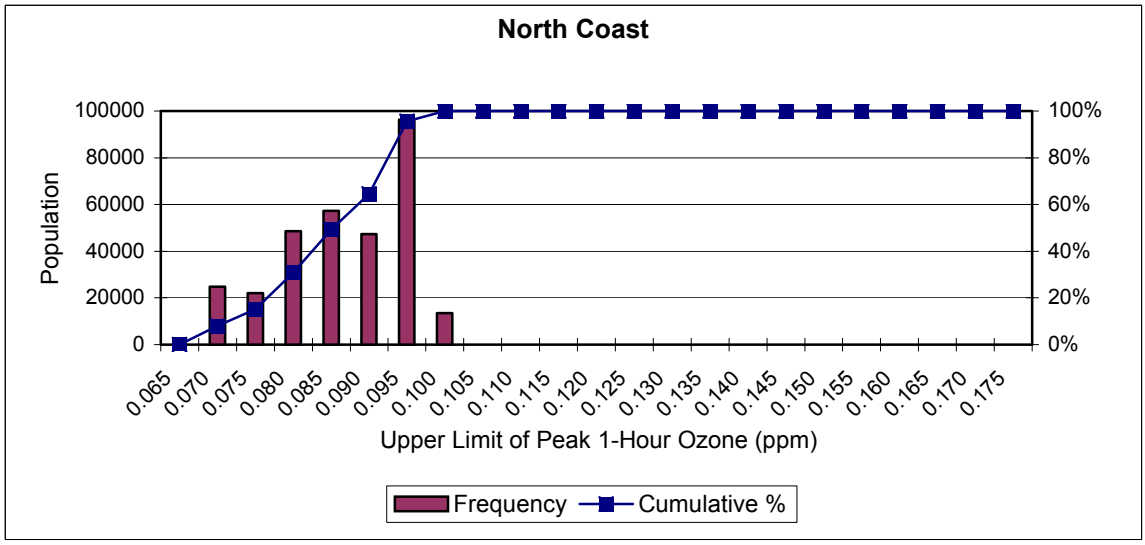


Figure 7-50

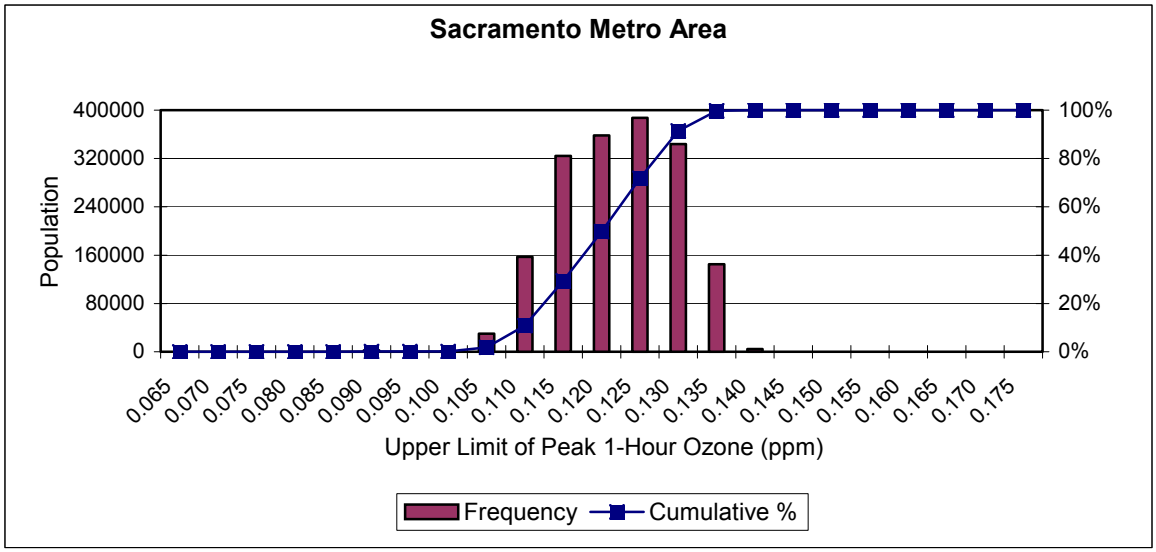


Figure 7-51

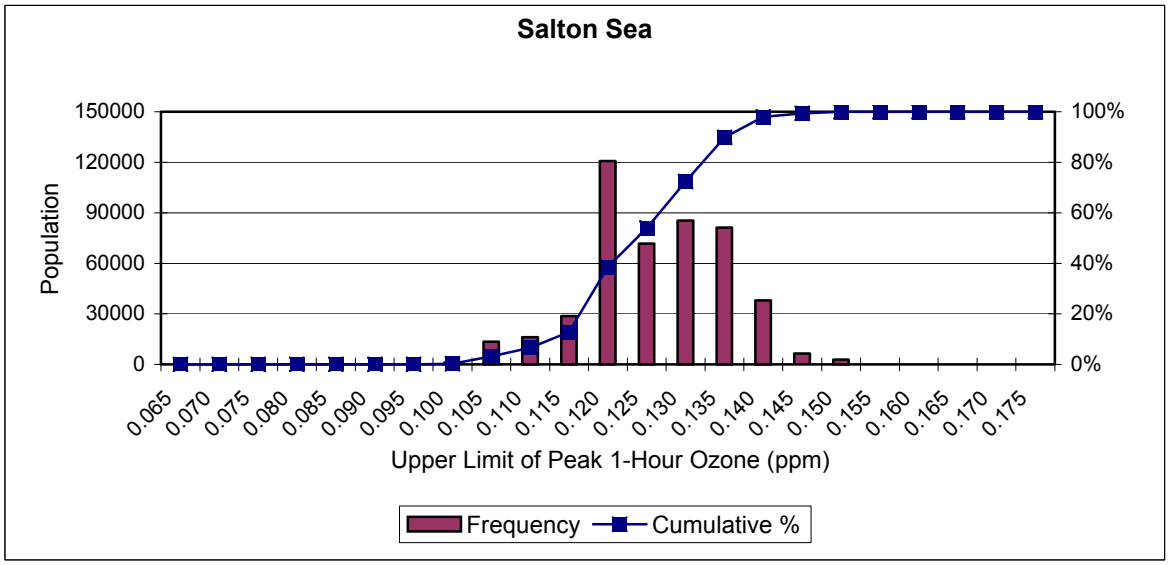


Figure 7-52

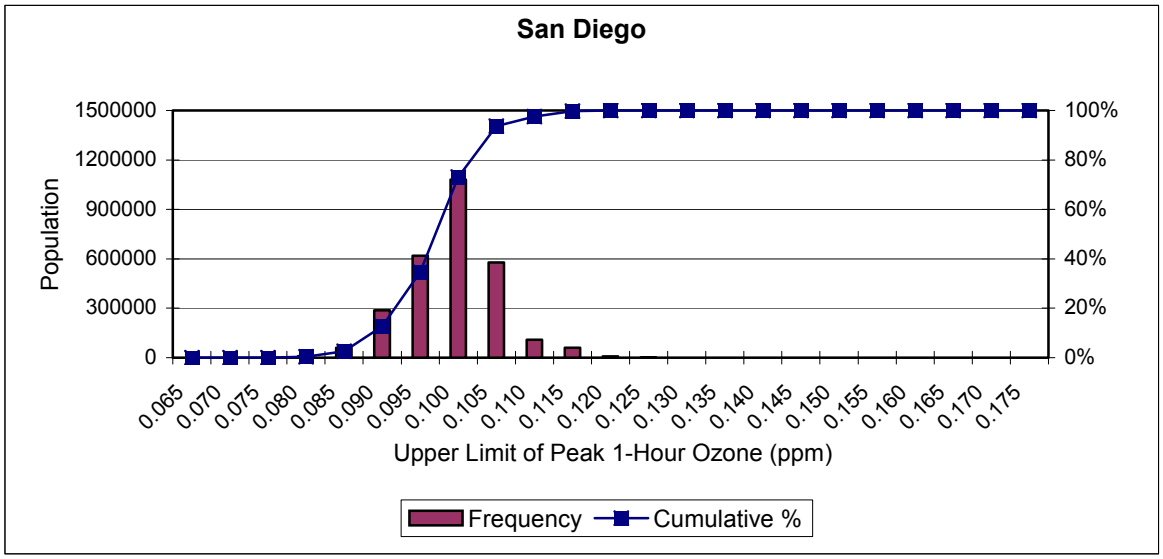


Figure 7-53

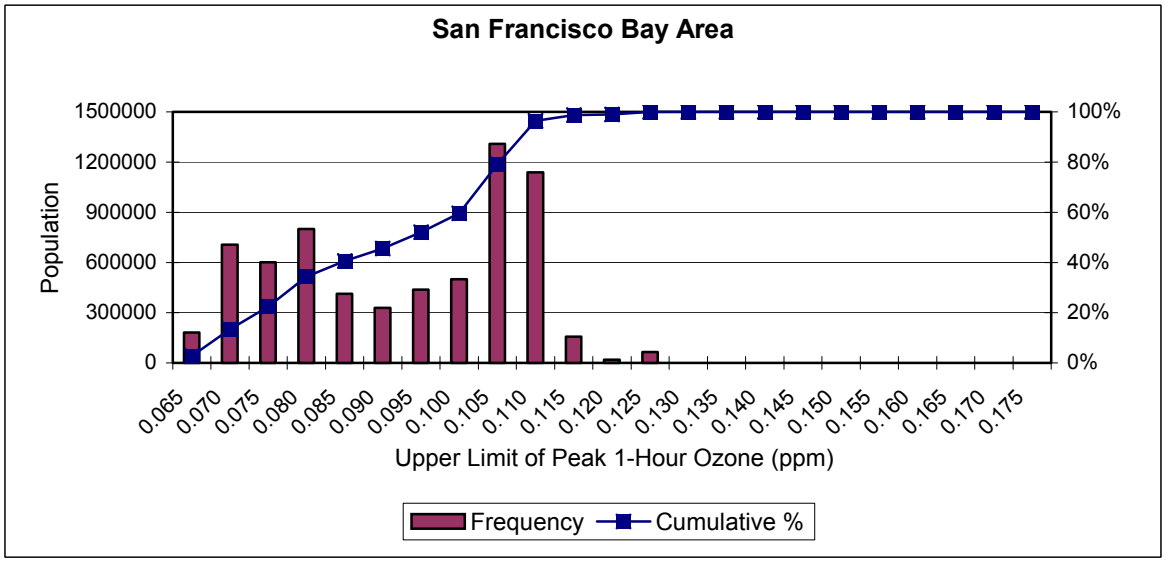


Figure 7-54

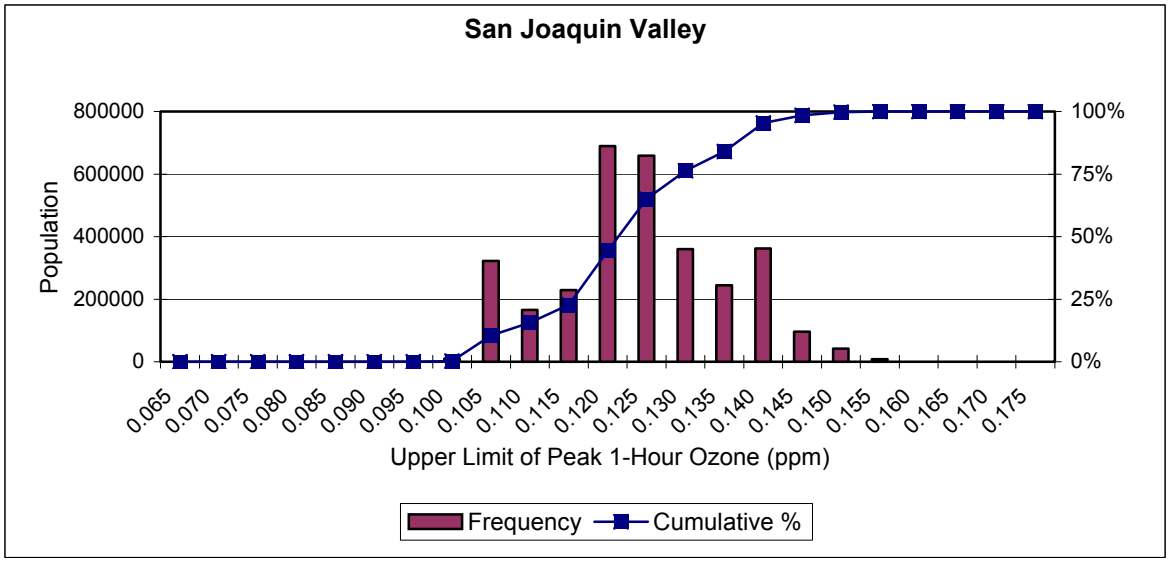


Figure 7-55

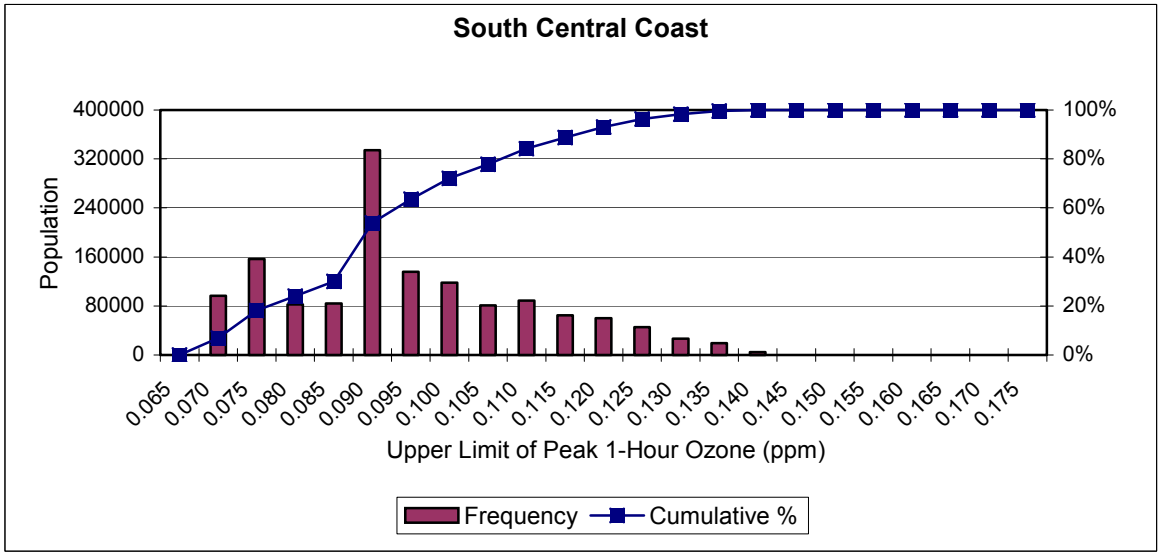


Figure 7-56

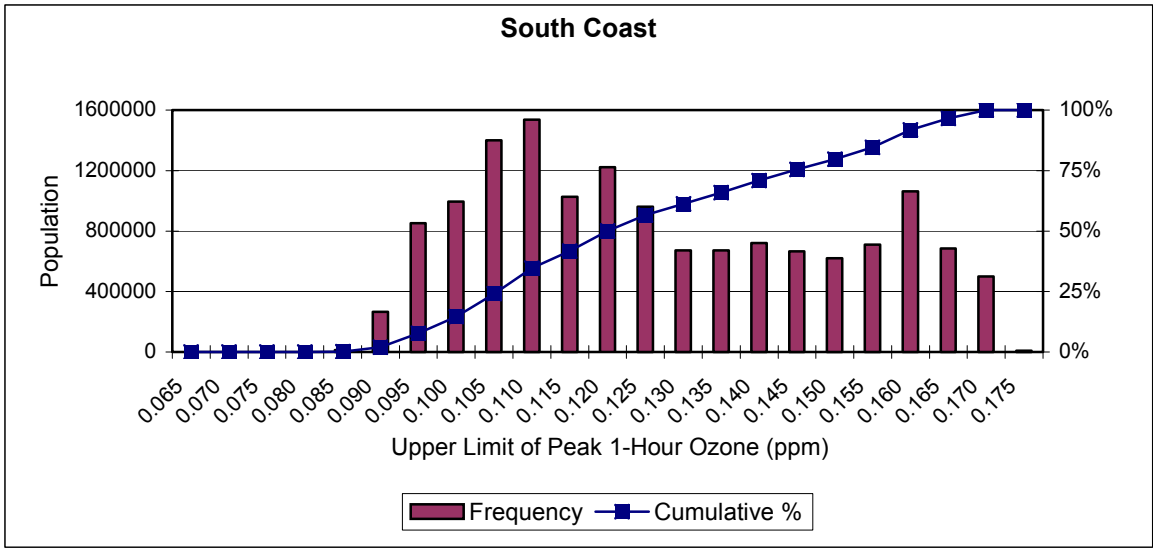


Figure 7-57

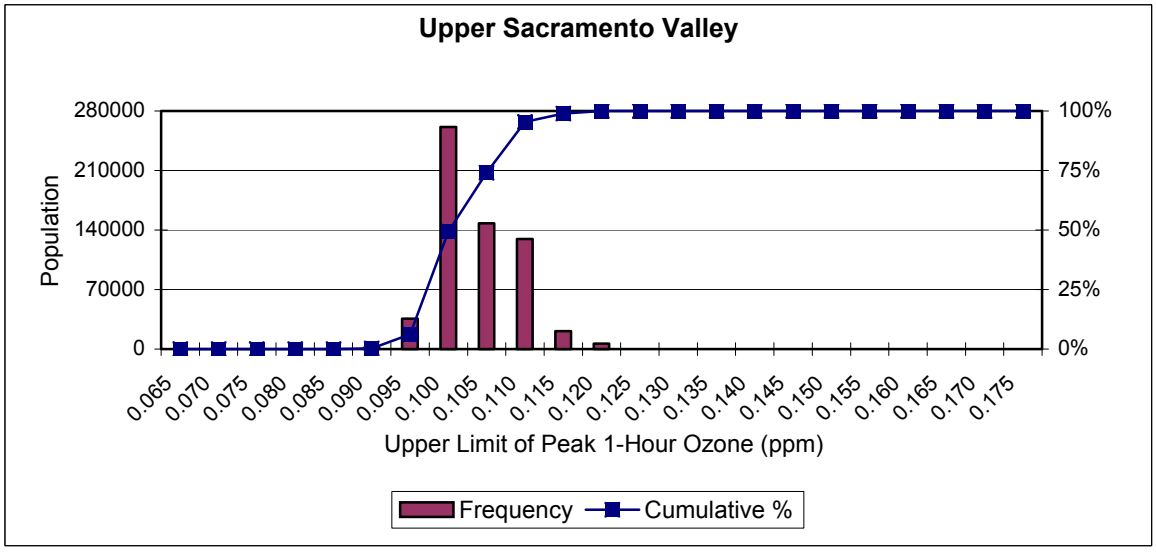


Figure 7-58

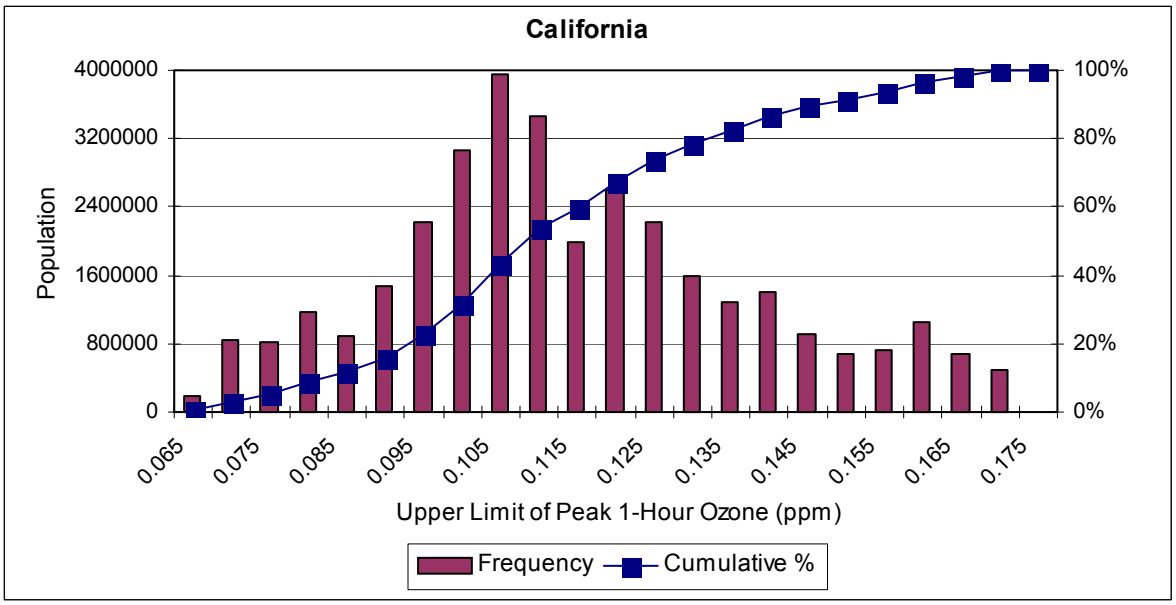

California
4000000
3200000
2400000
1600000
800000
0
Population
100%
80%
60%
40%
20%
0%
0.065
0.075
0.085
0.095
0.105
0.115
0.125
0.135
0.145
0.155
0.165
0.175
Upper Limit of Peak 1-Hour Ozone (ppm)
Frequency
Cumulative %

Table 7-14 Summary of Ozone Peak 8-Hour Indicator Population-Weighted Exposure

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.08 ppm
Great Basin Valleys	Representative data not available				
Lake County	0.065	0.070	10253	17.6%	
	0.070	0.075	45544	78.1%	
	0.075	0.080	2512	4.3%	
		LC Total:	**58309**	**Total >0.08**	**0.0%**
Lake Tahoe	0.070	0.075	1441	3.1%	
	0.075	0.080	13122	28.4%	
	0.080	0.085	4269	9.2%	9.2%
	0.085	0.090	2910	6.3%	15.5%
	0.090	0.095	9921	21.5%	37.0%
	0.095	0.100	6825	14.8%	51.8%
	0.100	0.105	7712	16.7%	68.5%
		LT Total:	**46200**	**Total >0.08**	**68.5%**
Mojave Desert	0.070	0.075	14619	1.8%	
	0.075	0.080	1858	0.2%	
	0.080	0.085	10423	1.3%	1.3%
	0.085	0.090	630	0.1%	1.4%
	0.090	0.095	51709	6.3%	7.7%
	0.095	0.100	49749	6.1%	13.8%
	0.100	0.105	24299	3.0%	16.8%
	0.105	0.110	32243	3.9%	20.7%
	0.110	0.115	167985	20.6%	41.3%
	0.115	0.120	411271	50.4%	91.6%
	0.120	0.125	45229	5.5%	97.2%
	0.125	0.130	5686	0.7%	97.9%
	0.130	0.135	1041	0.1%	98.0%
		MD Total:	**816742**	**Total >0.08**	**98.0%**

Table 7-14 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.08 ppm
Mountain Counties	0.070	0.075	9811	3.7%	
	0.075	0.080	6365	2.4%	
	0.080	0.085	18906	7.2%	7.2%
	0.085	0.090	11747	4.5%	11.6%
	0.090	0.095	63407	24.0%	35.7%
	0.095	0.100	72750	27.6%	63.3%
	0.100	0.105	9852	3.7%	67.0%
	0.105	0.110	70859	26.9%	93.9%
		MC Total:	**263697**	**Total >0.08**	**93.9%**
North Central Coast	0.060	0.065	50075	7.0%	
	0.065	0.070	302175	42.5%	
	0.070	0.075	244556	34.4%	
	0.075	0.080	47454	6.7%	
	0.080	0.085	66338	9.3%	9.3%
		NCC Total:	**710598**	**Total >0.08**	**9.3%**
North Coast	0.05	0.055	10409	3.4%	
	0.055	0.060	27178	8.8%	
	0.060	0.065	37665	12.1%	
	0.065	0.070	75408	24.3%	
	0.070	0.075	7855	2.5%	
	0.075	0.080	50707	16.4%	
	0.080	0.085	91266	29.4%	29.4%
	0.085	0.090	9580	3.1%	32.5%
		NC Total:	**310068**	**Total >0.08**	**32.5%**
Northeast Plateau	Representative data not available				
Sacramento Metro Area	0.075	0.080	3343	0.2%	
	0.080	0.085	6737	0.4%	0.4%
	0.085	0.090	127056	7.2%	7.6%
	0.090	0.095	330397	18.8%	26.5%
	0.095	0.100	387534	22.1%	48.6%
	0.100	0.105	522121	29.8%	78.3%
	0.105	0.110	317033	18.1%	96.4%
	0.110	0.115	59790	3.4%	99.8%
		SMA	**1754011**	**Total >0.08**	**99.8%**

Table 7-14 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.08 ppm
Salton Sea	0.080	0.085	11054	2.4%	2.4%
	0.085	0.090	33027	7.1%	9.5%
	0.090	0.095	77733	16.7%	26.1%
	0.095	0.100	35919	7.7%	33.9%
	0.100	0.105	81231	17.4%	51.3%
	0.105	0.110	60682	13.0%	64.3%
	0.110	0.115	41624	8.9%	73.3%
	0.115	0.120	111986	24.0%	97.3%
	0.120	0.125	12104	2.6%	99.9%
	0.125	0.130	526	0.1%	100.0%
		SS Total:	**465886**	**Total >0.08**	**100.0%**
San Diego	0.065	0.070	50101	1.8%	
	0.070	0.075	427672	15.2%	
	0.075	0.080	1051093	37.4%	
	0.080	0.085	1098796	39.1%	39.1%
	0.085	0.090	75922	2.7%	41.7%
	0.090	0.095	99909	3.6%	45.3%
	0.095	0.100	9266	0.3%	45.6%
	0.100	0.105	1011	0.0%	45.7%
		SD Total:	**2813770**	**Total >0.08**	**45.7%**
San Francisco Bay Area	0.05	0.055	763096	11.5%	
	0.055	0.060	957772	14.4%	
	0.060	0.065	1020174	15.3%	
	0.065	0.070	668135	10.0%	
	0.070	0.075	991820	14.9%	
	0.075	0.080	1384142	20.8%	
	0.080	0.085	431659	6.5%	6.5%
	0.085	0.090	361776	5.4%	11.9%
	0.090	0.095	82546	1.2%	13.2%
		SFBA	**6661120**	**Total >0.08**	**13.2%**
San Joaquin Valley	0.080	0.085	8579	0.3%	0.3%
	0.085	0.090	353061	11.1%	11.3%
	0.090	0.095	277622	8.7%	20.0%
	0.095	0.100	317234	9.9%	30.0%
	0.100	0.105	656639	20.6%	50.6%
	0.105	0.110	820218	25.7%	76.3%
	0.110	0.115	723099	22.7%	99.0%
	0.115	0.120	32933	1.0%	100.0%
		SJV Total:	**3189385**	**Total >0.08**	**100.0%**

Table 7-14 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.08 ppm
South Central Coast	0.060	0.065	204918	14.7%	
	0.065	0.070	134775	9.6%	
	0.070	0.075	141945	10.1%	
	0.075	0.080	362448	25.9%	
	0.080	0.085	135523	9.7%	9.7%
	0.085	0.090	125914	9.0%	18.7%
	0.090	0.095	118145	8.4%	27.1%
	0.095	0.100	68312	4.9%	32.0%
	0.100	0.105	62936	4.5%	36.5%
	0.105	0.110	28774	2.1%	38.6%
	0.110	0.115	14806	1.1%	39.6%
		SCC	**1398496**	**Total >0.08**	**39.6%**
South Coast	0.065	0.070	984	0.0%	
	0.070	0.075	827859	5.7%	
	0.075	0.080	1262909	8.7%	
	0.080	0.085	1973784	13.5%	13.5%
	0.085	0.090	2017916	13.8%	27.4%
	0.090	0.095	1643198	11.3%	38.6%
	0.095	0.100	1067479	7.3%	45.9%
	0.100	0.105	854422	5.9%	51.8%
	0.105	0.110	732178	5.0%	56.8%
	0.110	0.115	502485	3.4%	60.3%
	0.115	0.120	884039	6.1%	66.3%
	0.120	0.125	679312	4.7%	71.0%
	0.125	0.130	799747	5.5%	76.4%
	0.130	0.135	829078	5.7%	82.1%
	0.135	0.140	490458	3.4%	85.5%
	0.140	0.145	25034	0.2%	85.7%
		SC Total:	**14590882**	**Total >0.08**	**85.7%**
Upper Sacramento Valley	0.070	0.075	654	0.1%	
	0.075	0.080	796	0.1%	
	0.080	0.085	76960	12.8%	12.8%
	0.085	0.090	272315	45.1%	57.9%
	0.090	0.095	212207	35.2%	93.0%
	0.095	0.100	28913	4.8%	97.8%
	0.100	0.105	11673	1.9%	99.8%
		USV	**603518**	**Total >0.08**	**99.8%**

Table 7-14 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed	Cumulative % Pop >0.08 ppm
California	0.050	0.055	773505	2.3%	
	0.055	0.060	1043568	3.1%	
	0.060	0.065	1312832	3.9%	
	0.065	0.070	1241980	3.7%	
	0.070	0.075	2716684	8.0%	
	0.075	0.080	4188708	12.4%	
	0.080	0.085	3934294	11.7%	11.7%
	0.085	0.090	3397602	10.1%	21.7%
	0.090	0.095	2966794	8.8%	30.5%
	0.095	0.100	2043981	6.1%	36.6%
	0.100	0.105	2231896	6.6%	43.2%
	0.105	0.110	2061987	6.1%	49.3%
	0.110	0.115	1509789	4.5%	53.8%
	0.115	0.120	1440229	4.3%	58.0%
	0.120	0.125	736645	2.2%	60.2%
	0.125	0.130	805959	2.4%	62.6%
	0.130	0.135	830119	2.5%	65.1%
	0.135	0.140	490458	1.5%	66.5%
	0.140	0.145	25034	0.1%	66.6%
		State	**33752064**	**Total >0.08**	**66.6%**

Figure 7-59

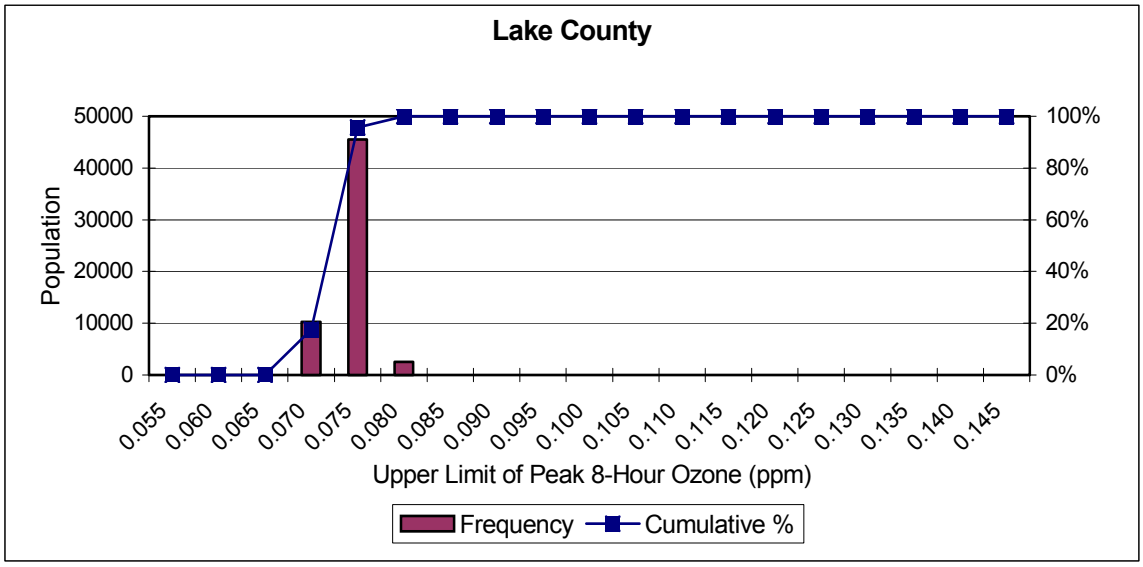
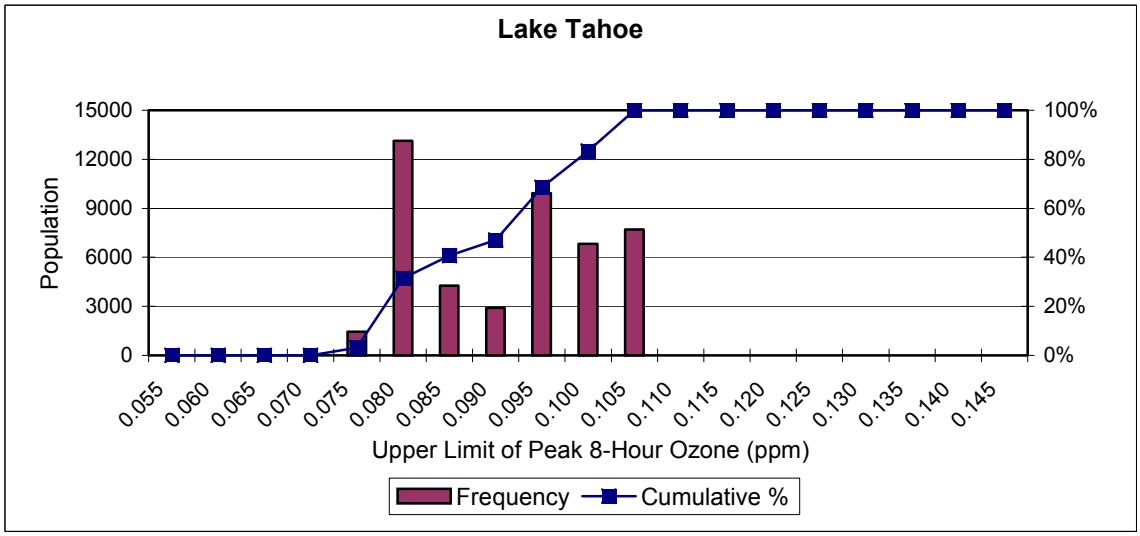


Figure 7-60



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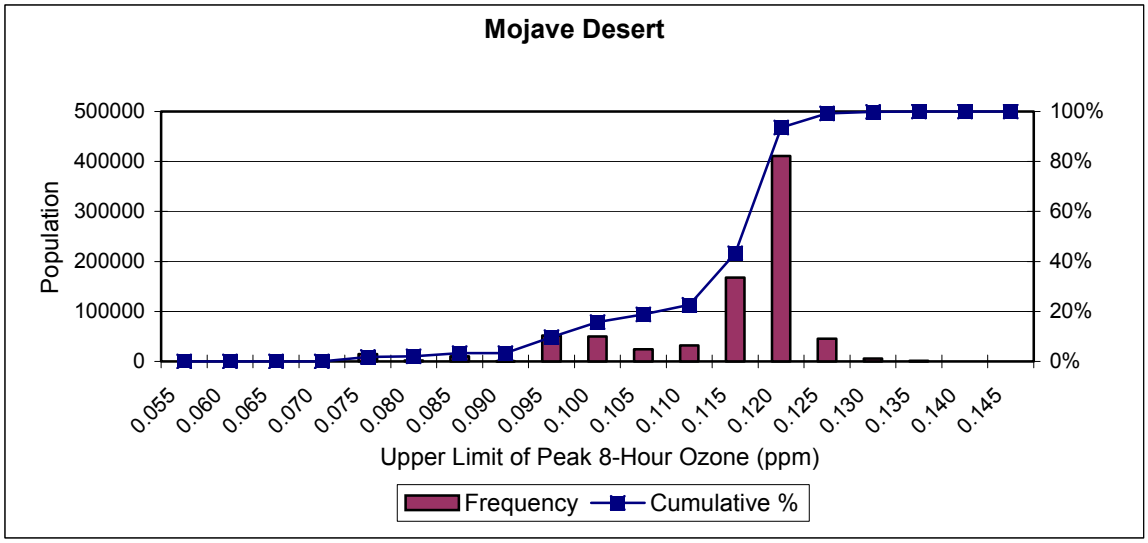

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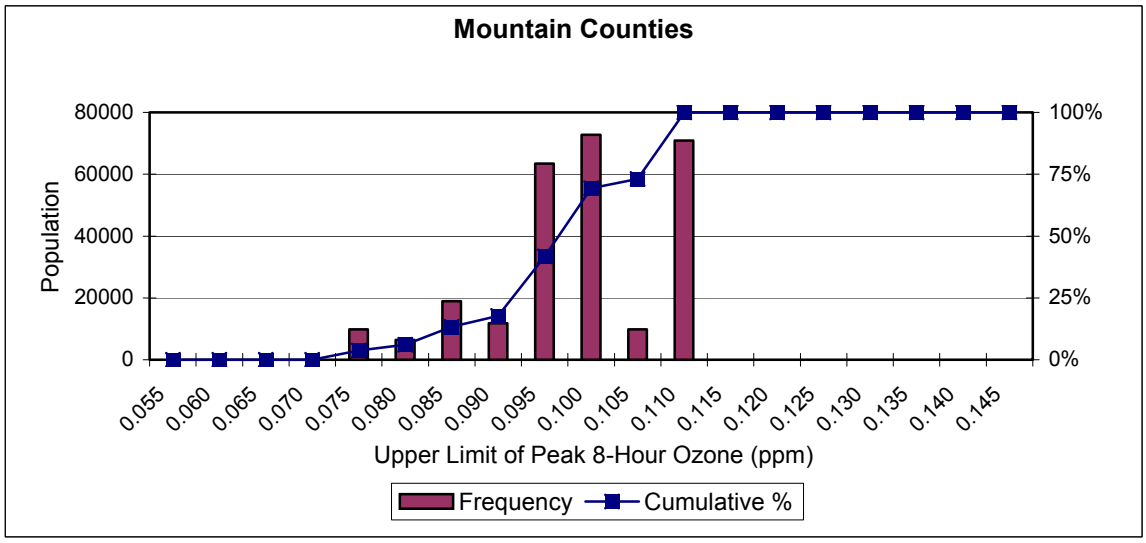

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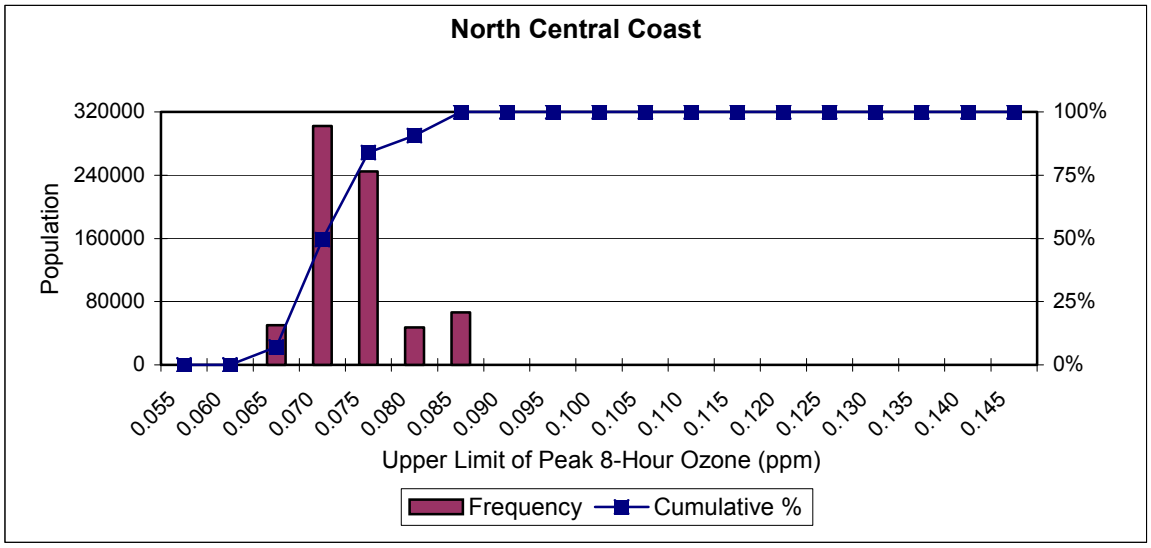

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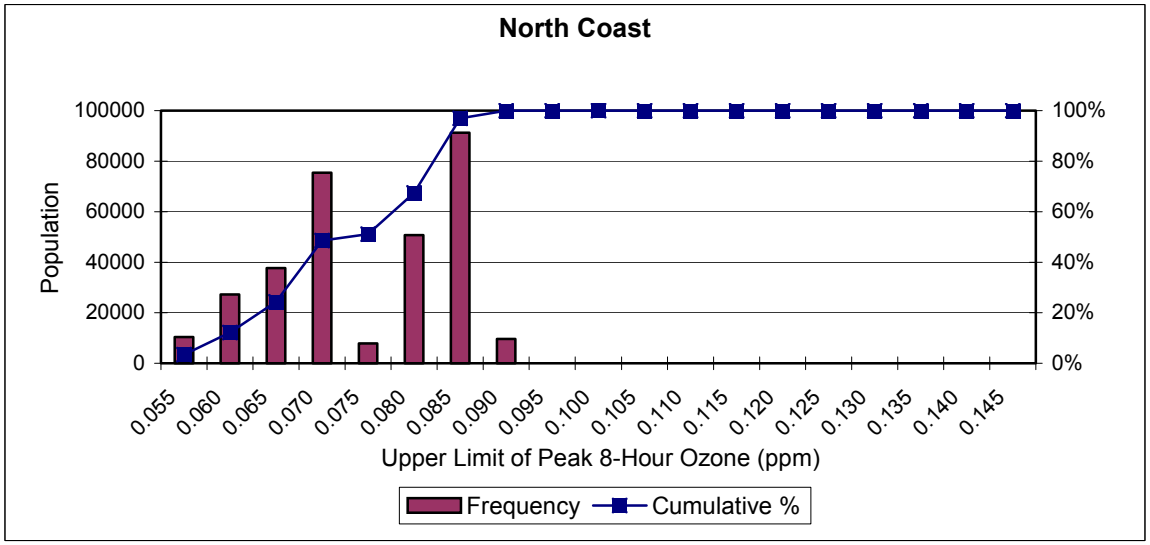

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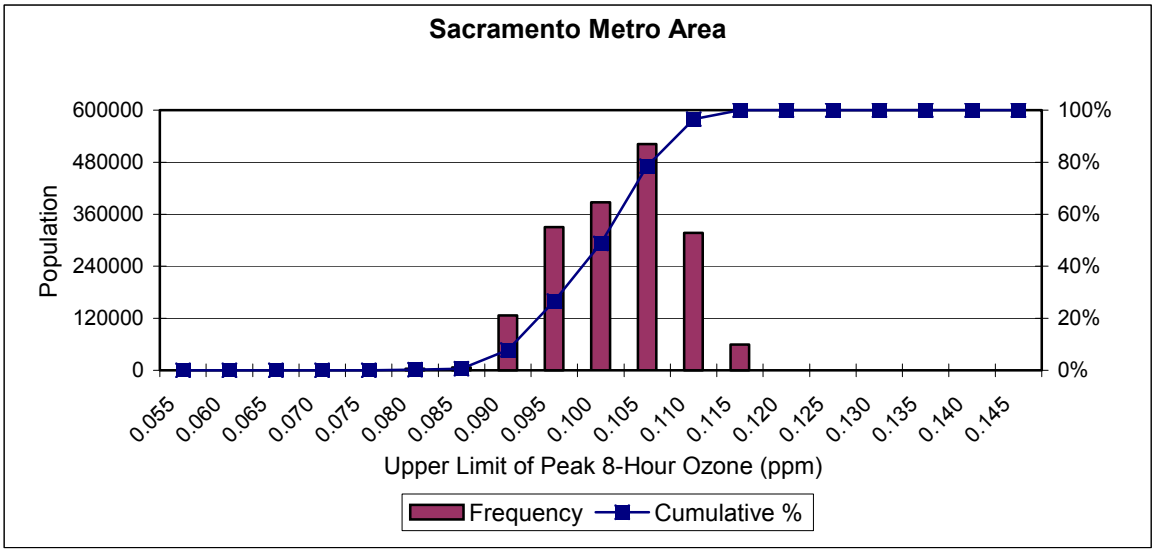

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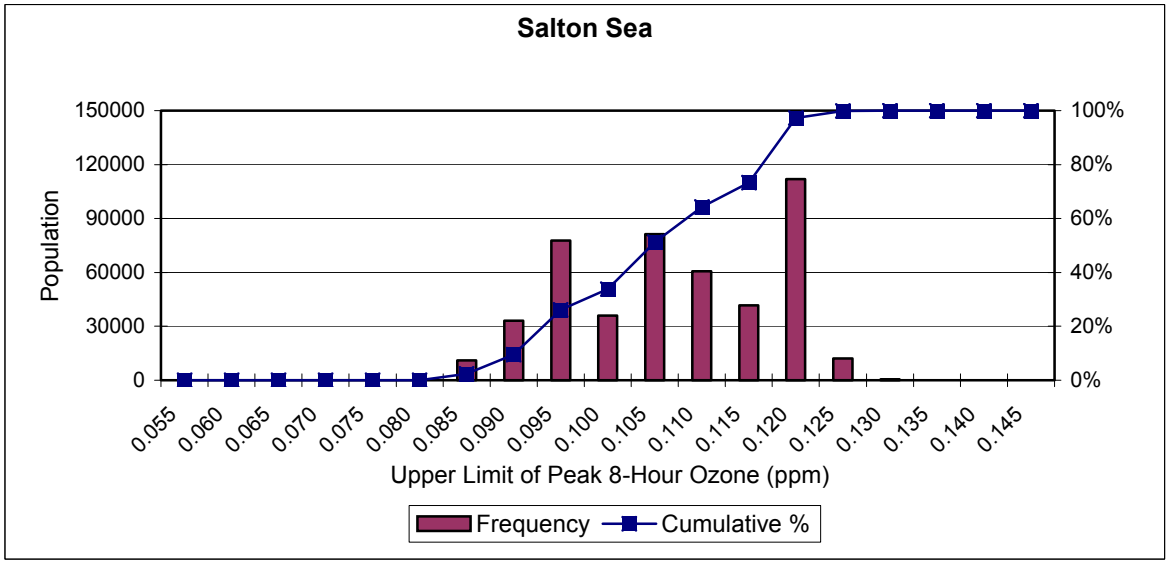

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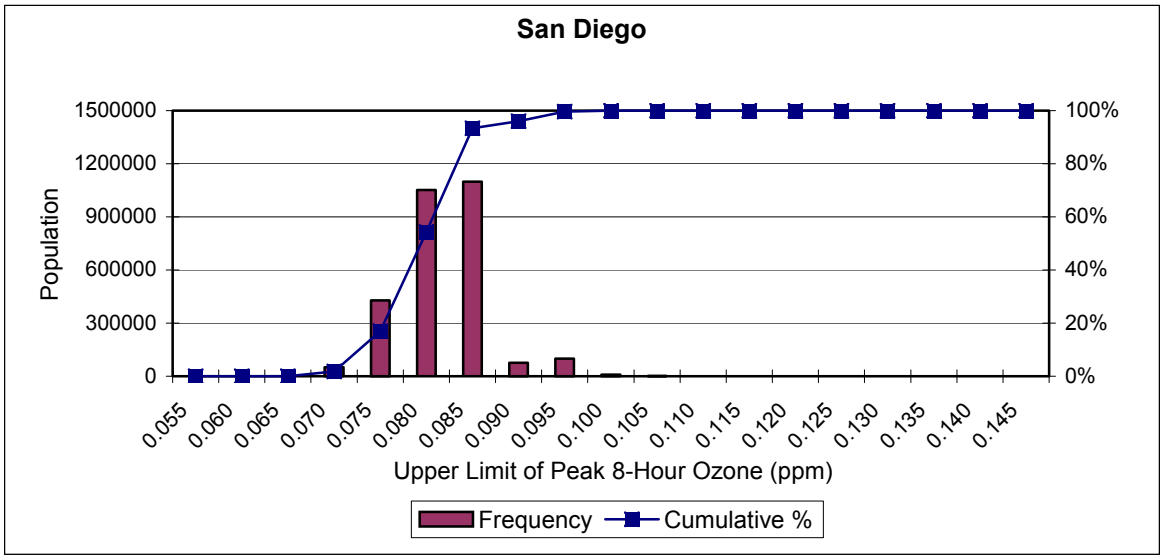

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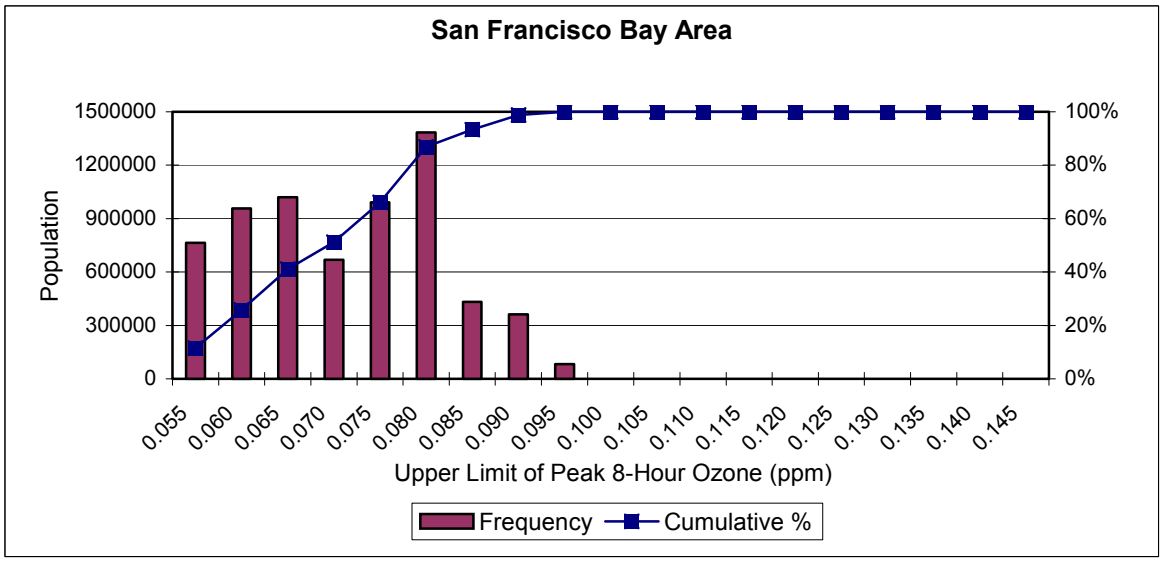

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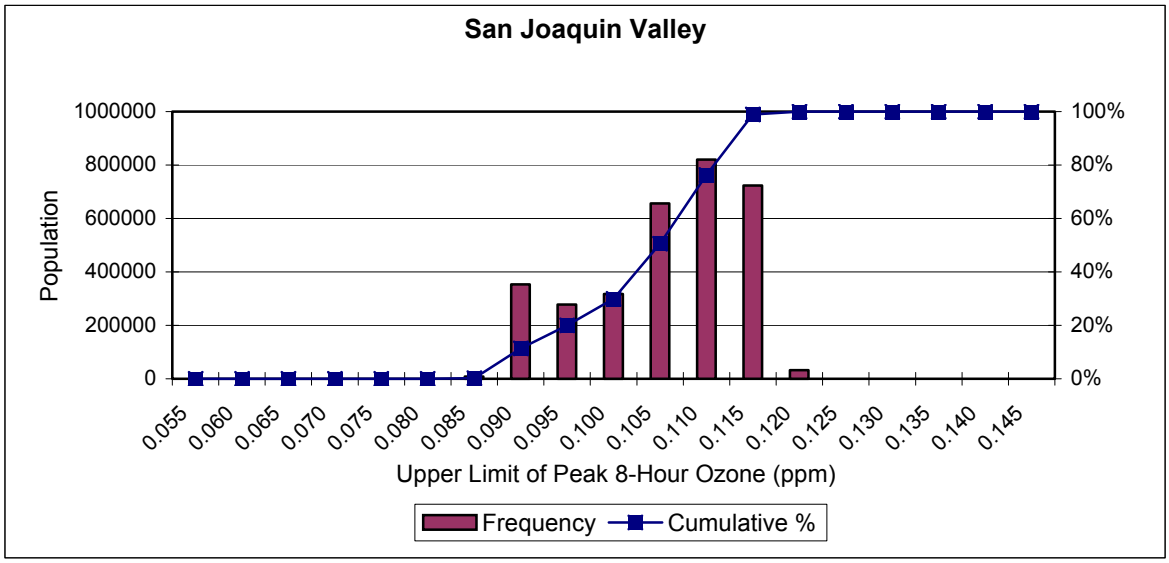


Figure 7-70

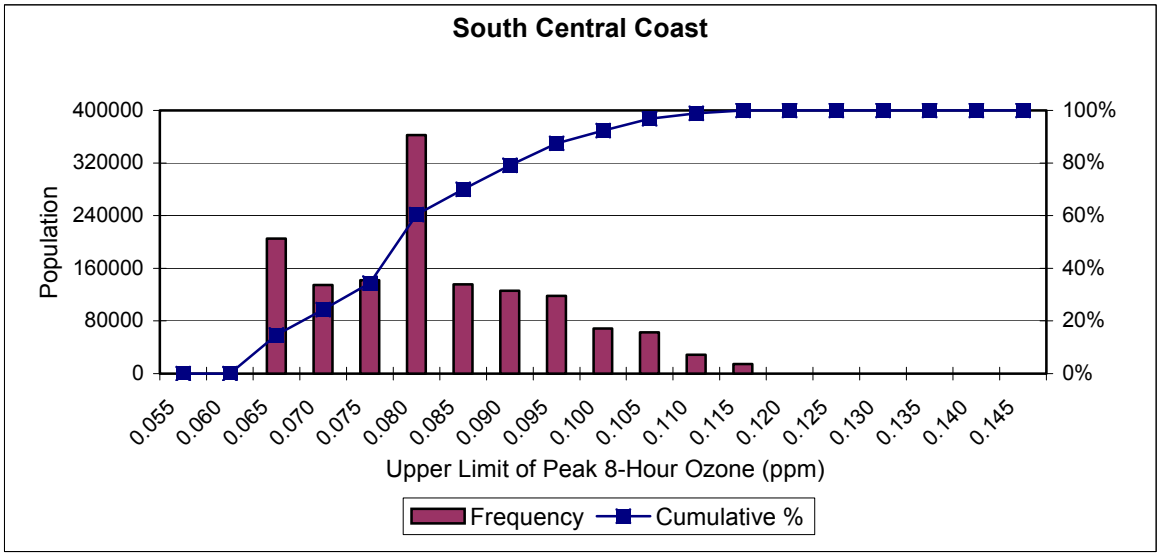


Figure 7-71

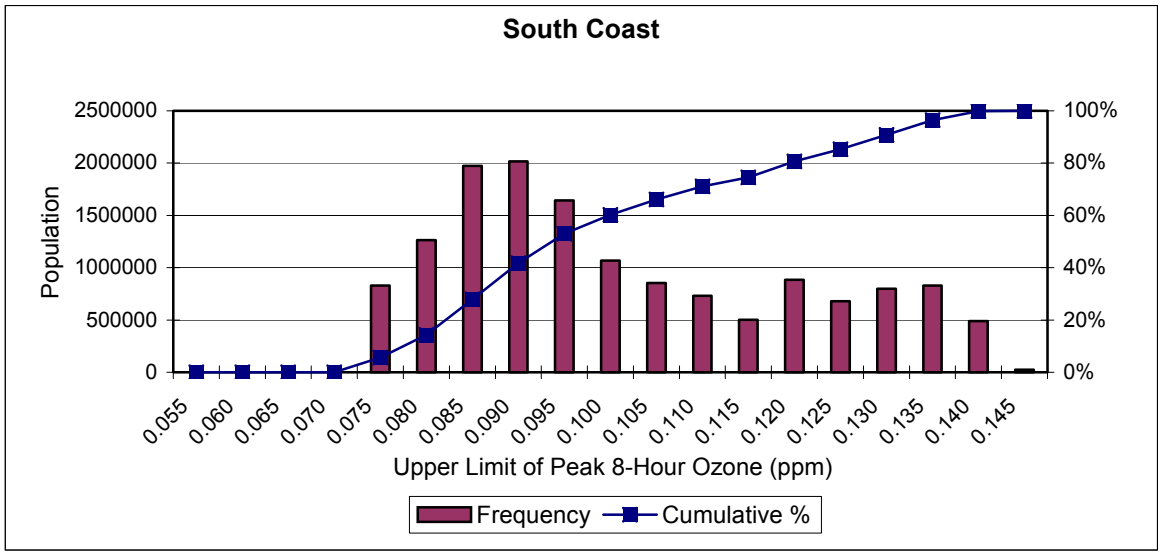


Figure 7-72

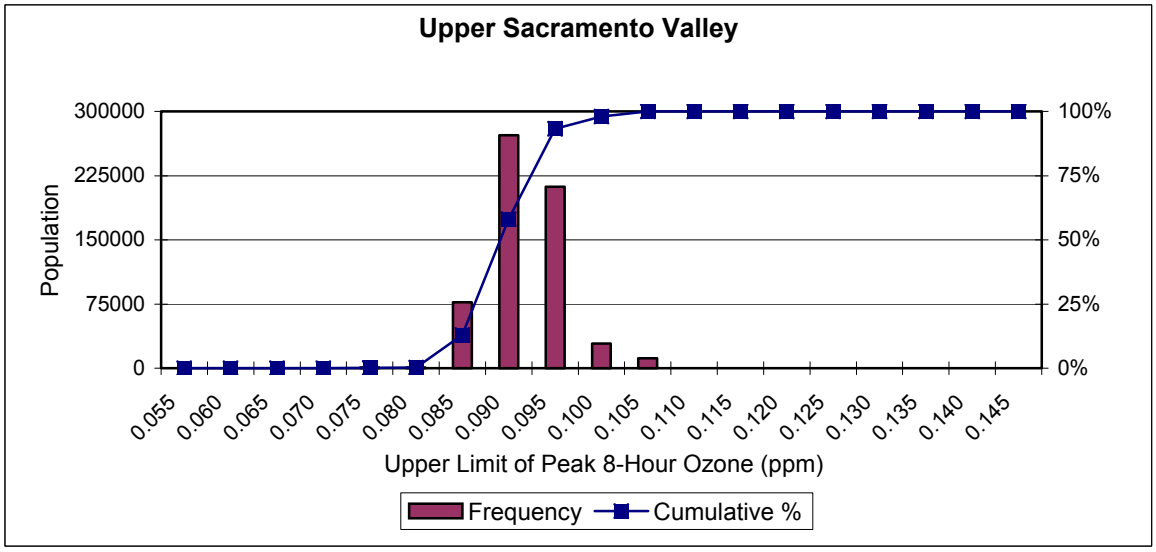
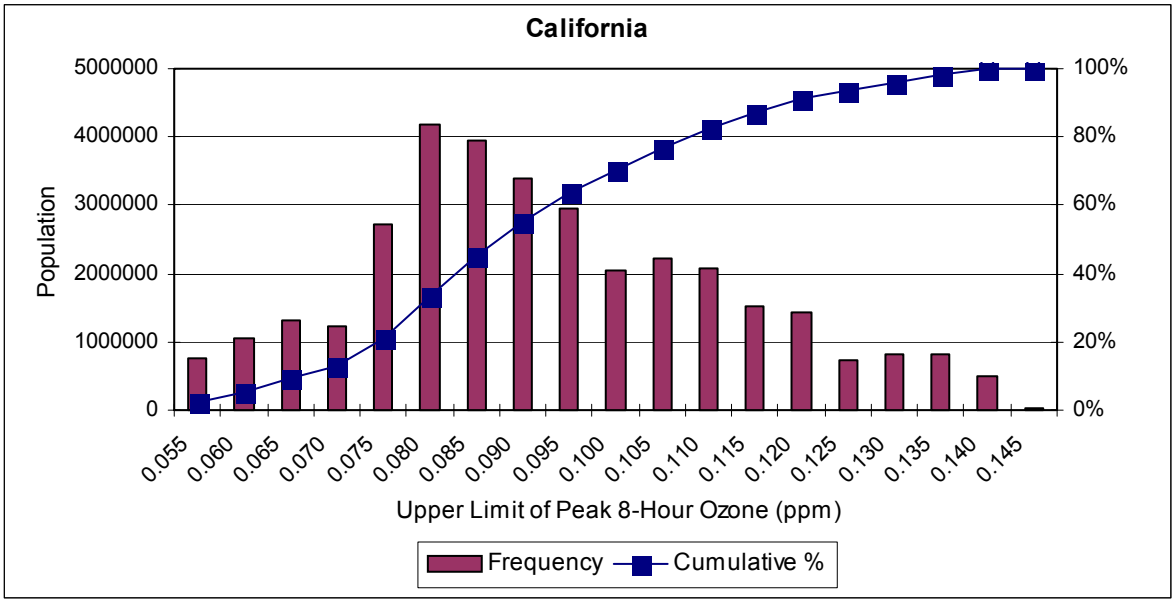


Figure 7-73



7.4 Indoor and Personal Exposures to Ozone

Californians' indoor and personal exposures to ozone are largely determined by the outdoor ozone concentrations in their community. Nonetheless, some Californians experience a substantial exposure to ozone indoors, due to the increasing use of certain types of appliances and equipment that emit ozone. Others, such as many children and those who are employed in outdoor occupations, may experience substantially greater exposures to ozone than the rest of the population, because they spend time outdoors during peak ozone periods.

This section discusses indoor ozone concentrations and sources of ozone, and briefly describes some of the chemical reactions know to occur in indoor environments when ozone is present. Information is also presented on Californians' personal exposures to ozone.

7.5 Indoor Ozone Concentrations

Indoor ozone concentrations are influenced by the outdoor ozone concentration, the rate of exchange between outdoor and indoor air (air exchange rate), the emission rates of indoor sources of ozone, removal by indoor surfaces, and reactions between ozone and other chemicals in the air (Weschler 2000). Temperature and relative humidity also affect indoor ozone concentrations, but to a lesser extent. As a result of these multiple interacting factors, indoor ozone levels tend to be highly variable across time, climate, location, and season, and may vary from building to building due to differences in building characteristics and emissions from indoor sources when they are present.

7.5.1 California Studies

Robust indoor data for California are limited. Measured average indoor levels have ranged from less than 10 ppb to greater than 25 ppb in studies where concurrent average outdoor levels ranged from 37 ppb to over 60 ppb (Avol et al. 1998; Geyh et al. 2000; Lee et al. 2002).

7.5.1.1 Homes

Several studies have examined indoor ozone concentrations in homes in California. Avol et al. (1998) studied indoor and outdoor ozone concentrations for 126 southern Californian homes. Indoor ozone levels (13 ± 12 ppb) were lower than outdoor levels (37 ± 19 ppb). Investigators with the Harvard Southern California Chronic Ozone Exposure Study measured indoor and outdoor ozone concentrations and personal ozone exposures over a 1-year period for 169 elementary school children and their homes in Upland and several towns in the San Bernardino mountains (Geyh et al. 2000). During the ozone season, average indoor concentrations were higher overall than during the non-ozone season, and were 3-17 ppb higher in the mountain homes than in the Upland homes due to natural ventilation and higher ambient concentrations in the mountain communities. The mean indoor and outdoor ozone levels in the Upland communities for the ozone season were 11.8 ± 9.2 ppb and 48.2 ± 12.2 ppb, respectively, vs. 21.4 ± 14.8 ppb and 60.1 ± 17.1 ppb for the mountain communities. Indoor and outdoor means in the non-ozone season were 3.2 ± 3.9 ppb and 21.1 ± 10.7 ppb for Upland, and 2.8 ± 4.2 ppb and 35.7 ± 9.3 ppb for the mountain communities. In 119 homes recruited from the Harvard Southern California Chronic Ozone Exposure Study, Lee et al. (2002) also found that average indoor ozone levels were higher in the mountain sites (16.3 ± 17.0 ppb) than the Upland sites (13.4 ± 8.5 ppb). Additionally, average outdoor concentrations (56.5 ± 22.3 ppb) were higher than indoor levels (14.9 ± 13.3 ppb).

7.5.1.2 Offices

One study in California has examined indoor ozone concentrations in an office setting. Indoor and outdoor concentrations of ozone, NO, and NO_2 were measured in real time for 14 months at a telecommunications office in Burbank, CA (Weschler et al. 1994). During the course of the study, the damper setting for the office ventilation system changed with fluctuations in outdoor and indoor air temperatures, resulting in a varying air exchange rate of 0.3 – 1.9 air changes per hour (ach). Consistent with previous work in California homes, indoor levels were higher during the ozone season (March through October) than during the non-ozone season (November through December), and were lower than outdoor levels. Findings from the Burbank site are presented in Table 7-15.

Table 7-15 95th Percentile and Median Ranges (ppb) for Indoor and Outdoor ozone at an Office in Burbank, CA.

Months	95% Indoor	Median Indoor	95% Outdoor	Median Outdoor
Mar – Oct	**24.6 – 55.7**	**0.4 – 8**	**53.3 – 106.2**	**3.4 – 21.1**
Nov – Feb	**7.3 – 18.9**	**0.2 – 1.2**	**21.6 – 32.8**	**1 – 4.5**

7.5.2 Other Studies

Studies in other areas of the country are illustrative of indoor concentrations that might also occur in parts of California. Liu et al. (1993) measured indoor, outdoor, and personal ozone concentrations for 23 children and their homes in State College, PA. As expected, the homes experienced significantly higher mean indoor concentrations during the day (19.2 ppb) than at night (10.5 ppb). The mean estimated outdoor daytime concentration (45.9 ppb) was much higher than the corresponding indoor level. As part of the Canadian Research on Exposure Assessment Modeling (CREAM) study, Liu et al. (1995) measured personal, indoor and outdoor ozone concentrations for 50 Toronto homes and offices in the winter and summer of 1992. For both homes and offices, mean indoor ozone concentrations were lower in the winter (home indoor: 1.6 ppb; workplace: 0.7 ppb) than in the summer (home indoor: 7.1 ppb; workplace: 10.0 ppb). The same seasonal effect was observed for home outdoor levels (summer: 19.1 ppb; winter: 15.4 ppb). Outdoor levels for homes were also higher overall than indoor levels, with greater concentrations during the day than at night.

7.5.3 Factors that Influence Indoor Ozone Concentrations

7.5.3.1 Outdoor Air

Studies have shown that indoor ozone levels generally follow the diurnal and seasonal patterns of outdoor ozone, with higher levels in the daytime and summer months (see above). Most indoor ozone originates from the outdoors (Weschler et al. 1989). Like outdoor concentrations, indoor ozone levels can remain elevated for long periods of time, generally eight hours or more, and display peak variations throughout the day.

7.5.3.1.1 Indoor/Outdoor Ozone Ratios

Researchers sometimes have characterized the relationship of indoor ozone concentrations to ambient concentrations with an I/O (indoor-outdoor) ozone ratio. Typically, indoor ozone concentrations range from 20% to 80% of outdoor values (Weschler et al. 1989), but vary substantially due to a number of factors, such as season, building ventilation rate, and microenvironment.

7.5.3.1.2 Season

Avol et al. (1998) measured seasonal variations in the indoor-outdoor ozone relationships for southern Californian homes. The I/O ratios were 0.43 ± 0.29 during the summer and 0.32 ± 0.21 for the non-summer months. In the CREAM study, I/O ratios also varied by season (Liu et al. 1995). A higher air exchange rate in the summer (1.04 ± 1.28 ach, mean) corresponded with a higher summer I/O ratio (0.40 ± 0.29).

7.5.3.2 Ventilation

For buildings with negligible indoor sources of ozone, the indoor-outdoor ozone relationship is largely dependent on the building ventilation rate. In general, studies show that open windows and doors allow outdoor ozone to enter, while closed windows and doors greatly reduce infiltration. In their study of a telecommunications office in Burbank, CA, Weschler et al. (1994) explained: “For

species originating outdoors, the air exchange rate is a major factor determining indoor concentrations (appearing in both source and sink terms); it influences the lag time between changes in indoor concentrations; it determines the amount of time available (residence time) for indoor chemical reactions."

Buildings with higher ventilation rates will yield the strongest indoor-outdoor ozone relationships (Weschler et al. 1989). During July, 1992, a commercial building in Burbank, CA, with an average air exchange rate of 0.4 ach had an I/O ratio of 0.31 (Weschler et al. 1994). A year later, when the air exchange rate was higher (0.7 ach, average), the I/O ratio was also higher (0.42). In a study of southern California homes, the I/O ratio was 0.10 in homes operating an air conditioner and 0.68 ± 0.18 in homes not using AC with windows open (Lee et al. 1999). In New Jersey homes without indoor gas combustion studied under different ventilation conditions, naturally ventilated homes also had higher I/O ratios than air-conditioned homes – 0.59 ± 0.16 and 0.28 ± 0.12, respectively (Zhang et al. 1994a). Gold et al. (1996) compared the relationship of indoor ozone concentrations with ambient concentrations for a school in Mexico City under three different ventilation conditions. With classroom windows/doors open, indoor ozone concentrations were 75% of outdoor concentrations, and with windows/doors closed, only 15% to 18% of outdoor concentrations. The third condition, which evaluated the use of an air cleaner with windows/doors closed, did not provide a significant reduction in indoor ozone levels. The air cleaner was a small portable floor unit, equipped with a charcoal filter for removing reactive gases and particles. When indoor sources of ozone are present, the air exchange rate has a smaller influence on the indoor-outdoor ozone relationship. After controlling for the air exchange rate, a significant association between the use of an electrostatic air cleaner—a potential source of ozone—and an increase in the I/O ratio was identified in 50 Toronto homes and offices (Liu et al. 1995).

7.5.3.3 Microenvironment

Building type and configuration can also impact I/O ratios. Jakobi et al. (1997) took measurements for different microenvironments—a classroom, a gym, offices, homes, and a car. The I/O ozone ratios for each microenvironment were as follows: office (0.40-0.90); classroom (0.54-0.77); gym (0.49-0.92); home (0.47-1.00); car (0.40-0.60). Hayes (1991) found similar peak indoor-outdoor ratios by using an indoor air quality model (IAQM) and making certain assumptions for different microenvironments and ventilation conditions.

7.5.3.4 Indoor Sources

Ozone emissions from a variety of sources have been measured in several studies. Some measured emission rates are surprisingly high, and would result in unhealthful air concentrations in most indoor environments. Within the last 10 to 15 years, the number of indoor sources of directly-emitted ozone has increased. Ozone generating "air cleaners," ionizing air cleaners, electrostatic precipitators, and several types of office equipment, including photocopiers and laser printers, are known to produce ozone. Computer terminals, ink/bubble jet printers, and fax machines also produce ozone, but limited emissions data exist for this type of equipment. Other electronic equipment and activities such as welding can also produce ozone emissions.

7.5.3.4.1 Ozone Generators

Appliances that purposely emit ozone to purportedly clean the air, also called ozone generators, appear to pose the greatest risk to human health relative to other indoor sources of ozone. Many government agencies recommend against the use of ozone generators (ARB, 2005; DHS 1997; Health Canada 1999). Ozone generators deliberately introduce ozone into the indoor environment, as opposed to ionizers and electrostatic precipitators, which are electronic air-cleaning devices that generate ozone as a by-product of the technology they use to remove particles from the air. Two tabletop models of ozone generators tested by Consumers Union (1992) were deemed unacceptable for occupied spaces. They had no effect on particulates in indoor air, they produced ozone at levels exceeding the FDA standard of 50 ppb ozone for medical devices, and they did not control or measure ozone output. Another investigator estimated that individuals within the immediate vicinity of an ozone generator can be exposed to peak exposure levels exceeding the FDA standard of 50 ppb (Kissel 1993). In a more recent study by US EPA, Mason et al (2000) tested ozone generators in a test home and found that ozone emissions in the test home resulted in indoor air concentrations that exceeded health-based ambient air quality standards. Despite their low emission rates relative to some other indoor sources such as large copiers, portable ozone generators can contribute largely to indoor concentrations and personal exposures, because they are often operated for long periods, and they are located in close proximity to people in their homes. Additionally, tests have shown that users of some small “personal air purifiers” intended for use in a person’s immediate breathing zone (near the nose and mouth) would also be exposed to levels well above health-based guidelines (Phillips et al., 1999).

Experimental investigations of ozone generator use reveal that ozone does not successfully remove contaminants or odor from indoor air, except perhaps at levels not safely tolerated by humans (Boeniger 1995; Kissel 1993; Chen and Zhang, 2004). Ozone generators produce ozone at rates faster than ozone can decay; compounds that react with ozone at an effective rate are largely alkenes (Boeniger 1995), and comprise less than 10% of gas phase pollutants (Weschler 2000). The exact number of people exposed to ozone from the residential use of ozone-generating devices is not known, but is estimated to be large due to the increased advertising and sales of air cleaners. In a recent report by Freedonia (2004), a business research organization, sales of air cleaning devices were found to have increased by an average of 5.4% per year for the last five years, and were predicted to continue to increase at that rate for the next five years.

7.5.3.4.2 Office Equipment

Studies have shown that the ozone produced by photocopiers is dependent on copy rates, light intensity, and the maintenance status of the equipment (USEPA 1995; Wolkoff et al. 1992). As a result, photocopiers have highly variable ozone emission rates – averaging 40 µg/copy with peak concentrations of 131 µg/copy, but with emissions as low as 4 µg/copy for advanced, recently serviced photocopying machines (USEPA 1995). A test method developed by Leovic et al. (1996) measured emission rates for four copiers in full operation mode ranging

from 1,300 to 7,900 µg/h. However, one dry-process copier tested extensively by Brown (1999) showed lower ozone emissions, averaging an estimated 0.4 ± 0.1 µg/copy, or 440 µg/h.

Older studies indicate earlier models likely had higher emission rates (Allen et al. 1978). The ozone emissions from 69 photocopiers were in the range of 0-1,350 µg/min with a mean of 259 µg/min, and the ozone concentration in the breathing zone of 19 operators was between ≤0.001 to 0.15 ppm (1 to 150 ppb) at its maximum (Hansen et al. 1986). Ten photocopiers emitted ozone at rates of <1 to 54 µg/copy, resulting in concentrations of < 4 to 300 µg/m^3 (2 to 153 ppb) (Selway et al. 1980).

Laser printers can also be an important source of indoor ozone. Tuomi et al. (2000) tested 4 laser printers for ozone and VOC emissions. Three laser printers equipped with the corona discharge technology had emission rates of 0 to 3,700 µg/h ozone, with resulting air concentrations of 0 to 360 µg/m^3 (0 to 180 ppb). The laser printer without corona discharge rods, representing the newest technological advances, emitted negligible amounts of ozone.

Ozone concentrations resulting from use of laser printers, photocopiers, and other office equipment have been estimated to exceed health standards, especially under low ventilation conditions (Allen et al. 1978; Selway et al. 1980; USEPA 1995). The emission rates as well as the duration of ozone production, location of office equipment within the building, and environmental factors, such as temperature and lighting, influence the amount of indoor ozone found within occupants' breathing zones (USEPA 1995). In an early study, investigators concluded that the use of photocopiers under conditions of poor ventilation or naturally ventilated areas in the summer where outdoor values exceed 50 ppb can pose a health hazard indoors (Allen et al. 1978). Office equipment can be modified to significantly reduce ozone emissions. For photocopiers, using charging rollers in place of corona wires can decrease ozone levels during charge and transfer processes. Ozone filters, which catalytically convert ozone to oxygen, but do not remove ozone from the air, also reduce ozone emissions. Laser printers without a filter showed average ozone emissions of 440 µg/min as opposed to laser printers with a filter, which averaged 100 µg/min (USEPA 1995). The efficiency of ozone filters is relative to filter thickness, air velocity across the filter, beginning ozone concentration, and the cleanliness of the filter. Activated carbon filters tend to be the most effective.

7.5.3.5 Indoor Chemistry

7.5.3.5.1 <u>Heterogeneous Reactions</u>

Ozone that has been deposited onto indoor surfaces can react with chemicals that constitute, or are adsorbed onto, surface materials; these reactions are referred to as heterogeneous reactions (Weschler, 2000). Deposition onto surfaces may be the most important removal process for indoor ozone (Jakobi et al. 1997; Weschler et al. 1994). The removal rate is dependent on the surface-to-volume ratio, airflow, relative humidity, surface composition, and exposure history of the indoor environment (Lee et al., 1999; Reiss et al., 1994; Weschler 2000). In general, smaller rooms and rooms with fleecy surfaces have faster rates of

indoor ozone removal. Ozone decay also occurs at a faster rate when temperature and humidity levels are high, because the deposition velocities of ozone to different surfaces increase with higher temperature and relative humidity (Weschler 2000).

In addition to removing ozone from indoor air, ozone deposition can also influence the emissions of potentially harmful chemicals from building products (Kleno et al., 2001). Natural rubbers and neoprene, compounds associated with latex paint, linoleum and carpet, unsaturated components of waxes and polishes, and unsaturated semi-volatile organics adsorbed on indoor surfaces react with ozone to produce highly volatile chemicals harmful to human health and damaging to materials such as rubbers, dyes, film, and books (Weschler 2000). Reiss et al. (1995a) studied the heterogeneous reaction of ozone with latex paint in an environmental chamber. Significant amounts of formaldehyde and lesser concentrations of acetone and acetaldehyde were produced with the exposure of latex paint surfaces to ozone. In the presence of indoor ozone, the unsaturated fatty acids from paints using linseed oil as a drying agent reacted readily with ozone to produce aldehydes and organic acids (Weschler 2000). Olefins or other chemicals contained in surface materials such as plywood and plaster also react with ozone to produce formaldehyde (Moriske et al., 1998). Ozone was also shown to impact indoor levels of VOCs in a carpeted environmental chamber (Weschler et al., 1992). Nonvolatile substances in the carpet fibers reacted with ozone to produce the higher molecular weight aldehydes. Carpet can act as a reservoir for the aldehydes, adsorbing them once they have formed and releasing them long after the ozone exposure ends (Weschler, 2000).

7.5.3.5.2 <u>Homogeneous (Gas-phase) Reactions.</u>

In contrast to heterogeneous reactions, homogeneous reactions occur in the gas phase when ozone reacts with other airborne chemicals (Weschler, 2000). Ozone reaction rates for inorganic gases commonly found in indoor air (i.e., formaldehyde, acetaldehyde, ammonia, sulfur dioxide and carbon monoxide) are too slow to effectively compete with air exchange rates for removal of indoor ozone. However, some volatile organic chemicals (VOCs) with unsaturated carbon-carbon bonds react with ozone equal to or faster than the rate of air exchange. Most notably, these chemicals include the terpenes: d-limonene, α-pinene, isoprene, and styrene. Terpenes are used in a variety of cleaning products and other consumer products to impart a scent to the product, or to contribute desirable solvent properties. The reaction of terpenes with ozone leads to the production of irritant chemicals such as formaldehyde and ultrafine particles (Wilkins et al., 2001; Weschler and Shields, 1999; Atkinson and Arey, 2003). Studies examining the effects of ozone on VOCs emitted by carpet and in environmental tobacco smoke (ETS) showed similar results (Shaughnessy et al. 2001; Weschler et al. 1992).

Reiss et al. (1995b) used a model to examine the homogeneous removal of ozone in several studies. They found that the homogeneous reaction of ozone with d-limonene, α-pinene, and styrene in residential environments accounted for 85%, 14%, and 1% of the ozone removal, respectively. Additionally, Reiss et al.

(1995b) noted that homogeneous chemistry comprised 20% of the total ozone removal in another study of homes.

Ozone-terpene reactions can also be a significant source of sub-micron particles. Weschler and Shields (1999) examined particle formation and indoor ozone concentrations in an office setting. In the first experiment, ozone from an ozone generator and a selected terpene were deliberately introduced into one office, while a second office served as a control. Particles ranging from 0.10-0.20 µm in aerodynamic diameter were 20 times more prevalent in the first office than in the control environment. In the second experiment, d-limonene was intentionally added to an office where outdoor air was the main source of ozone. Particles closely tracked indoor ozone concentrations, and formed at a rate 10 times greater in the presence of d-limonene. Wainman et al. (2000) also demonstrated that limonene can influence indoor particle concentrations. In a series of experiments conducted in a dynamic chamber system, ozone was reacted with limonene at 30%, 50% and 70% relative humidity. Particle formation with aerodynamic diameters from 0.1 to 0.3 µm was measured for all experiments, and was shown to increase with larger surface areas and smaller air exchange rates.

Another important reaction in indoor air chemistry is the ozone-nitric oxide reaction. The reaction does not require photochemical initiation, and occurs when both precursors are present in indoor air (Zhang et al. 1994b). When gas appliances are operating, ozone will react with the NO produced, leading to a decrease in ozone concentration (Weschler 2000). In an office setting in Burbank, California, the reaction rate for the ozone-nitric acid reaction varied with time, and was most apparent in the late morning and early evening (Weschler et al. 1994). In a southern Californian home, the operation of a gas stove decreased the ratio of indoor-outdoor ozone from 0.82 to 0.15 within 7 minutes (Lee et al. 1999). However, gas combustion is not a recommended means for removing ozone from indoor air, and is not as effective as removal by surfaces (Zhang et al. 1994a).

The product of the ozone-nitric oxide reaction – nitrogen dioxide – also reacts with ozone, forming the nitrate radical. The nitrate radical is highly reactive, and converts quickly to nitric acid, a strong oxidizing agent that can contribute to numerous respiratory ailments. The nitrate radical may also initialize another pathway leading to the formation of formic acid from formaldehyde (Zhang et al. 1994b). Formic acid can also be formed indoors through the reaction of ozone with unsaturated VOCs (Zhang et al. 1994a).

7.5.4 Personal Exposures to Ozone

People's personal exposures to ozone vary directly by their personal locations and activities throughout the day, especially the time they spend outdoors. As shown in Table 7-16, California adults spend about 87 percent of their time indoors, on average, and about 6 percent of their time outdoors, and 7 percent of their time inside vehicles (Jenkins et al, 1992). California children, on the other hand, spend an average of 10% of their time outdoors. However, older children spend more time outdoors: children 6-11 years of age spend 13 percent of their

time outdoors, on average, and some children spend even more time outdoors (Phillips et al., 1991).

Table 7-16 Average Percent of Time Californians Spend in Major Locations

AGE	AVERAGE PERCENT OF TIME[1,2]			
	Inside the Home	Other Indoors	Outdoors	Inside a Vehicle
7.5.4.1.1 Children 0 - 2	85	4	7	4
3 - 5	76	9	10	5
6 - 11	71	12	13	4
All Children (0 - 11)	76	10	10	4
Adults and Teens	62	25	6	7

[1]From: *Study of Children's Activity Patterns* (Wiley et al., 1991a, ARB Contract no. A733-149; Phillips et al., 1991).

[2]From: *Activity Patterns of California Residents* (Wiley et al., 1991b, ARB Contract no. A6-177-33; Jenkins et al., 1992a).

Most importantly, children are often outdoors during the time of day when ozone levels tend to be highest. In the California Children's Activity Pattern Study (Phillips et al., 1991), 82.8 percent of the children ages 0 -11 spent time outdoors during their diary day, and those children spent, on average, 70 percent of their time outdoors during the hours of 12 noon to 8 p.m. Ozone concentrations peak anytime from noon to late evening – the time when children are most likely to be outdoors. Research indicates that the pulmonary function of children is affected by their peak ozone exposure each day and total ozone exposure (Lioy et al. 1989).

7.5.4.2 California Personal Exposure Studies

Information on personal ozone exposures comes from the use of passive samplers, such as the Harvard ozone monitor, and from predictive results obtained from microenvironmental models. Models have been developed to explain up to 72% of the variability in personal ozone exposures (Liu et al. 1995). Liu et al. (1995) concluded that: "With a sufficient number of ambient monitoring sites and time-activity information, the weighted mean ambient measurements may be suitable for predicting personal exposures."

In the Harvard Southern California Chronic Ozone Exposure Study, Geyh et al. (2000) found that personal exposures were more closely associated with indoor levels than outdoor levels (Table 7-17), and were significantly influenced by

Table 7-17 Personnal Ozone Exposure Studies

Reference	Location and population	No. of subjects	Study period	Age	Averaging time	Concentration: arithmetic mean; all in ppb			r[b]
						Personal[a]	Indoor[a]	Outdoor[a]	
California Studies									
Geyh et al. 2000	Upland, CA	84 children 61 homes	06-09/95, 05/96	6-12	6 days (144 h)	18.8 ± 10.1	11.8 ± 9.2	48.2 ± 12.2	NA[c]
			10/95-04/96			6.2 ± 5.4	3.2 ± 3.9	21.1 ± 10.7	NA
	Mountain towns	85 children 54 homes	06-09/95, 05/96	6-12	6 days (144 h)	25.4 ± 13.4	21.4 ± 14.8	60.1 ± 17.1	NA
			10/95-04/96			5.7 ± 5.1	2.8 ± 4.2	35.7 ± 9.3	NA
Liu et al. 1997	San Diego, CA	22	05-07/94	10-47	12-h day	13.6	NA	63.1 (APCD)	NA
		18	09-10/94	9-38		10.5	NA	54.5 (APCD); 45.1 (p[e]); 44.0	NA

								(a[f])	
Delfino et al. 1996	San Diego, CA	12	09-10/93	9-16	12-h day	11.5 ± 11.2	NA	43 ± 17 (APCD[d])	0.5
Other Studies									
Liu et al. 1995	Toronto, Canada; CREAM	34 people, 50 homes and offices	01-03/92	NA	weekly	1.3 ± 2.9	1.6 ± 4.1 (home); 0.7 ± 0.7 (work)	15.4 ± 6.0 (home); 11.4 ± 3.0 (p[g]); 9.3 ± 4.2 (c[h])	NA
		89 people, 50 homes and offices	06-08/92	NA	12-h day	8.2 ± 8.7	7.1 ± 12.6 (home); 10.0 ± 11.6 (work)	19.1 ± 10.8 (home); 18.5 ± 9.6 (p); 18.4 ± 12.6 (c)	0.2
					12-h night	NA	6.2 ± 9.5 (home)	9.4 ± 10.2	NA
					24-h	NA	NA	15.6 ± 8.5 (p); 15.5 ± 9.2 (c)	NA
					weekly	NA	NA	13.2 ± 4.0 (p); 13.0 ± 2.7 (c)	NA

Liu et al. 1993	State College, PA	23 children and their homes	07-08/91	10-11	12-h day	23.9 ± 16.2	19.2 ± 11.3	45.9 ± 21.3 (home); 56.4 ± 16.2 (p[g]); 55.3 ± 14.7 (c[h])	0.4
					12-h night	NA	10.5 ± 7.2	19.1 ± 8.9 (p); 20.1 ± 10.1 (c)	NA
					24-hr	NA	NA	29.8 ± 14.3 (home); 37.8 ± 10.7 (c)	NA
a-All personal, indoor, and outdoor samples were taken with the Harvard passive sampler unless otherwise noted.									
b-Correlation of personal ozone exposures to the stationary ambient monitoring (SAM) site									
c-Information not available									
d-continuous measurement made at the San Diego County Air Pollution Control District (APCD) monitoring site(s)									
e-passive (collocated with 4 active monitors)									
f-active (used at 12 outdoor locations)									
g-passive (collocated with continuous monitor at SAM site)									
h-continuous measurement taken with a UV photometric ozone analyzer and/or chemiluminescence monitor at SAM site									

community and gender. Study participants in the mountain communities were exposed to 35% more ozone on average than the Upland community. In particular, boys, who averaged more time outside than girls, had higher personal exposures than girls did – a difference that was more pronounced during the summer months. In the spring and fall of 1994, Liu et al. (1997) conducted personal and outdoor ozone monitoring for cohorts of 22 and 18 subjects over an 8-week period in San Diego, California, using the Harvard passive and active samplers. As shown in Table 7-17, Liu et al. (1997) found that outdoor concentrations were four to five times higher than personal exposure levels for both seasons.

Delfino et al. (1996) measured outdoor and personal ozone concentrations during peak exposure hours for 12 asthmatic subjects from 9 to 16 years of age in San Diego, California. Results showed little correlation between personal and outdoor ozone; personal ozone was 27% of outdoor ozone (see Table 7-17).

7.5.4.2.1 Indoor Activity

While the source of indoor ozone is mostly outdoor air, indoor ozone exposures (concentration x time) tend to be larger than outdoor exposures, because most people spend nearly 90% of their time indoors (Weschler et al. 1989). In the study of 23 children and their homes in State College, PA, Liu et al. (1993) identified indoor ozone concentrations (19.2 ppb, mean) as the most important predictors of personal exposures (23.9 ppb, mean). Zhang et al. (1994a) estimated that exposure in indoor residential environments in New Jersey accounted for 67% of the estimated potential dose of ozone (Table 7-18). The potential dose is equal to the concentration multiplied by the contact rate and time.

Table 7-18 Potential Dose from New Jersey Homes.

Exposure Factor	Outdoor Mean	Indoor Mean
Concentration (mg/m^3)	0.19 (95 ppb)	0.035 (18 ppb)
Contact Rate (m^3/hr)	1.4	0.83
Time (hr/day)	0.88	15.37
Potential Dose (mg/day)	0.22	0.45

7.5.4.2.2 Outdoor Activity

In addition to indoor concentrations, research indicates that the amount of time spent outdoors is also an important variable in predicting ozone exposure. Brauer et al. (1995) conducted personal exposure monitoring for three groups of healthy adults with specified activity patterns – 25 office workers, 25 camp counselors, and 15 farmworkers. Because camp counselors and farmworkers spent more time outdoors than office workers did, their personal exposure levels were closer to the ambient concentration. The mean differences between ambient and

personal concentrations were 12 ppb, 8.5 ppb, and 2.5 ppb for office workers, camp counselors, and farm workers, respectively. During episodes of high ozone in Philadelphia and Los Angeles, children and retired adults were estimated to experience higher personal-to-outdoor ratios under the assumption that they spent more time outdoors than other population groups (Hayes et al. 1989). Individuals accustomed to spending more time outdoors on the weekends will likely experience higher personal exposures to ozone (Liu et al. 1997). In their study of San Diego residents, Liu et al. (1997) found that the mean personal exposure was 22.6 ppb on Saturday, and 17.3 ppb on weekdays.

7.5.4.3 Role of Outdoor Ozone

Although it may be a useful indicator of peak exposure, the outdoor ozone concentration by itself is not a reliable indicator of personal ozone exposures. Outdoor measurements tend to overestimate personal measurements. Delfino et al. (1996) found that personal levels for 12 children were less than one-third the outdoor levels measured at the Air Pollution Control District (APCD) monitoring sites in San Diego, CA (Table 7-17). In the study of 50 Toronto homes and offices, Liu et al. (1995) also measured personal levels that were significantly lower than outdoor levels. There was also very little correlation between personal ozone exposures and measurements taken from the stationary ambient monitoring (SAM) site in a study of Pennsylvanian children and their homes (Liu et al. 1993). Table 7-17 shows the Pearson correlation coefficients (r), which range from 0.22 to 0.45, for personal-ambient levels for three recent personal ozone exposure studies.

7.5.5 Summary

Indoor ozone concentrations are highly variable, and typically range from about 20% to 80% of outdoor values. The relationship of indoor air to outdoor air is largely dependent on the building air exchange rate, except when indoor sources are present. Indoor sources of ozone include air cleaners, such as ozone generators and ionizers, and office equipment, such as copiers and laser printers. The ozone emitted from these sources can react with indoor surfaces and compounds in indoor air, and produce harmful chemicals, such as aldehydes, organic acids, and fine aerosols.

Time-activity patterns are key determinants of personal ozone exposures. Those who spend substantial time outdoors, such as children who play outdoors and workers in outdoor occupations, experience substantially greater exposures than most of the population. Although indoor ozone concentrations are typically much lower than outdoor levels, the greatest exposure to ozone for most individuals occurs indoors, because of the amount of time spent indoors. However, outdoor ozone exposures are generally more reflective of peak exposures, which may be more relevant in determining health impacts.

7.6 Conclusions

The State area designation process has several provisions for excluding high values that are not reasonable to control through the regulatory process. Under State law, there are three types of highly irregular or infrequent events: extreme concentration events, exceptional events, and unusual concentration events.

While a concentration identified as a highly irregular or infrequent event "exceeds" the level of the State standard, such an exceedance is not considered a "violation" of the standard. This is important because only a "violation" can trigger a nonattainment designation. Although the State ozone standard is expressed as a concentration that is "not to be exceeded," the designation criteria allow some leeway for excluding exceedances that are not reasonable to control.

Both the California 1-hour ozone standard of 0.09 ppm, and the proposed California 8-hour standard of 0.070 ppm are more stringent than the federal 1-hour standard of 0.12 ppm, and the federal 8-hour standard of 0.08 ppm. In addition, attainment designation criteria for California standards add stringency in that they are based on one exceedance per year, on average, compared to federal area designation processes, which are based on the fourth highest ozone concentration over three years, which allows four exceedences per year.

Indoor ozone concentrations are highly variable, and typically range from about 20% to 80% of outdoor values. The relationship of indoor air to outdoor air is largely dependent on the building air exchange rate, except when indoor sources are present. Indoor sources of ozone include air cleaners, such as ozone generators and ionizers, and office equipment, such as copiers and laser printers. The ozone emitted from these sources can react with indoor surfaces and compounds in indoor air, and produce harmful chemicals, such as aldehydes, organic acids, and fine aerosols.

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8 Welfare Effects

8.1 Agricultural Effects – Introduction

The Health & Safety Code (§39014) defines an "ambient air quality standard" as:

> … specified concentrations and durations of air pollutants which reflect the relationship between the intensity and composition of air pollution to undesirable effects established by the state board or, where applicable, by the federal government.

In establishing state ambient air quality standards, the Air Resources Board is called upon to divide the state into air basins (i.e., regions of similar meteorological and geographical conditions), and to adopt standards in consideration of public health and welfare (Health & Safety Code §39606). While the primary purpose of establishing an ambient air quality standard for ozone is public health protection , studies have also shown that impacts to vegetation often occur at exposures below those that would result from the concentrations and durations specified by the existing health-based ambient air quality standards (e.g., Peterson et al., 1987). Despite the volume of published reports documenting ozone impacts to plants, a previous effort in California to establish a secondary standard to protect vegetation from the adverse effects of ozone was not successful (ARB, 1987).

In the following three subsections, the impacts of ozone on agricultural crops, forest trees, and materials are reviewed, respectively. In broad terms, impacts to crops are generally more severe than for forest trees owing to their inherently more vigorous rates of physiological activity and growth. In the subsection on crops, methods used to expose plants to ozone are first examined to clarify various issues and concerns that have arisen with respect to the validity of data collected using experimental systems that allow for controlling aerometric conditions and ozone concentrations to plants. This is followed by a critical examination of the physiological basis of ozone damage to plants, with special emphasis on carbon metabolism and the resulting impacts on crop growth and yield. The subsection on effects to forests focuses on data collected since the 1950s on mixed conifer forests in the San Bernardino Mountains and the Sierra Nevada. These data, collected over a multi-decade time frame, indicate that increasing numbers of ponderosa and Jeffrey pines exhibit ozone-specific needle damage due to the pollutant's cumulative effects. The third subsection discusses the impacts of ozone on materials, including building materials, rubber, paint, and fabrics. It should be noted that the material presented in this chapter is intended to give a brief overview of ozone-related effects other than those related to human health. Since the ambient air quality standard for ozone is based on

human health endpoints, we have not estimated potential welfare benefits that may be associated with attainment of the proposed standards.

8.2 Ozone Impacts on Crop Production In California

8.2.1 Introduction and Summary

The previous Technical Support Document dealing with the effects of tropospheric ozone on crop productivity in California, entitled “Effects of Ozone on Vegetation and Possible Alternative Ambient Air Quality Standards, was issued by ARB in March, 1987 (ARB, 1987).

Considerable new research has been conducted and published in the peer reviewed literature in the intervening period (Heagle, 1989). This material includes two Air Quality Criteria Documents for Ozone and Related Photochemical Oxidants (USEPA 1986, 1996a) and an interim document (USEPA 1992), which are mandated by the federal Clean Air Act to be developed by the U.S. Environmental Protection Agency at prescribed intervals. A series of ARB-initiated research programs and resulting ARB reports have also been generated during this period that generalize ozone-induced crop yield losses across California. A review of ozone-induced crop loss focused mainly on the National Crop Loss Assessment Network (NCLAN) studies (Heagle, 1989). Another provided a multidisciplinary evaluation of the NCLAN program (Heck et al., 1988a). While advances have been made in mechanistic studies, the conclusions from the early efforts to quantify the economic damage caused by ambient ozone (ozone) remain largely valid.

The regional distribution of ozone makes it the most damaging air pollutant for agricultural productivity, both globally and in California (Heagle, 1989). Current impacts of ozone (ozone) on crop production, including alteration of basic physiological processes, functional aspects of native ecosystems, and suppression of crop yield and quality, are significant under current ambient conditions. Despite substantial investment in regulatory initiatives and mitigation technologies, ozone concentrations in rural crop production areas of California continue to exceed phytotoxic levels. These concentrations are increasing in many rural areas driven by growing population and associated emissions of ozone precursors from industrial and transportation sources (NARSTO, 2000; Taylor et al., 1994). For background information see Heck et al. (1982, 1988a), Davison and Barnes (1998), Heggestad et al. (1988), Miller (1988), Heggestad and Lee (1990), Temple et al. (1993), Skelly et al. (1983), Materna (1984), Miller and McBryde (1999), USEPA (1996a,b), Schulze et al. (1989).

The following review considers the mechanisms of ozone phytotoxicity, the physiological injury to plant function, and quantifiable damage to agronomic and horticultural yields. It is important to demonstrate plausible mechanisms of yield reduction, in order to attribute confidence to available yield loss data. These data are necessarily variable, as they arise from a hugely diverse range of [genotype x environment x ozone exposure conditions]. Only a few of the potential combinations can ever be investigated. Process modeling will extend this to somewhat more cases in which suitable mechanistic information is available.

Yield losses for most possible combinations of local relevance, particularly in California's diverse agricultural economy, will inevitably be predicted in only semi-quantitative terms from available exposure-response relationships combined with incomplete knowledge of response mechanisms. Demonstration and communication of plausible mechanisms, and of non-monetary injury to plant systems, is essential in the absence of precise estimates of yield loss in specific production situations.

The conclusions of this review do not differ appreciably from those of previous similar documents (e.g., ARB, 1987; USEPA 1986, 1996a). It is clear from available evidence that ozone at ambient concentrations damages crop yield under many situations. It is also clear that the extent of damage depends on a variety of environmental, exposure, and genetic parameters that are poorly understood for a few crops and completely unknown for many others. The uncertainty in exposure vs. yield loss relationships is larger than the uncertainty in ambient ozone concentration data. The post-NCLAN conclusions of Adams et al. (1988), i.e., that further research to improve the precision of yield loss functions does not proportionally reduce the uncertainty in economic loss forecasts, may be more accurate for large-scale aggregate analyses, such as for the eight major crops over the entire U.S. than for the highly diverse California agricultural production system. Lack of information on individual species and genotypes, some of high production value in California, remains a serious limitation in California ozone exposure-yield loss assessment.

Much of the available information has been determined using field exposure chambers, particularly in the U.S., the NCLAN-type open top chamber (OTC; Heagle et al., 1973). This technology, largely because of the enormous role it has played in producing the available data sets, has come under intense recent scrutiny (e.g., Manning and Krupa, 1992; Krupa and Kickert, 1997; Grunhage et al., 2001). It is therefore important to evaluate the reliability of exposure-response data obtained in OTCs, and its relevance to crop loss estimation under ambient conditions. As such considerations underpin currently available conclusions, this review first considers the range of available exposure technologies.

Given the wide range of known crop sensitivities to ozone, and the narrow range of crops that have been investigated, some uncertainty will remain in any regulatory threshold, due to the inherent compromises and averages over many crops and conditions. The precision of estimates of ozone impacts on yield are also subject to the limitation that ozone, itself, is rarely the dominant yield suppressing factor under ambient conditions. It is one of many such factors, albeit potentially a significant one. The multiple areas of focus in the following review are intended to address the common argument that scientific uncertainty in assigning specific yield loss valuations implies any uncertainty regarding the reality of crop damage due to current ambient concentrations of ozone. These impacts ultimately affect both producers and consumers of agricultural produce.

8.3 Methods Of Exposing Plants To Ozone

8.3.1 Exposure Technologies.

The previous document (ARB, 1987) reviewed the types of exposure technologies then available for crop loss assessment. Four classes of exposure technologies were considered, with some variation within the major categories: controlled systems, managed systems, seminatural systems, and natural systems. These technologies are also considered by USEPA (1996a), Hogsett et al., (1988), Grunhage and Jager (1994), and Manning and Krupa (1992). This list of available technologies remains adequate today, though considerable refinements in methodology have taken place. Characteristics of these systems, drawn from many of these sources, are presented in Table 1.

An important, if implicit, conclusion of the earlier document (ARB, 1987) was that results obtained from most of the available exposure technologies could be integrated with results obtained using other technologies. Results were considered to differ quantitatively, rather than qualitatively, and a unified body of conclusions could be drawn with respect to ozone impacts on crop yield.

In contrast, literature published since 1987 has questioned this conclusion. As most quantitative exposure-response functions have been developed using Open Top Chambers (OTCs), these techniques have come under particularly critical re-evaluation. An evaluation of the limitations and assessment of the reliability of relationships derived using OTC techniques is central to current understanding of ozone impacts on crops.

Many of the exposure technologies in Table 2 are no longer considered appropriate for crop yield assessment. The currently most acceptable technologies are open top field exposure chambers (OTCs) in the field and a variety of chamberless field designs. Chemical exclusion (principally EDU; Manning and Krupa, 1992) has been considered for crop loss assessment. On balance, however, this remains a technique for the future. The effects of such compounds on plant physiological processes, both in the presence and absence of ozone exposure, remain to be more fully elucidated. This conclusion regarding chemical exclusion confirms that of the previous document (ARB, 1987).

8.3.1.1 Free Air Exposure Systems

Recent literature has called for greater attention to non-chamber designs, partly in response to perceived limitations of OTC-derived response relationships. Such designs are attractive in many ways, but they are not new (Table 1). Zonal Air Pollution (ZAP) exposure and air exclusion systems allowed exposures to sub-ambient and elevated concentrations of ozone, both in their original (Jones et al., 1977; Olszyk et al., 1986b; Olszyk et al., 1986b) and more recent (Runeckles et al., 1990) designs. The recent innovations largely incorporate modernized computer control and more realistic ozone exposure dynamics.

A circular free air system for chamberless ozone exposure, similar to the now familiar Free Air Carbon Dioxide Enrichment (FACE) systems for CO_2 enrichment, has been deployed in several countries (McLeod et al., 1992; Shaw,

1986). Uniform average concentrations are imposed on the circular area at the center of each chamberless plot. A similar system is now operational in the U.S. incorporating exposures to both CO_2 and ozone (Karnosky et al., 1999).

Theoretical rationales and experimental difficulties associated with such studies have been reviewed by Oren et al. (1989) and were considered in the summary publications of NCLAN (McLeod and Baker, 1988; Ormrod et al. 1988). These chamberless designs do not allow strict replication over time, as wind speed direction and turbulence determine the actual exposure. With zonal systems multiple treatments may be imposed with increasing distance from the emitting manifold. With sufficient treatments within a locality, a statistical regression analysis may be used to relate damage to exposure.

8.3.1.2 Field gradient studies

Planned gradient studies utilize plants of uniform genotype planted across a naturally occurring gradient of ozone concentration. Optimally the ozone gradient can be isolated from gradients in other parameters. Such a protocol was considered promising in the previous document (ARB, 1987). More recent evaluations suggest that this technique may be nearly ideal in theory (e.g., Manning and Krupa, 1992), but limited in practice to the southern California experience (e.g., Oshima et al., 1976, 1977a,b; Grulke, 1999). There is a general lack of other demonstrated gradients of sufficient magnitude. A gradient study was conducted downwind of the London metropolitan area (Ashmore et al., 1988). In this case it proved impossible to isolate the effect of ozone among the multiple covarying pollutants and environmental factors. A study using uniform potting medium and two clones of white clover was performed across the U.S.A. (Heagle et al., 1995). Although climate differed considerably a relationship with ozone exposure was discernable.

In addition to co-occuring gradients in other anthropogenic air pollutants, ambient gradient studies are also frequently confounded by covariation in microclimate, soil type, and exposure dynamics (i.e., diurnal and seasonal timing of peak concentrations), along with the planned gradient in ozone concentration. Analysis in these cases must ultimately rely upon multifactor regression analyses (Oshima et al., 1976, 1977b). The utility of experiments conducted along ambient gradients will be facilitated by further development of process models that adequately reflect physiological interactions of microenvironmental and pollution variables. Despite these limitations, the utility and past successes of gradient studies, and their unique applicability to remote areas including those in developing countries, has led to their recent favorable review (Bell and Marshall, 2000).

Field survey methods represent a subset of ambient gradient studies, in which unplanned epidemiological or natural history studies are conducted (Oren et al., 1989) using available distributions of crops and ozone exposures. Correlations may become apparent between measures of damage such as crop loss and metrics of ambient ozone concentration. These studies suffer from the same limitations as planned field gradient experiments, in addition to problems of

unplanned spacing of observations along the gradient, and possibly inadequate levels of (pseudo)replication at similar points along the gradient.

The shortcomings of chamberless experiments of all types differ from those of OTC experiments. In some cases they may be equally severe.

8.3.1.3 Open Top Chambers

Development of the OTC allowed exposure to a range of ozone concentrations at, above, and below ambient levels. OTCs exclude intrusion of ambient pollutants by maintaining positive pressure of filtered air. The pollutant of interest may be added to clean air in varying concentrations.

The cylindrical NCLAN chamber was three meters in diameter by 2.4 meters tall. In some cases a conical frustrum was installed to minimize wind intrusion and in some cases a rain cover was installed. The design and micro-environmental impact of the NCLAN OTC (Heagle et al., 1973) was evaluated at the time of publication of the original NCLAN results (Heck, 1989; Heagle et al., 1988), and in detail in USEPA (1986). An overview of the microenvironmental impacts of the OTC is provided in Table 2.

Use of small, partially enclosed chambers, such as OTCs, required some experimental compromises. Some of the potential limitations of exposure-crop loss relationships obtained using OTC techniques are considered below. These OTC technologies were considered a major technological advance at the time of their adoption, over the conventional closed top chambers then in use for air pollution exposure studies (Bell and Marshall, 2000).

8.3.2 Altered Environmental Interactions

The NCLAN study (1980-1988) established protocols and exposure technologies to ensure compatible response data were obtained at a variety of locations with different crops. At the time of final summarization of the NCLAN results, the limitations of the study were recognized and the needs for further study using the same techniques with more comprehensive measurements or different techniques as they became available was acknowledged (Holt, 1988). Concerns were raised in the NCLAN summary (Heck et al., 1988a) regarding the unknown implications of the demonstrably altered plant microclimate.

These microclimate impacts of OTCs have been detailed and evaluated by Heagle et al. (1973, 1979b, 1988a), McLeod and Baker (1988), Unsworth et al. (1984a,b), USEPA (1986, 1996), and Hogsett et al. (1988). They were also considered in the previous document (ARB, 1987). In the intervening years, these altered microclimatic parameters caused by enclosure of plants in OTCs have been raised as serious objections to extrapolation of yield loss relationships developed in them to ambient field conditions (e.g., Manning and Krupa, 1992). These are reviewed below. It appears that plant growth may differ between OTCs and adjacent ambient plots outside, but that sensitivity of yield loss to ozone may be less affected.

A succinct statement of the goals of experimental ozone fumigation has been provided by Colls and Baker (1988): "…all parameters are fixed, one variable is

controlled at a particular value, and a response is observed…appropriate if the variable (which could be the pollutant concentration) is large enough in value for the effects due to it alone to be much greater than any other effects due, for example, to unnatural lighting or soil conditions."

These authors note that there is no *a priori* justification for assuming that changes (e.g., in yield) under unnatural conditions will be observed under ambient conditions. However, in discussing the potential advantages and pitfalls of open field fumigation systems, they further consider the role of OTCs in plant exposure experiments (Colls and Baker, 1988): "The principle of open top chambers is to control only the air supply… This is hard to put into practice [i.e., various micrometeorological parameters are altered]. All these factors affect the growth of plants to some degree; it has not yet been shown whether such unavoidable environmental modifications make a quantitative difference to pollutant sensitivity. "

8.3.2.1 Ozone Concentration

Ozone concentrations are typically monitored and recorded within each OTC. Thus actual exposures are known but may differ from the nominal (planned) exposure, and may differ from ambient values outside the OTCs. Differences between actual and nominal ozone concentrations are due to measurement noise, incursion of ambient ozone and to ozone degradation in the delivery system or against the surfaces inside the OTC. A study reviewed extensively by ARB (1987) found that between 65-81% of ambient ozone concentrations were excluded from the OTC over two years of measurement (Thompson, 1985).

The NCLAN style OTC injected air into the chamber at or below canopy height, in contrast to ambient field exposure in which ozone arrives from above. This effectively reverses the normal gradient between ozone concentration and height above and within the canopy. While the gradients in the NCLAN OTCs are statistically significant, they may be of limited biological significance since they are rather small and since light penetration and physiological activity of lower leaves is generally less than of those in the exposed upper canopy. These potential artifacts were reviewed by Heagle et al. (1989a).

Of greater significance than altered within-canopy gradients, is the gradient in the field between the reference height at which ozone is monitored and the canopy top. The OTC technology imposes a known ozone concentration at canopy level. Yield response relationships are thus parameterized with the concentration at the top of the canopy, whereas under ambient conditions the ozone concentration is not monitored at canopy height, but rather at the usually higher concentration at reference height (2 m, 10 m, or other). This inconsistency could overestimate crop loss under ambient conditions. Grungage et al., (2001) have considered the mathematical/micrometeorological transformation of reference height ozone concentrations to canopy height.

The standardization of OTC design (Heagle, 1973, 1979b; Mandl et al., 1973), and formal consideration of the gradients existing under ambient sampling conditions (DeSantis, 1999; Grunhage et al., 1999, 2001) suggests that

correction for these gradients of ozone concentrations could be applied to minimize potential artifacts. The concern in recent literature regarding potential confounding of OTC-derived results may be reduced substantially by further developments in current efforts to quantify ozone exposures in terms of effective ozone flux (Grunhage et al., 2001), incorporating transformation of ozone concentration at reference height to ozone at canopy height.

8.3.2.2 Temperature

Temperature is often slightly higher inside OTCs than outside, generally by about 1-3° C both day and night. It has been suggested that this temperature increase could be exploited experimentally as a surrogate for expected global warming, making studies of ozone responses more relevant to future atmospheres.

Interactions between low temperatures and ozone sensitivity are less significant for crop responses in most of California than in many other areas. Ozonation during winter may reduce chilling resistance (Foot et al. 1996, 1997) and exposure during the growing season may induce winter injury following subsequent sub-freezing temperatures (e.g., Chappelka and Freer-Smith, 1995; USEPA, 1996). ozone in the Mediterranean climate of California is primarily a summer phenomenon, as is most, but not all, crop production.

The importance of allometric relationships among plant component organs affects interactions of ozone with temperature. Growth of *Raphanus sativus* (radish) was inhibited by ozone when roots were chilled to 13° C but not when they were maintained at 18° C (Kleier et al., 2001). Root to shoot biomass ratio (R:S) was reduced by ozone under both root temperatures. ozone increased the sensitivity to root temperature of leaf area (reduced by chilling) and of root to shoot ratio (increased by chilling), though the effects of temperature in these experiments were generally non-significant. Modeling results vary with respect to the importance of [temperature x ozone] interactions. Temperature explained some of the variability, but was rarely a dominant factor, in model treatments of multifactor exposure-response relationships (e.g., Mills et al., 2000; van Oijen and Ewert, 1999). In most cases the combination of temperature and relative humidity (RH) or the closely related evaporative demand (VPD) accounted for a considerable fraction of the observed variability in the ozone-end point relationships. Unfortunately, temperature and both RH and VPD are closely related under many environmental conditions.

8.3.2.3 Humidity

RH will likely be increased in the OTC relative to outside under most conditions (e.g., 3-4% in the study of Thompson, 1985). This will depend on ambient humidity, the transpirational activity and density of plants within the OTC, and prevailing wind speed and humidity outside. Generalizations regarding a consistent OTC effect are not possible.

Humidity, and its alternate expression, evaporative demand (VPD), may directly influence crop sensitivity to ozone by influencing stomatal conductance (Grantz, 1990). In humid air of low VPD, stomata open more widely than in dry air with

higher VPD, thereby increasing the flux of ozone into the leaf interior and increasing potential damage and injury. A multi-site and multi-crop study in Europe led to development of Critical Levels of ozone for short term (5 day) exposures (AOT 40) depending on VPD (500 ppb-hr for dry conditions when VPD > 1.5 kPa; 200 ppb-hr for humid conditions when VPD < 0.15 kPa; Benton et al., 2000).

There is considerable variability among genera, species and cultivars in the significance of the interaction of humidity and ozone sensitivity, with modeling results diverging (Balls et al., 2000; Mills et al., 2000; van Oijen and Ewert, 1999). In the multi-year experiments performed in the Po River Valley in Italy during humid but mild spring growing conditions, an apparent interaction between humidity and ozone sensitivity was identified between years. Other factors, including the phenological timing of ozone dynamics, also differed. This interaction between the progression of growth stages of the plant, which may vary with weather and other conditions, and the timing of peak or sustained moderately elevated concentrations of ozone is increasingly understood to have a large influence on the extent of plant injury.

Assessment of ozone impacts on California agriculture may depend on the relevance of exposure-response relationships determined in humid areas to the summer-dry, Mediterranean climate in California. This issue has parallels with the situation in Europe, where the AOT40 metric was developed using northern European studies, but extrapolated to the Mediterranean climates of the southern European countries (De Santis, 1999, 2000). In the case of Europe, the issue has been addressed using OTC exposures, showing substantial yield losses of a wide variety of crop species in Mediterranean regions (Fumagalli et al., 2001a,b). Nevertheless, the magnitude of damage predicted by the available response equations from Northern Europe was not observed in the south. This is most likely attributed to genotypic differences in cultivars as well as to hardening of plants in response to environmental conditions prevailing in the Mediterranean summer growing season.

A consequence and sometimes correlate of humidity is leaf surface wetness. Leaves in OTC environments are subject to reduced periods of dew formation due to the fan-driven turbulent air. Dew presence on leaves of grape (Grantz et al., 1995) and mixed pasture (Pleijel et al., 1995b) enhanced ozone deposition, though not through the stomatal uptake pathway, but rather through heterogeneous aqueous reactions on the leaf surface. In contrast, in the amphistomatous leaves (i.e., with stomata on both upper and lower surfaces) of cotton, the presence of dew reduced ozone uptake, probably by occlusion of stomatal pores on the underside of the leaves (Grantz et al., 1997). In both cases, and perhaps generally, the impact of leaf wetness on ozone damage and injury, as opposed to deposition, may be minimal.

The conclusion of the previous document (ARB, 1987), and of USEPA (1996a), that increased humidity or reduced VPD is generally associated with greater plant sensitivity to ozone, remains valid. This is due to effects on stomatal conductance rather than to leaf wetness. The general effect of altered humidity

on crop responses to ozone measured in OTCs is unclear. It is probably dominated by irrigation or rainfall, which itself modifies the humidity of the canopy environment, both within and outside of OTCs.

8.3.2.4 Water Status

OTCs reduce effective rainfall that reaches plant roots inside OTCs. This is typically less important in the rain-free, summer growing season for most crops in California and other regions with Mediterranean climates. A tendency to maintain experimental plantings more abundantly irrigated than ambient control plots or reference commercial fields will influence plant growth and could alter sensitivity to ozone. However, effects of soil moisture on growth are much more consistent than on sensitivity to ozone.

The effects of soil moisture deficit on crop response to ozone have remained ambiguous. Drought clearly closes stomata, which reduces flux of ozone into the plant interior. With other factors held constant (e.g., antioxidant defense compounds), this effect will reduce damage caused by ozone. However, drought may induce defense compounds, which increase ozone tolerance. Drought reduces plant growth by inhibiting cell expansion, and also alters development. These impacts on growth and yield may dominate small increases in ozone tolerance.

Soybean exhibited some protection by soil moisture deficit (Vozzo et al., 1995), wheat exhibited only additive effects of ozone and moisture deficit (Fangmaier et al., 1994a,b; Bender et al., 1999; Ommen et al., 1999). Miller et al. (1988) found no [ozone x water deficit] interaction in plant growth of cotton at a moderate level of drought which reduced leaf area and yield but did not affect total biomass production. However, for yield a strong interaction was observed. The water stress reduced yield by 16% at low ozone (20 ppb) but increased yield by 28% at 70 ppb ozone, relative to well-watered plants at the same high ozone concentration. Clearly drought reduced the impact of ozone considerably (Heagle et al., 1988b), though at more moderate levels of ozone the protection afforded by drought did not protect yield nor even restore ozone-exposed yields to control levels in filtered air.

Cotton in California (Temple et al., 1988a) was rendered less sensitive to ozone by both mild and severe water deficits. However, yield was inhibited at even moderate water deficit to such an extent that it fell below well-watered levels at all ozone concentrations at which comparisons were made. Thus the widely cited “protective” effect of water stress was largely illusory, as the impact of water management to protect against ozone was more damaging than the ozone itself. The same was true for total plant biomass productivity in alfalfa (Temple et al., 1988b). Chemical soil drought (i.e., salinity) was mostly additive with ozone in reducing growth of rice (*Oryza sativa*; Welfare et al., 1996).

The conclusion from the multi-site and multi-crop NCLAN study (Heagle et al., 1988a) was that any protective effect of soil drought against ozone damage is inconsistent and dependent in unknown ways on the magnitude and timing of the drought.

An important observational study was conducted in the Ohio River Valley of the Midwestern U.S. (Showman, 1991) in which visual injury was observed on a wide variety of native vegetation. Injury was more substantial in a year of relatively low ozone concentrations but adequate rainfall, than in a subsequent year of higher ozone concentrations but drought. This study is strong evidence for the protective effect of some level of water deficit, though the environmental conditions required to lead to this result remain uncharacterized.

The NCLAN and observational results, above, are supported by modeling results with TREEGRO (Retzlaff et al., 2000). Simulated protection against ozone was found in white fir (*Abies concolor*) at high levels of soil moisture deficit, but additive effects of moisture stress and ozone were predicted at more moderate levels of soil drought. More recent experimental studies support these conclusions, but have not added to the quantitative or mechanistic characterization of the effect (Mills, 2002).

8.3.2.5 Turbulence

Among the most notable microclimate alterations in OTCs is the uniformity of wind speed. A typical constant OTC wind velocity of about 2.5 km h^{-1} is in striking contrast to the diurnal varying wind speed in the ambient environment. Air movement is more constant within the OTC, but may be greater than or less than ambient levels outside the OTC, depending entirely on location. In many environments this may represent a reduction in daytime wind speed, but in the dominant cropping area of California, the San Joaquin Valley, it is often an increase.

Turbulence has three primary impacts on plants. Dew persistence declines as noted above. Thigmotropic (contact or movement sensing) stimulation increases. This may lead to various types of hardening responses that reduce sensitivity to further stimulation, mostly poorly characterized but potentially leading to increased tolerance to ozone exposure. Leaf and canopy boundary layer resistance to penetration of gases present in the mixed atmosphere declines.

In the ambient environment turbulence reduces the gradient between the ozone concentration measured at reference height and that measured at canopy height, and also the gradient between the top of the canopy and the leaf surface. This increases stomatal coupling (Jarvis and McNaughton, 1986) with several consequences. It increases stomatal control of transpirational water loss, increases the CO_2 concentration available at the leaf surface for photosynthesis and that of ozone for uptake. In the turbulent OTC environment, with well mixed, ozonated, air blown directly into the canopy, canopy and leaf boundary layer resistances are minimized. In practical terms, this allows the experimenter to impose a known ozone concentration directly at the leaf surface. It also allows ozone flux into the plant to be calculated from stomatal conductance and ozone concentration.

Another factor of relevance to the OTC is the leaf boundary layer resistance. Fan-generated turbulence in the OTC minimizes this resistance, and stabilizes it throughout the course of the day. In the natural canopy leaf boundary layer

resistance is dependent upon thermal and mechanical (wind-driven) turbulence, which typically varies during the day.

Unsworth (1984a,b) found that boundary layer resistance was reduced by the OTC in soybean canopies, but that fluxes of ozone were similar to measured values in extensive soybean fields.

8.3.2.6 Radiation

The OTC environment is lower in light intensity than the ambient environment. The plastic walls of the OTC generally impose a 10-15% decrease in photosynthetically active photon flux density. This will depend on dust accumulation on the plastic walls, and on the age and weathering of the plastic itself.

Sanders et al., (1991) found a 20% reduction in radiation and a slight (0.8 C) increase in temperature inside OTCs relative to ambient conditions outside. They observed that broad beans (*Vicia faba*) plants yielded more and developed more rapidly under these conditions than outside. There was no indication that impacts of ozone observed inside the OTCs (not reported) would have been substantially altered by the OTC environment (i.e., a significant interaction).

In the northern hemisphere, including California, plants grown on the northern half of OTCs may grow more vigorously and yield more than plants on the southern side (Heagle et al., 1988a). In response to this radiation environment, different quadrants of an OTC may exhibit different yield levels, with significant spatial correlation (within an OTC; across quadrants) between plant performance and microclimate (Heagle et al., 1989a). These obvious differences in plant vigor are not linked to differences in ozone sensitivity (Heagle et al., 1988a) as shown by the lack of spatial correlation (within a quadrant; across chambers) of the relative response of yield to ozone.

[Light x ozone] interactions remain incompletely characterized. Results in multi-layer forest canopy trees (Chappelka and Samuelson, 1998) are complex due to within plant gradients in age and light environments. In many cases ozone sensitivity is greatest near optimal light intensity, varying between shade and sun species or leaves (e.g., Tjoelker et al., 1995b; Fredericksen et al., 1996a,b; Topa et al., 2001; Mortensen, 1990). The previous conclusion of USEPA (1996a), that low light intensities and short photoperiods enhance ozone sensitivity, appears to be an over-generalization, as various contrasting effects have been observed (Tjoelker et al., 1995a; Fredericksen et al., 1996b).

Very high light environments induce photoinhibition (itself a form of oxidant damage). An [ozone x high light] interaction involving both photochemistry and dark biochemistry was apparent in leaves of bean. ozone exposure caused effects largely on dark reactions (Guidi et al., 2000; Dann and Pell, 1989). Some parameters, derived from chlorophyll fluorescence, (e.g., F_o) were significantly affected by light in bean leaves but not by ozone. Others (e.g., $1-q_p$) were affected by ozone but not by light, while still others (e.g., quantum yield for CO_2

fixation) were affected by both to a similar extent, but more than additively when exposed to both ozone and light.

The inconsistent experimental evidence is fully reflected in available modelling treatments. Using two models and the dataset of the European Stress Physiology and Climate Experiment (ESPACE), Bender et al. (1999) found that ozone impact on yield of wheat increased with light intensity. In contrast, the similar modeling study of Ball et al. (1998, 2000) found that the effects of moderate ozone concentrations on clover declined with increasing light intensity. Effects of OTC technologies on light-induced changes in crop sensitivity to ozone exposure are likely to be equally inconsistent.

8.3.2.7 Nutrition

There is considerable interaction between ozone sensitivity and soil fertility. In some cases, ozone sensitivity may be greatest at optimal levels of general soil fertility. However, ozone sensitivity was reduced in *Plantago* and birch by adequately fertile soil (Whitfield et al., 1998; Landolt et al., 1997) and manganese deficiency increased ozone sensitivity of beans.

[Nitrogen x ozone] interactions are the best characterized, and environmentally the most relevant. N may enter plants either via soil deposition or by foliar uptake (Grantz et al., 2003. In long-lived natural communities, N deposition may exacerbate the impacts of ozone on plant health and productivity (Fenn et al., 1996; Takemoto et al., 2001; Grulke, 1999). The complex of tropospheric pollutants in California contains NH_3, derived from dairy and fertilizer emissions, acidic HNO_3 vapor, and NO plus NO_2. This complex leads to formation and deposition of NH_4NO_3 particulate matter, especially in the wintertime in the San Joaquin Valley. HNO_3 vapor exhibits a particularly high rate of deposition in areas in which it is present, particularly the Los Angeles air basin and surrounding mountains (Takemoto et al., 2001).

Ozone sensitivity of a variety of deciduous forest tree species, including aspen, poplar, and birch (Greitner et al., 1994; Bielenberg et al., 2001; Paakkonen and Holopainen, 1995), was minimized at near-optimal levels of N nutrition.

Current rates of ambient N deposition are minor compared with application rates of N containing fertilizers to most crop production systems in California. Increasing nitrogen fertility increased ozone sensitivity of wheat (Cardoso-Vilhena and Barnes, 2001). In cotton, high levels of N fertility protected growth and yield against ozone (Heagle et al., 1999). There was some [N x ozone] interaction in partitioning of carbon within leaves (Booker, 2000). At moderate levels of N fertility ozone exposure increased leaf soluble sugars, but at lower and higher N levels, ozone decreased soluble sugars. The expected protection against ozone by elevated CO_2 (e.g., in leaf starch or soluble sugar concentrations) was reduced at high levels of N fertility.

Natural and managed ecosystems are subject to increasing concentrations of atmospheric CO_2. ozone impacts will be interactive with both increasing temperature and with CO_2 concentration. A retrospective study of [ozone x CO_2]

interactions (on vegetative growth rather than economic yield) concluded that elevated CO_2 would substantially alleviate the ozone-induced reduction in plant growth (Poorter and Perez Soba, 2001). Similar conclusions have generally been drawn from a number of exposure studies of [CO_2 x ozone] effects on crop yield.

There is insufficient evidence at present to predict the impact of nutritional status on plant sensitivity to ozone. Predicted effects of enhanced N nutrition are species dependent. In the case of well-fertilized crop species which are treated with abundant N under commercial agronomic conditions, the typical OTC cultivation regime may have little impact on sensitivity to ozone. Rising CO_2 is likely to reduce the deleterious impacts of ozone on crop yield. However, an equally valid perspective is that any growth and yield enhancement expected due to rising CO_2, will be substantially reduced by ozone.

8.3.2.8 Pest Management

Frequently the intensive cultivation of crops in OTCs leads to finer control of biotic interactions than would be possible commercially in the ambient field environment. The large number of insect, fungal and bacterial species of agronomic and horticultural importance, and the variability in potential ozone exposure dynamics and microenvironmental conditions, renders a complete experimental characterization of all possible interactions unlikely. While generalization from the relatively few experimental treatments will be required, the contrasting conclusions among the few available studies makes even this task uncertain. Further experimental work is required on such [biota x ozone] interactions. This reaffirms previous conclusions (USEPA, 1996a; Docherty et al., 1997; Flukiger et al., 2002).

Insects remain a serious challenge to crop production. Aphid infestation remains the best characterized interaction with ozone exposure. Unfortunately, ozone has been reported to enhance and to inhibit aphid development (Flukiger et al., 2002). In contrast to unambiguous responses of aphids to other air pollutants (SO_2 and NO_2), responses to ozone in the literature are about evenly divided between positive, negative, and null changes (Holopainen, 2002).

ozone impacts on the aphids appear to be indirect, mediated by effects on the host plant. Experimental variability is likely linked to nutritional, genetic and environmental differences in the plant sensitivity to ozone. Relative growth rate of individual Russian wheat aphids (*Diuraphis noxia*) in California increased by over 40% at 12 hm (hourly mean) ozone concentration of 102 ppb (Summers et al., 1994). As with other aspects of plant response to ozone, development of process models that incorporate responses of the various organisms to ozone, and their mutual and environmental interactions will be required to fully generalize the multitude of interactions. A step in this direction, with respect to the aphid interaction, has been the suggestion that aphid performance may be enhanced by ozone when temperatures are near the optimum for the insect, and reduced with both warmer and cooler temperatures (Whittaker, 1994).

Growth of *Lygus rugulipennis* was inhibited by ozone on Scots pine trees. As other *Lygus* species are serious insect pests in cotton, dry beans and other crops

in California, this observation may be of significance to California agriculture. However, as *Lygus* populations evolving in the San Joaquin Valley are doing so under ozone pressure, increased resistance to ozone may be expected in the future.

Spider mite infestation of ozone-sensitive clover and peanut was increased by ozone exposure (Heagle et al., 1994; Hummel et al., 1998). Mites are of concern to California tree crops, particularly citrus, and to cotton, suggesting a potentially deleterious interaction with ozone in California. The possibility of evolving resistance to ozone, as has occurred with miticides in the San Joaquin Valley, cannot be ignored.

Infestation of an ozone-resistant clover by spider mites did not increase following exposure to ozone. This suggests, as above, that the interaction is mediated by the plant response to ozone. This level of mechanistic detail will be required to develop the necessary process models of ozone impact that will allow generalization from limited experimental data to the ambient crop production environment.

California agricultural production systems are now heavily dependent upon low-impact Integrated Pest Management (IPM) techniques. These involve extensive use of beneficial predatory insects and spiders in lieu of synthetic pesticides. ozone has been reported to impede the prey acquisition behavior of at least one parasitoid, *Asobara tabida* (Gate et al., 1995). The widespread use in IPM of pheromone-baited attraction traps, and pheromone-based mating disruption strategies, may also be impacted by ozone. The male pheromone of fruit flies was significantly degraded by ozone (Arndt, 1995) as demonstrated by chemical analysis and by reduced bioactivity as an aggregation stimulus.

While ozone exposure may alter the biology of the insect component of these plant-pest complexes, the reverse may also be true. For example, whitefly has become an important pest in California agriculture. Infestation with the greenhouse whitefly (*Trialeurodes vaporariorum*) increased the sensitivity of common bean (*Phaseolus vulgaris*) to chronic exposure to moderate concentrations of ozone (Rosen and Runeckles, 1976).

Pathogens may exhibit considerable sensitivity to ozone. Postharvest handling of fruit in California has made some use of ozone exposure at higher than ambient concentrations (continuous 300 ppb), as a storage fumigant. This treatment (at 5 ^{o}C and 90% RH) reduced aerial mycelial growth, sporulation, and infection intensity of brown rot (*Monilinia fructicola*) on peaches. Gray mold (*Botrytis cenerea*) was also inhibited on grapes (Palou et al., 2002). A variety of other molds and rots including *Rhizopus stolonifer* (Rhizopus rot) infections were slowed but infection was not reduced. It should be noted that this concentration of ozone, while currently above ambient in California, was not always so, and is not unknown now in urban environments in developing nations.

Infection with facultative pathogens (i.e., those that can feed upon plant material while it is alive or after its death) was increased, or symptoms exacerbated, by prior exposure to realistic ozone concentrations in controlled environment

exposure chambers. Grey mold (*Botrytis cinerea*) infection of bean (*P. vulgaris*) was also increased (Tonneijck, 1994; Tonneijck and Leone, 1993) following inoculation with conidia (asexual spores) but not with mycelia (vegetative tissue) (Tonneijck, 1994) at these moderate concentrations. This contrasts with the effect of 300 ppb, above. Leaf spot (*Marssonina tremulae*) of poplar was enhanced by low concentrations of ozone. Conidial germination was inhibited at higher concentrations of 200 ppb for 8 hours/day over 2 weeks (Beare et al., 1999). White mold of bean (*Sclerotinia sclerotiorum*) was enhanced by ozone (Tonneijck and Leone, 1993), as were tan spot (*Pyrenophora triticirepentis*; Sah et al., 1993) and blotch (*Septoria nodorum)* of wheat (*Triticum aestivum*). The sensitivity of 12 wheat genotypes to ozone was correlated (r^2=0.86) with sensitivity to tan spot, suggesting that similar mechanisms may be involved in response to ozone and to pathogens (e.g., Bahl et al., 1995).

Infection with obligate biotrophic pathogens, (i.e., those that require living plant tissue to feed upon) was similarly variable in their interactions with ozone. Powdery mildew (*Sphaerotheca fulginea*) on cucumber (*Cucumis sativa*) was enhanced at low but decreased at high ozone concentrations (Khan and Khan, 1999). Powdery mildew (*Erysiphe polygoni*) on pea (*Pisum sativum*) was inhibited by ozone exposure (Rusch and Laurence, 1993). However, plant sensitivity to ozone was reduced by the infection, as was the case with cucumber powdery mildew at higher ozone concentrations, and with rust (*Uromyces viciae-fabae*) on broad beans (*Vicia faba*; Lorenzini et al., 1994).

[ozone x nematode] interactions are suggested by the reduced allocation of carbohydrate to roots caused by ozone (Cooley and Manning, 1987). Infection of tomato (*Lycopersicon esculentum*) with root knot nematode (*Meloidogyne incognita*) was exacerbated by ozone exposure, and ozone impacts were enhanced synergistically by nematode infection (Khan and Khan, 1998). This and other nematodes are a substantial factor in California agriculture. The [nematode x ozone] interaction may increase in importance as methyl bromide usage for preplant fumigation is phased out to protect the stratospheric ozone layer.

Weed competition is a leading cause of crop loss, but there is little known about ozone interactions in weed-crop competition. Grass-legume mixtures tend to simplify toward pure grass in response to ozone exposure (USEPA, 1996a). This has been observed in an open air fumigation system (Wilbourne et al., 1995) as well as an OTC exposure system (Nussbaum et al., 1995) with a grass-clover system (*Lolium perenne-Trifolium repens*). Dieback of clover left barren patches that encouraged introduction of weedy species.

Modest alterations of the microenvironment were documented within and above a multi-species pasture inside OTCs, relative to the ambient plots outside (Fuhrer, 1994). Of greatest importance was a reduction in global radiation (22%, averaged over two years) and an increase in temperature (1.3 C in both years). Differences in wind speed, precipitation and vapor pressure deficit were noted, but are extremely site-specific. A significant reduction in the ratio of biomass of clovers to grasses was noted inside relative to outside the OTCs. Weedy species replaced clover, particularly during the second year of the experiment. Clover

decline is often observed in experimental studies of mixed swards, but these were clearly accelerated in this study. It was not demonstrated that responses to ozone were altered (i.e., interaction, which was not considered in this study) though both the OTC environment and ozone reduce the clover contribution to total biomass. Similar effects of both factors on clover were noted by Heagle et al., (1989b), without evidence of interaction.

ozone exposure of an alfalfa-timothy mixture (*Medicago sativa-Phleum pratense*) caused a competitive disadvantage to the alfalfa, probably due largely to the ozone impact on the extensive alfalfa root system (Johnson et al., 1996a). This may be of particular relevance to California production of alfalfa hay and seed, as increased competition from grass weeds is a common cause for retirement of perennial alfalfa fields.

Current capability to predict the interaction between ozone exposure and plant competitive interactions is limited. Outcomes cannot be inferred from the relative ozone sensitivities of the individual species involved (Evans and Ashmore, 1992). For example, an early successional community dominated by sumac (*Rhus copallina*) was replaced during OTC fumigation by a community dominated by blackberry (*Rubus cuneifolius*), even though blackberry is quite ozone sensitive. A potential difficulty in extrapolating ozone exposure-response relationships from OTC to ambient environment may be the greater attention likely given to weed control in OTCs relative to extensive agronomic fields.

8.3.3 Intercomparisons of Available Exposure Technologies.

8.3.3.1 Plant Growth

It is clear that plant growth, and in some cases morphology, may differ between plants grown in OTCs and in ambient plots outside or in nearby commercial fields. This has been documented numerous times (e.g., for winter grown lettuce and wheat; Thompson, 1985; Olszyk et al., 1986a) in southern California. These differences were associated with an increase in temperature ranging from 0.1 to 4.2 C, and a reduction in light intensity of about 25%. More recently, Elagoz and Manning (2002) found that growth of beans (*P. vulgaris*) differed in chambers from plants grown outside under Massachusetts conditions. However, while yield of an ozone tolerant line was suppressed in the OTC relative to the ambient plots outside, there was no effect of ozone on yield in either cultivar (Elagoz and Manning, 2002). Olszyk et al., (1992) found changes in plant growth habit of Valencia oranges in OTCs that had little effect on ozone sensitivity.

Plants of alfalfa exposed to ozone in southern California in a mechanical field exclusion system were shorter than those grown in nearby OTCs. Plants in the OTCs exhibited similar biomass (Olszyk et al., 1986b). Significantly, plants in the field exclusion system were more similar in appearance to plants grown in ambient field plots than to those grown in the OTC. Plant height was one of the few (< 10%) of endpoints that differed across exposure technologies. Height of plants grown inside OTCs and subjected to non-filtered ambient air, were generally taller during the NCLAN experiments than plants grown in ambient plots outside (Olszyk et al., 1980; Heagle et al., 1979b). Plant height was the only

growth parameter that exhibited a consistent difference (Heagle et al., 1988a). A retrospective analysis of multi-location, multi-crop NCLAN studies (Krupa et al., 1994; Legge et al., 1995) found that 39 of 56 yield observations were not significantly different between non-filtered and ambient outside plots.

The altered microenvironment associated with OTC exposure protocols is associated with slight alterations in plant growth. Changes in physiological parameters are less well documented. The increase in humidity (reduction in VPD) under some OTC conditions is predicted to increase stomatal conductance. This would increase ozone flux and plant damage. However these interactions are inconsistent across experiments. Heagle et al. (1988a) reviewed reports of reductions, increases, and unchanged values, in stomatal conductance and carbon assimilation rates in OTC-grown plants of varying species in a variety of NCLAN locations.

The OTC effect on stomatal conductance is important because recent literature suggests that fluxes of ozone to plant internal receptors may be the most closely related exposure parameter to injury or damage. Flux to oat (*Avena sativa*) was compared in OTCs and in an open field (Pleijel et al., 1994b), using a mass balance-resistance analog model and a concentration gradient method, respectively. The results demonstrate clearly that no consistent trend in stomatal conductance was observed that could explain large differences in calculated ozone flux to the plants in the two contrasting conditions. In the case of the oat crop, ozone concentration in the OTC (imposed at canopy height) was 25% lower than in the field (at 2 m reference height). In contrast, flux to the plants plus chamber walls was 40% greater in the OTC. This was not attributed to leaf boundary layer resistance, which was similar under both conditions in the upper canopy, though larger in the OTC at deeper, less active, layers of the canopy. It appears to be (partially) due to the loss of the gradient between 2 m and the outside of the individual leaf boundary layer, and (mostly) to uptake by non-vegetative elements in the OTC such as soil and plastic walls. These data do not seem to cast serious doubt on results obtained using OTC technology. The concerns of Legge et al. (1995) are mostly addressed by a flux based metric as proposed by Grunhage et al. (2001) and particularly if chemical defense mechanisms are included, as proposed by Massman et al. (2000).

8.3.3.2 Sensitivity to Ozone

Several validation tests of the OTC technology have been published. Oshima et al. (1976) used the natural gradient available across southern California with alfalfa in a uniform soil mix and 5 gal pots and found only ozone among a number of microenvironmental parameters, was correlated with yield suppression and leaf abscission (i.e., detachment of the leaf along a biochemically prepared separation zone). In this early study any possible co-occuring gradients of other pollutants were not considered. In the same area of southern California (Olszyk et al., 1986b) a comparison of ambient air plots (unchambered), OTC plots (chambered), closed field chambers, and field exclusion system plots (unchambered) was undertaken, also with alfalfa. Comparisons using each technology were co-located. Comparisons were made using ambient air in

Southern California (high but unspecified ozone concentration) and charcoal filtered air. Yields and plant growth were similar, though plant height was greater in the OTCs. The ozone-induced yield suppression in ambient air in OTCs was similar to that predicted by the chamberless gradient study. This study provides particularly strong evidence that yield responses obtained in OTCs may be reliably extrapolated to field conditions, and with even more confidence, compared with yield responses obtained using other exposure technologies.

Grunhage and Jager (1994) suggest that chamber exposures may overestimate the impact of ozone on crop yield due to invariant deposition velocity. This reflects both atmospheric conductivity (above; Grunhage et al., 2001) and stomatal dynamics which are more constant in an intensively managed OTC than in less optimally managed ambient field settings. Unexpectedly small responses of tobacco to high concentrations of ambient ozone are likely explained by the non-contemporaneous occurrence of stomatal conductance and ozone availablility.

In cool periods of low incident radiation, the increase in temperature but further reduction in light intensity observed inside the OTC may enhance plant sensitivity to ozone. This is not relevant to much of California agriculture, though production of winter vegetables is important in some areas. Microenvironmental effects of OTCs under such conditions were demonstrated in California (Thompson, 1985).

Sensitivity to ozone is not generally affected by growth in OTCs (Heagle et al., 1988a; Olszyk et al., 1986b). The ratio of sensitive to tolerant clover biomass was similar in OTC and ambient plots (Heagle et al., 1996). Comparisons of contrasting ozone exposure technologies have been conducted with clones of trembling aspen. Plants were exposed in OTCs (Karnosky et al., 1996), across an open air exposure gradient in three locations (Karnosky et al., 1999), and in a chamberless FACE system (Karnosky et al., 1999; Isebrands et al., 2000, 2001). In these various comparative studies there was little indication that the relative ranking of ozone sensitivity of these diverse clones (Karnosky et al., 1996, 1999) was altered by the exposure technology. There was similarly no evidence of any systematic increase in ozone sensitivity in those plants exposed in the OTC experiments. Indeed, symptom development may have been more pronounced in the chamberless experiments (Karnosky et al., 1999). Yield of oilseed rape in Britain was reduced by 14% and the number of yield bearing branches by 38% in a free-air (i.e., non-chamber) exposure system. AOT40 levels were 3774 ppb-hr (fall) and 8960 ppb-hr (spring) relative to ambient controls of 239 ppb-hr and 690 ppb-hr, respectively (Ollerenshaw et al., 1999). Peak hourly concentrations were about 77 ppb and 80 ppb relative to control values of 30 ppb and 31 ppb. A similar free-air exposure of winter wheat (Ollerenshaw and Lyons, 1999) to AOT40 = 3472 ppb-hr (fall) and 6178 ppb-hr (spring), with daily mean values of 75 ppb and 81 ppb, reduced yield by 13%, relative to ambient controls of 135 ppb-hr and 469 ppb-hr; 30 ppb and 33 ppb. This is similar to other estimates of the ozone sensititivity of wheat obtained in OTCs (Table 8).

OTCs with raisable side panels were used to reduce the impact of growth environment on plants subjected to ozone (Wiltshire et al., 1992). Apple trees

were grown with the plastic sides raised except when ozone was added, during periods when local ambient atmospheric conditions were conducive to high concentrations of ozone, (high radiation, high temperature and low wind speed) and for only 8 h/day and no more than three consecutive days. Trees exhibited no visible ozone injury and were only intermittently exposed to OTC conditions, yet exhibited substantial ozone-induced leaf abscission and reduced leaf area duration (potential productivity estimated as the product of green leaf area and time of leaf display). Yield was not reported but would be expected to be reduced along with leaf area duration.

The limitations of crop yield loss assessments conducted in OTCs was considered in USEPA (1996a). Concerns included small plot sizes, altered microenvironmental parameters, and effects of charcoal filtration on air quality. The importance of characterizing such factors and their influence on extrapolation and scaling of these controlled exposure experimental data to estimates of regional (e.g., California) crop loss assessment was noted at that time. However, it was concluded that:

"…These uncertainties are not quantified, although there are preliminary data establishing their existence…There is an urgent need to estimate these uncertainties so that the OTC data can be used fully, with little doubt as to how well the data represents (sic) real crop losses."

and that

"…at the current time, OTCs represent the best technology for determination of crop yield responses to ozone; concentration and duration of the gas are well controlled, and the plants are grown under near-field-culture conditions."

These conclusions, similar to those of ARB (1987) and to those explicitly stated by Olszyk et al. (1986b), remain valid today. The definitive studies may not be possible to design, and certainly remain to be reported at this time. There is little scientific justification for the categorical discounting of ozone yield-response relationships obtained using OTC technology.

8.4 Sites and Modes of Ozone Damage to Crops

8.4.1 Ozone Uptake

The previous document (ARB 1987) concluded that ozone present near the plant canopy must overcome physical resistances prior to entry into plant leaves. Entry into the leaf interior was considered prerequisite to injury and damage. Research since 1987 has not invalidated these important conclusions. Possible effects of OTC exposures on these resistances are considered above.

Surface conductance (1/resistance) for deposition of ozone from the atmosphere consists of plant and non-plant components, and depends on interacting physical, physiological and biochemical factors (Baldocchi et al., 1987; Grantz et al., 1997; Hicks et al., 1987). These resistances are associated with a boundary layer of poorly mixed air, and a stomatal resistance that is under physiological control. In contrast to uptake through stomata, external deposition may cause

minor damage to cuticles (the water impermeable leaf coating) and trichomes (leaf hairs) on leaf surfaces, which may increase non-stomatal, cuticular water loss (i.e., through imperfections in the waxy cuticle that covers the epidermis of leaves and other plant parts) (Barnes et al., 1998; Kerstiens, 1989a,b). Heterogeneous reactions leading to ozone destruction occur throughout the canopy (Leuning et al., 1979a,b) and cause little injury but complicate interpretation of ozone fluxes, as the distribution of sinks for ozone within the canopy remains uncertain. In addition, degradation of ozone by bare soil may approach 40% of total deposition under some conditions (Leuning et al., 1979b; Turner et al., 1973). These factors also confound flux measurements in OTCs, as noted above.

The dominant and most damaging route is through stomatal pores of photosynthetically active leaves. Internal leaf sites represent significant regional sinks for ozone, controlled by the physiologically modulated stomatal response (Baldocchi et al., 1987; Grantz et al., 1995; Massman and Grantz, 1995; Roper and Williams, 1989; Thomson et al., 1966; Temple, 1986). In daylight the canopy stomatal conductance is usually smaller than the atmospheric conductance and therefore controls the rate of ozone uptake from the atmosphere, deposition to vegetation, and damage (Grantz et al., 1994, 1995, 1997; Massman and Grantz, 1995; Massman et al.,1994; Wesely et al., 1978). The primacy of stomatal uptake is suggested by a moderate regression, among several highly diverse species, between ozone-induced growth decline and increased stomatal conductance (Volin et al., 1998). Further inclusion of carbon assimilation (as the ratio of assimilation to stomatal conductance) improved this regression substantially.

ozone fluxes between the atmosphere and an extensive cotton canopy in California increased with increasing canopy stomatal conductance, whether the canopy was dew-wetted or dry (Grantz et al., 1997). In isolated leaves, stomatal closure substantially reduced ozone uptake and largely prevented ozone damage to foliage (Butler and Tibbits, 1979). Perennial crops typically exhibit lower gas exchange and stomatal conductance than annual agricultural crop species (Reich 1987). This reduces ozone uptake and damage relative to physiologically more active crop species. These differences were reflected in the eddy covariance determinations of ozone uptake to cotton and grape in California (Grantz et al., 1994). Canopy conductance is thus dominated by stomatal conductance, which constitutes a first line of plant defense (Taylor et al., 1982a; Grantz et al., 1995; Massman and Grantz, 1995).

Normalization of ozone flux, usually measured by micrometeorological techniques, by ambient ozone concentration yields the ozone deposition velocity (V_d = flux/concentration). V_d is typically more strongly related to canopy conductance than is ozone flux (Grantz et al., 1997). This indicates that ambient ozone concentration is not well correlated with stomatal opening. This might be expected both from the inhibitory role of ozone on stomatal opening and from the lack of correspondence between atmospheric conditions that are conducive to stomatal opening (e.g., high humidity) and periods of high ozone concentration and vigorous atmospheric mixing (Grunhage et al., 1997). This means that

neither timecourses of ambient ozone concentration (the current definition of "dose") nor models of stomatal conductance, will yield a general prediction of ozone uptake or plant impacts. The interaction of the two, along with the dynamics of plant sensitivity to ozone (e.g., Massman et al., 2000) may rationalize does-response relationships in OTCs with those obtained in the field.

8.4.2 Metrics for Evaluating Crop Productivity Losses.

Various indices of ozone exposure have been evaluated in the literature, generally using statistical regression techniques that determine how well they relate ozone monitoring data (ambient or experimental) to crop loss. Yield loss indices vary in methods of averaging, weighting peak exposures or discounting sub-threshold concentrations (e.g., SUM06, AOT40) in which higher concentrations receive more mathematical weighting in terms of the response of the vegetation (Lefohn and Runeckles, 1988; Lee et al., 1988), summing concentrations over various periods, and weighting of phenological stages (i.e., time-dependent periods of plant development). None of these various metrics has been linked to mechanistic models. It has not been possible to identify the "best metric" nor to use them to predict interactions of ozone with environmental parameters such as temperature or humidity. Weighting of peak concentrations may predict visible injury indices over short exposures, yet perform less well over over longer, chronic exposures, during which stomatal conductance and ozone uptake may decline. The NCLAN program evaluated four possible ozone exposure statistics. These generally involve long-term means or peak analysis. It remains unresolved whether the average concentration as suggested by the results of Heck et al., (1984) or peak concentrations which were found to be site-specific (and thus exposure dynamics-specific) (Heck et al., 1984) will better predict injury and damage caused by ozone.

Current research suggests that a long-term cumulative metric (i.e., a sum of concentrations over a substantial portion of the growing season) may best predict ozone impacts on agricultural yields (Lee et al., 1994). This is the basis of the AOT40 metric currently identified in Europe and the SUM06 metric frequently used in the U.S. No attempt is made here to resolve these issues. Results of published studies are reported using the original ozone metric. Both recent and older research, as observed previously (e.g., Fuhrer et al., 1992; Massman et al., 2000), indicate that quantitative estimates of effective ozone dose are likely to relate most accurately to resulting injury and damage. Effective ozone dose is appropriate toxicological terminology for the oxidant that reaches the sensitive biochemical receptors within the leaf, following penetration of physical resistances identified above and biochemical resistances associated with extracellular detoxification compounds. Characterization of effective ozone dose remains imprecise, requiring simultaneous quantitative consideration of ambient ozone concentration, atmospheric turbulence, stomatal conductance, and antioxidant metabolic capacity within the leaf (Musselman and Massman, 1999; Massman et al., 2000). It will also require more quantitative treatment of diurnal (Lee and Hogsett, 1999) and phenological (Soja et al., 2000; Tingey et al., 2002)

patterns of ozone tolerance than are currently available. It is not possible at the present time to implement this strategy due to insufficient available data.

8.4.3 Ozone Attack.

ozone injury and its relationship with environmental conditions is poorly characterized. Acute ozone stress is associated with high atmospheric concentrations, even for short periods of time, while chronic stress is associated with exposure over long periods of time, even at low concentration (Koziol and Whatley, 1984; Heath, 1988, 1994a). However, the definition of high and low concentrations differ by genotype, exposure history, and micrometeorological conditions. Exposure leads to initial, or primary, injury which may be followed by secondary injury. The initial site of damage may depend upon plant species, exposure dynamics and the resulting cellular location of oxidant attack. Secondary reactions appear to be similar to those involved in generalized plant stress responses (Pell et al., 1997; Kangasjärvi, 1994; Miller and McBride, 1999).

The biochemical and physiological targets of ozone attack were tabulated in the previous document (ARB, 1987), and are summarized in Table 3. Research since 1987 has not invalidated the conclusions regarding these processes as key targets of ozone impacts. However, considerably greater understanding of the mechanism and characteristics of these impacts has been developed over this period.

The internal concentration of ozone within the intracellular gas phase of the leaf had been considered to be near zero (Laisk et al., 1989). Recent evidence (Moldau and Bichele, 2002) suggests that a substantial concentration of ozone may remain, as expected for sustained damaging exposures. Reactivity of cell wall sites leads to gradients within the substomatal space and the surrounding cell wall space. A Henry's Law equilibrium (i.e., the condition in which the partial pressure of ozone in the gas phase remains proportional to the mole fraction of ozone in the liquid phase) occurs at the interface where the gaseous species dissolves into the aqueous phase. Reductive detoxification by antioxidant metabolites and enzyme systems, including ascorbate, glutathione, and superoxide dismutase, may occur in aqueous solution, often following stimulation of their synthesis in response to ozone exposure. While ozone reacts with unsaturated organic molecules (forming carbonyl and peroxide groups) and with exposed sulfhydryl groups of proteins (forming sulfones and disulfide bridges) (Mudd, 1996), the primary biochemical or physiological target of ozone attack, the mechanism of ensuing ozone phytotoxicity, and the key components of plant resistance to ozone, all remain to be characterized (Heath, 1988).

Ozone is decomposed at internal plant sites at the expense of oxidizing a plant constituent. When this is an antioxidant defense molecule, direct damage may be avoided. However, ozone effects on overall plant productivity include the metabolic costs to synthesize and regenerate such defense compounds. This may be considered a hidden cost of resistance to ozone damage. Rapid entry of oxidant can overwhelm the antioxidant response.

Cell wall and plasma membrane-bound proteins may be early targets of ozone attack, particularly those with exposed cysteine, methionine, or tryptophan. Proteins with enzymatic activity including membrane transport systems (e.g., for cations, sugars) may be physiologically significant early receptors. Inactivation of the K^+-activated ATPase (generally believed to be involved in K^+ transport) by *in vivo* exposure to ozone was linked to a sensitive sulfhydryl group. Altered plasma membrane fluidity, permeability (Elkiey and Ormrod, 1979), K^+-exchange via ATPase reactions (Dominy and Heath, 1985) and Ca^{2+} exclusion (Castillo and Heath, 1990a,b), are among the first detectable responses to ozone exposure. The plasma membrane Ca^{2+} efflux pump was inhibited by ozone exposure and the influx permeability increased (Castillo and Heath, 1990). Fumigation of bean plants inhibited the outward directed, ATP-requiring, Ca^{2+} pump and increased the passive permeability of Ca^{2+} (Dominy and Heath, 1985). The loss of K^+ from the cytoplasm and increase in Ca^{2+} concentration, along with a shift in apoplastic pH could trigger a series of diverse cellular responses in mesophyll cells, leading to photosynthetic disruption, or in phloem companion cells, to disruption of phloem loading. This role of Ca^{2+} as a second messenger with broad impacts on metabolism has been considered a potentially key component of ozone-induced plant damage (Castillo and Heath, 1990).

The similarities between these putative Ca^{2+}-mediated responses to ozone and wounding responses suggest that ozone may trigger wound-regulated genes (Mehlhorn et al., 1991). In many cases ozone attack resembles pathogen challenge followed by a hypersensitive response with localized cell death (Sandermann, 1996). Pathogen attack activates Ca^{2+} channels which triggers the active oxygen defense mechanism which causes localized apoptosis (programmed cell death). Ozone-induced passive inward flow of Ca^{2+} could initiate the same defense system (Castillo and Heath, 1990a,b). Nevertheless, it remains to be demonstrated that Ca^{2+} influx is the first significant event in ozone injury.

8.4.4 Visible Injury Caused By Ozone To Crops

Visible lesions on the leaf surface are the most commonly observed symptom of ozone damage in both native vegetation and crop species. These include a variety of apparently abnormal appearances, depending on species. These may include the appearance of bronze colored lesions or speckling (stippling) on the adaxial (upper) surface of exposed leaves (Flagler, 1998). It may also include the appearance that the leaf tissue is water-filled, with a darkened and faintly wet appearance (often called “waterlogging). The lower surface may also acquire a bronze-colored hue, and the upper surface may develop a silver and slightly shiny coloration. In addition to these highly species-specific discolorations (for pictorial examples see Flagler, 1998), the leaf tissue may become chlorotic (development of a yellow coloration with fading of the dominant green pigmentation of chlorophyll as its concentration declines. With continuing chronic exposure, or acute exposure to very high concentrations of ozone, the tissue may become necrotic (as cells and tissues begin to die). Acute exposure to high concentrations of ozone leads to greater ozone-induced foliar injury symptoms

than chronic exposures to more moderate long-term concentrations. However, damage to crop yield, except for leafy vegetables in which visible injury is economic damage, may be greater over season-long chronic exposures. Chronic exposure even to very low concentrations of ozone accelerates foliar senescence, leading to visible pigment loss and earlier leaf abscission.

The diversity of symptom presentation makes it difficult to attribute foliar injury specifically to ozone. The development of chlorotic mottle and necrotic areas of foliage, and frequently accelerated abscission of leaves following acute episodes or prolonged chronic exposure, represent a loss of photosynthetically active leaf area that may decrease whole-plant carbon assimilation. Chlorosis and necrosis, however, are common responses to a variety of stresses and the various pigmentation reactions may resemble nutrient deficiencies and both microbial and arthropod pest pressure. Nevertheless, these visible injury patterns can be semi-diagnostic to a trained observer (Flagler, 1998).

A trained, international panel was able to identify ozone symptoms in plantings of several uniform genotypes of important crops across the length and breadth of Europe (Benton et al., 2000). This important study demonstrated that ozone is injurious to crops at ambient concentrations across a wide sampling of European crop production areas. Symptoms were observed on beans, tomatoes and watermelon, of particular relevance to California, in one or more sites. Test species were grown in locations only as appropriate, but symptoms were also widely observed in garden vegetation of (presumably) locally adapted crop cultivars.

Unfortunately, even authentic visible ozone injury is not well correlated with physiological, horticultural or ecological endpoints (Davison and Barnes, 1998). In some cases, visible injury indicates an advanced state of metabolic disruption and tissue degradation. Plants may then lack the ability to respond appropriately to environmental cues. In other cases visible symptoms may either precede or follow impacts on physiological processes such as photosynthesis or stomatal response. However, as these physiological indicators require specialized instrumentation to detect, they may go unnoticed in the absence of visible injury.

The mechanism of visible damage appears to involve leakage of cellular electrolytes and production of pigments such as anthocyanins associated with wounding. Ozone and other wounding stimuli induce production of ethylene and a family of polyamine compounds. Inhibition of ethylene synthesis with aminoethoxyvinyl glycine (AVG) inhibited ozone-induced visible injury (Mehlhorn et al., 1991), without reducing effects on damage.

8.4.5 Growth and Photosynthesis

Following a half-century of research, the mechanism of ozone action remains unknown. What remains striking in the available literature is the observation that ozone often causes large impacts on plant growth that cannot be related quantitatively to the relatively small effects on physiological processes such as photosynthetic carbon assimilation. A regulatory system operating at the level of the intact plant must be invoked.

Economic yield in grain, fruit and vegetable crops is only indirectly related to plant growth, linked through the concept of Harvest Index (ratio of marketable to total plant biomass). Thus economic damage due to ozone is mechanistically several steps removed from physiological impacts of ozone.

It is clear that ozone exposure reduces plant biomass production. Uptake of ozone by upland cotton (Oshima et al., 1979; Miller, 1988; Olszyk et al., 1993; Temple et al., 1988a; Temple, 1990a,b) and Pima cotton (Grantz and Yang, 1996), and a variety of diverse plants (Pell et al., 1994). Reductions in biomass occur among all plant components, with root system biomass reduced the most severely (Cooley and Manning, 1987), and occasionally to very low levels (e.g., in Pima cotton exposed in greenhouse chambers; Grantz and Yang, 1996).

A literature search revealed 97 published experimental studies in which ozone effects on growth were considered. Relative Growth Rate (RGR; see List of Abreviations for definition) was reduced in 90% of these observations, with 53 of these statistically significant (Table 4). In contrast, only 5% of observations yielded increases in RGR, and all were non-significant. Reductions in RGR were general among a large array of plants (Appendix Table 1), characterizing 89%, 86%, and 100% of observations with herbaceous (i.e., non-woody) dicotyledonous (generally broad-leaved plant species, with two cotyledons or “seed leaves”), monocotyledonous (generally narrow-leaved or grass-like plant species, with a single cotyledon) and perennial tree (long-lived, woody) species, respectively (Table 4). RGR of herbaceous dicotyledonous species was most sensitive to ozone exposure, with significant declines reported in 63% of experiments and 79% of species. Many crop plants grown in California are herbaceous dicotyledonous species. Averaged over all plants studied (Table 5), RGR was reduced by ozone exposure by 4-17% among the various taxa.

These observations of growth reductions are not limited to experiments conducted in greenhouse chambers and OTCs (Appendix Table 2). Clones of trembling aspen were reduced in stature and stem volume (average 20% reduction), by exposure in a chamberless (FACE) system to a twice-ambient treatment in Wisconsin.

Reduced growth may be caused by ozone inhibition of primary carbon acquisition. ozone is principally deposited to leaves. Visible symptoms occur in leaves. ozone molecules that reach the leaf interior may directly affect photosynthesis in mesophyll cells (Spence et al. 1990).

Carbon assimilation is generally reduced following ozone exposure. Carbon assimilation in seedlings of Pinus taeda was reduced by ozone concentrations up to 95 ppb, 7 hm (Adams et al., 1990a). Carbon assimilation of recently mature leaves of Pima cotton was reduced by up to 20% by brief (45 minute) pulses of high (200 – 800 ppb) ozone concentrations (Grantz and Farrar, 1999). Similar 60 minute pulses of 600 ppb ozone in tomato (Lycopersicon esculentum) reduced photosynthesis by 43%, and in bean (Phaseolus vulgaris) by 29%. 90 minute pulses of 400 ppb ozone reduced assimilation in tobacco (Nicotiana tabacum) by 78% (Hill and Littlefield, 1969).

Early research into the mechanism of ozone impacts demonstrated that plasmalemma and chloroplast membranes were vulnerable to oxidant attack (Heath, 1980). Ozone exposure causes a loss of K^+ and Ca^{2+} from cells (Heath, 1994a; Castillo and Heath, 1990a,b) that could be mediated by membrane-bound signal molecules, and that could indirectly impact chloroplast function. Photosynthetic partial reactions have also been suggested as primary targets of ozone attack (Koziol and Whatley, 1984; Reich and Amundson, 1984; Heath, 1988). Direct effects of ozone on photosynthesis occur over quite short time periods (Farage et al., 1991) and can be severe following both chronic (Wiese and Pell, 1997; Rieling and Davison, 1994) and acute exposures (Darrall, 1989; Forberg et al., 1987; Farage et al., 1991; Farage & Long, 1995; Guidi et al., 1997, 2000; Grantz & Farrar, 1999, Zheng et al., 2000). ozone caused a decline in the activity and quantity of Rubisco (Ribulose bisphosphate carboxylase; E.C. 4.1.1.39), the primary carbon fixation enzyme of photosynthesis (Pell et al., 1992, Farage et al., 1991; Farage and Long, 1995) and a decrease in the regeneration of RuBP (Ribulose bisphosphate, the substrate for carbon fixation) (Farage and Long, 1995). Leaf chlorophyll concentration, carboxylation efficiency and photosystem II activity also declined (Farage et al., 1991; Pell et al., 1994, 1997; Pell and Pearson, 1983; Kangasjarvi et al., 1994; Dann and Pell, 1989; Reich, 1983).

The half-time for the decline in mRNA for the small subunit of Rubisco is a few hours (Krapp et al., 1993), reduced by ozone to about an hour or less by inhibition of transcription and by accelerated degradation. Similar observations hold, over slightly longer time frames, for Rubisco protein and activity. Photoxidation of chlorophyll may occur following ozone exposure as electron transfer from H_2O to NADPH is inhibited with build up of oxidative intermediates or inhibition of the dark reactions that consume small carbohydrate intermediates.

Sakaki et al. (1990) found that chloroplast envelope proteins with potentially oxidant-sensitive sulfhydryl groups were not affected by ozone fumigation, in vivo. The absence of ozone damage to these exposed sulfhydryls suggests that neither ozone nor its oxidizing reaction products penetrate sufficiently to inhibit enzymes located within the envelope. That chloroplastic enzymes, including Rubisco (Pell et al., 1997) are eventually inhibited, suggests transfer of information via a signal cascade, rather than penetration of ozone or its unstable reaction products to the chloroplast.

Photosynthetic responses to ozone are observed in OTC and chamberless exposure settings. In two clones of aspen (tolerant and sensitive to ozone) exposed in a FACE exposure facility, photosynthetic assimilation of carbon declined, particularly in older leaves (Noormets, 2001a). ozone also causes stomatal closure (Farage et al., 1991; Pell et al., 1992; Darrall, 1989; Farage et al., 1991; Farage and Long, 1995; Rieling and Davison, 1994; Minnocci et al., 1999; Torsethaugen et al., 1999; Martin et al., 2000) without affecting calculated intercellular CO_2 concentration or stomatal limitation of photosynthesis (Noormets, 2001a). ozone responses are thus consistent with other

environmental impacts on carbon assimilation. Stomatal closure does not generally limit CO_2 assimilation, due to parallel and apparently coordinated declines in mesophyll photosynthetic capacity and stomatal guard cell function.

Over long term exposures changes in photosynthetic capacity are consistent with the observed acceleration of leaf senescence caused by ozone (Pell et al., 1997; Alscher et al., 1997). Senescence may be accelerated by a primary attack on photosynthesis.

ozone-impacts on photosynthesis are often not large enough to explain effects on growth of plants (e.g., wheat; Meyer et al., 1997). On a whole shoot basis, ozone reduction of source strength in individual leaves may be offset by a compensatory increase in rate of leaf production and higher photosynthetic activity per unit leaf area in young leaves (Pell et al., 1994; Farage and Long, 1995; Pell et al., 1992; McCrady and Anderson, 2000). Despite these compensatory responses ozone often reduces area and longevity of photosynthetically active leaves (Beyers et al., 1992).

8.4.6 Carbohydrate Allocation and Translocation

Recently assimilated carbohydrates must be allocated among plant organs and partitioned among multiple biochemical constituents. Only then can they be integrated into structural and non-structural biomass and enter into metabolism. This is required to sustain plant growth and normal patterns of development (Amthor and Cumming 1988), to mount defense mechanisms (Lechowicz 1987), and to repair wounding (McLaughlin and Shriner 1980; Dickson and Isebrands 1993). These allocation patterns are altered by exposure to ozone. Transport of newly acquired photosynthate to developing sink tissues may be particularly severely reduced by acute or chronic exposure to ozone.

Many reports indicate the general observation that ozone exposure reduces R:S (Cooley & Manning, 1987: Darrall, 1989; Dickson et al., 1997; Reinert & Ho, 1995, Grantz and Yang, 1996; Reinert et al., 1996; Landolt et al., 2000; Landolt et al., 1997; Olszyk and Wise, 1997; Chappelka et al., 1988; Chappelka and Chevone, 1988; Anderson et al., 1991; Bennett et al., 1979; Oshima et al., 1979; Darrall, 1989; Barnes et al., 1998; Laurence et al., 1994; Tingey et al., 1971; Miller, 1988; Kostka-Rick et al., 1993; Taylor and Ferris, 1996). Of 20 diverse plant species surveyed by Cooley and Manning (1987), 17 exhibited a decline in R:S.

Morphological changes in root systems may occur in response to ozone (Warwick and Taylor, 1995) that are not clearly associated with altered biomass allocation (Taylor and Davies, 1990). ozone impacts on biomass allocation to reproductive structures (Oshima et al., 1977b; Bennett et al., 1979; Bergweiler and Manning, 1999) and to stolons (shoot runners that produce roots) (Wilbourn et al., 1995; Barnes et al., 1998) are also observed.

It is not currently known how ozone alters carbohydrate allocation. Root:shoot allometric relationships (R:S) are highly conserved in plants (Hunt, 1990; Gunn et al., 1999), with carbohydrate reserves buffering periods of reduced carbon

assimilation. Resources are diverted to leaves for defense/repair of ozone-induced wounding (Amthor and Cumming, 1988; Barnes, 1972; Barnes et al., 1990a; Skärby et al., 1987; McLaughlin and McConathy, 1983; McLaughlin et al., 1982; Grulke 1999; McLaughlin and Shriner, 1980; Evans and Ting 1973; Ting and Mukerji, 1971) and detoxifying reactive oxygen species produced by the dissolution of ozone (Lee and Bennett 1982, Mehlhorn et al. 1986). This occurs following both experimental exposure to ozone (Cooley and Manning, 1987; Miller, 1988; Oshima et al., 1978; Kasana and Mansfield, 1986; Kostka-Rick and Manning, 1992) and across natural exposure gradients (Grulke and Balduman, 1999; Taylor and Davies, 1990). Resistance to further challenge by biotic or abiotic (e.g., ozone) stressors is probably reduced under these conditions (Laurence et al. 1994; McLaughlin et al., 1982; USEPA, 1986, 1996a; Pell et al., 1993). The energetic costs of ozone-induced repair and resistance have not been quantified.

For annual crops the net effect of reductions in C-allocation to roots may be large, particularly during seedling development. In perennials, such as trees and vines, altered allocation over many years may impose large constraints on growth, even though single year effects observed in many experimental studies may be modest (Retzlaff et al., 1997, 2000). This may be economically significant particularly for almond trees and grape vines that are dominant commodities in California.

In loblolly pine, ozone caused retention of carbon and biomass in shoots, reducing allocation to roots (Kelly et al., 1993; Spence et al., 1990). Ozone reduced allocation of carbohydrate to the fine roots in seedlings of Pinus taeda (Adams et al., 1990a). Ozone reduced allocation to leaves and roots in Populus tremuloides and in two C_3 grasses (Agropyron smithii and Koeleria cristata) but not in Quercus rubra or C_4 grasses (Bouteloua curtipendula and Schizachyrium scoparium). The root biomass ratio (root:plant) and R:S were reduced by ozone in upland cotton (Olszyk et al., 1993; Oshima et al., 1979; Temple, 1990a,c). In Pima cotton allocation of biomass to roots decreased by 40% (Grantz and Yang, 1996, 2000), from 13-14% of plant dry weight in charcoal-filtered air to only about 6-8% at 111 ppb ozone 12 hm. Reduced allocation of biomass to root systems was observed across a wide range of N treatments in poplar (Populus tremuloides) seedlings (Pell et al., 1995). Occasional reports indicate no change or increased allocation to roots has been observed following exposure to ozone (Davison and Barnes, 1998; Reiling and Davison, 1992a; Barnes et al., 1998).

The allometric coefficient (k) is derived from the exponential growth equation (after Troughton, 1955) and relates the relative growth rates of competing plant parts such as root and shoot (Farrar and Gunn, 1998; Gunn et al., 1999). A change in k indicates that the fundamental developmental program of the plant has been altered. While it is common for the rate of plant development to be altered by environmental conditions, with a consequent change in R:S at a given plant age, it is less common for similar changes in k to be observed. In the case of plant response to ozone, the concern is that the highly conserved balance of root system development and shoot development, which enables the shoot to be

physically supported and supplied with sufficient water and nutrients to foster rapid photosynthesis and growth, may be disrupted. The resulting plants with inadequate root systems may exhibit reduced productivity due to physiological disruptions of various types.

A recent literature search revealed 108 experimental exposures of plants to ozone from which k could be derived (Appendix Tables 1, 2). The 108 exposures represented 44 different plant species, of which 50% exhibited significant changes in k (44% of 25 dicotyledonous species) of which 91% exhibited a decrease in k. A number of individual species exhibited both positive and negative effects of ozone on k, reflecting the conserved nature of allometric relationships (Hunt, 1990; Gunn et al., 1999). Most observations yielded negative changes in k, and the majority of significant effects were negative (Table 4). However, many observations of ozone effects on k yielded inconclusive results. Overall, only 29% (24% among herbaceous dicotyledonous species; Table 4) reported a significant effect on k, of which 74% were reductions.

The mean % change in k and RGR, over all observations, trended negatively for all species (Table 5), and was significantly below 0 for the herbaceous dictotyledonous plants. ozone impacts on carbohydrate allocation were not always associated with effects on growth. Several herbaceous dicotyledonous species (*Chenopodium album, Epilobium hirsutum, Rumex acetosa*), five herbaceous monocotyledonous species (*Arrhenatherum elatius; Bromus erectus; Brachypodium pinnatum; Lolium perenne; Triticum aestivum*) and two tree species (*Picea abies; Quercus petraea*) exhibited significant ozone-induced changes in k without exhibiting any response of RGR to ozone.

Reduced biomass allocation to roots by ozone implies a disruption of integrated plant function that could represent a principal effect of ozone exposure (McLaughlin et al., 1982). Differences in the sensitivity of grain yield of cultivars of sweet corn (*Zea mays*) to ozone have been attributed to differential responses of root system development and hydraulic properties (Harris and Heath, 1981). Similarly, cotton plants with restricted root development exhibited reduced productivity (Browning et al., 1975). Sustained reductions in carbon allocation to roots in loblolly pine (Kelly et al., 1993; Spence et al., 1990) were projected to adversely affect water and nutrient acquisition, especially on droughted sites (Kelly et al. 1993).

Lee et al. (1990) demonstrated an increase in root hydraulic conductance per unit root dry weight in seedlings of red spruce (*Picea rubens* Sarg.) exposed to ozone. This reflected ozone-induced reduction of root biomass. There are numerous reports of substantial effects of ozone on fine root development in a number of plant species, including beet (*Beta vulgaris*) and Pima cotton (*Gossypium barbadense*; Grantz and Yang, 1996, 2000). Despite a similar increase in biomass-specific hydraulic conductance, whole root system hydraulic conductance of Pima cotton declined by 94% at 0.111 ppm 12 hm. Hydraulic conductance of Pima cotton declined by 41% on a per unit leaf area basis over this range of ozone exposures, a key measure of root:shoot integration. Hydraulic conductance was also reduced by ozone in upland cotton cultivars

(*Gossypium hirsutum*) selected under ozone pressure in the San Joaquin Valley (Grantz et al., 1999).

The reduction of hydraulic conductance on a leaf area basis could lead to severe water deficits and enhance cavitation in the xylem vessels, induce systemic vascular failure due to embolism, and endanger plant survival under drought conditions (Tyree and Sperry, 1988). ozone increased the impact of water deficit in soybeans (*Glycine max* L.; Heggestad et al., 1985) and red spruce (*Picea rubens* L.; Roberts and Cannon, 1992), inducing a greater loss of biomass productivity and a lower (drier) leaf water potential.

The reverse situation has also been observed, sometimes in the same species. Maintenance of leaf water status despite degraded root hydraulic efficiency reflects a coordinated decline in stomatal conductance (Grantz and Yang, 1996; Meinzer and Grantz, 1989, 1990). ozone causes substantial reductions in stomatal conductance in general, and in Pima (Grantz and McCool, 1992; Grantz and Yang, 1996) and upland (Temple, 1986) cottons, specifically. In the study of Grantz and Yang (1996) stomatal conductance of Pima cotton was reduced by 41%, whereas hydraulic conductance decreased by only about 35% over a range of ozone concentrations from 0 to 0.111 ppm. Leaf water potential of ozone-exposed seedlings of Pima cotton was similar to or greater (wetter) than ozone-free controls (Grantz and Yang, 1996), similar to reports for upland cotton in which stomatal conductance was reduced by about 40% and leaf water potential increased (Temple, 1986, 1990a,b; Temple et al., 1988a). Similar observations have been made in alfalfa (*Medicago sativa* L.; Temple et al., 1988b), and red spruce (Lee et al., 1990). In more variable open field environments the conservation of shoot water status observed in these studies may not prevail. Orange trees exposed to high ozone concentrations exhibited substantially lower leaf water potentials than control trees on some days, but similar leaf water potentials on most days (Olszyk et al., 1991). The characteristics of these contrasting days were not explored.

ozone-inhibited allocation of biomass to roots could thus lead to reduced stomatal conductance and inhibited photosynthesis, a hidden cost of ozone exposure in lost productivity. Hydraulic linkage between edaphic (soil environment) conditions and stomatal response, and chemical communication from roots to shoot (e.g., Dodd et al., 1996) both have integrating roles in whole plant function, as indicated by the model of Grantz et al. (1999).

There is some evidence that ozone may exert direct effects on allocation (e.g., Grantz and Yang, 2000). Direct ozone-inhibition of carbon efflux from source leaves could reduce allocation to roots and indirectly inhibit carbon assimilation in the shoot. Loading of sugars into the phloem (i.e., the specialized, tube-like tissue that carries sugars between different plant organs) was reduced by ozone in several plant systems (McCool and Menge, 1983; McLaughlin and McConathy, 1983; Mortensen and Engvild, 1995; Grantz and Farrar, 1999). While carbon assimilation declines, translocation of carbon is inhibited to a greater extent. This was considered a possible mechanism to reduce R:S in the earliest research on ozone impacts (Tjoelker et al., 1995b). Indirect effects following from a putative

primary lesion in carbon translocation could include feed-back inhibition from accumulation of sugars in leaves, and degraded leaf water relations from constricted root development (Meyer et al., 1997; Grantz et al., 1999). End-product inhibition of photosynthetic carbon metabolism due to accumulation of soluble carbohydrates following ozone exposure alters metabolic activities and could reduce assimilation (Einig et al., 1997).

Exposure of foliage to ozone often results in accumulation of carbohydrate in source leaves due to reduced translocation to distant sinks (e.g., Hanson and Stewart, 1970; Spence et al., 1990; McCool and Menge, 1983; McLaughlin and McConathy, 1983; Gorissen and van Veen, 1988). Translocation of ^{13}C in *Phaseolus vulgaris* was rapidly inhibited by ozone (Okano et al., 1984). Uptake and accumulation of ^{11}C in stem tissues was inhibited by ozone in loblolly pine (*Pinus taeda* L.), with the total amount translocated reduced by about 45% (Spence et al., 1990). A delay in efflux was observed in this species for about 5 h, followed by an apparent acceleration in the loss of ^{14}C, attributed to ozone-enhanced respiratory losses (Friend and Tomlinson, 1992). Exposure to ozone reduced carbohydrate export from source leaves of *Plantago major* (Zheng et al., 2000), reduced the amount of ^{14}C transported to roots of clover (Blum et al., 1983), and at higher concentrations nearly abolished export of recent photosynthate from source leaves of cotton (Grantz and Farrar, 1999).

Rates of export from source needles and velocities of movement down the stem were not significantly affected in loblolly pine or wheat, though both trended downward (Mortensen and Engvild, 1995; Spence et al., 1990). The amount of carbohydrate translocated per unit time declined (Mortensen and Engvild, 1995; Fangmeier et al., 1994b), suggesting that phloem loading was inhibited.

In wheat (Meyer et al., 1997), birch (*Betula pendula*; Einig et al., 1997), and *Plantago major* (Zheng et al., 2002) ozone-impacts on carbon assimilation were attributed by the authors to such feedback inhibition. Photosynthetic capacity was reduced by end product inhibition due to girdling (cutting around the stem to disrupt the phloem) rather than ozone exposure in a variety of plants, particularly those such as cotton and cucurbits (e.g., melons, cucumbers and their relatives), in which carbohydrates are stored as starch (e.g., Goldschmidt and Huber, 1992). Feeding of glucose to a sucrose-storing species (spinach, *Spinacia oleracea*) reduced the activity and content of photosynthetic enzymes and pigments (Krapp et al., 1991).

In tomato (Ho 1976) and upland cotton (Hendrix and Peelen 1987), export of newly assimilated carbon in the light was proportional to leaf sucrose content in the absence of ozone exposure. In tomato, ozone increased retention of ^{14}C in source leaves (McCool and Menge, 1983). In wheat (Balaguer et al., 1995), ozone had no effect on carbon efflux in the light, but virtually abolished export in the subsequent dark period, resulting in enhanced retention of label in the source leaf. This is consistent with the ozone disruption of starch remobilization and carbohydrate translocation observed by Hanson and Stewart (1970). Efflux of label in darkness was generally related to starch content (Hendrix and Grange, 1991; Hendrix and Peelen, 1987), but this correlation was disrupted by exposure

to ozone. Export of label in the light was unrelated to starch content, and little starch was degraded in the light (Hendrix and Grange, 1991). In wheat (Balaguer et al. 1995), efflux and soluble sugar content covaried across a range of treatments in the absence of ozone. This relationship, too, was disrupted by exposure to ozone. Soluble sugars accumulated in Pima cotton subjected to chronic (Grantz and Yang 2000) and acute (Grantz and Farrar, 2000) ozone exposure, while translocation declined. ozone increased foliar concentrations of starch and fructans in wheat (Barnes et al., 1995) and other species. In poplar (*Populus* spp.) prior to visible senescence, soluble sugar contents increased with ozone (Fialho and Bucker, 1996), while starch was unaffected. With the onset of ozone-accelerated senescence, however, total foliar starch concentration declined and concentrated along the minor veins in bundle sheath cells, the sites of phloem loading. In seedlings and mature branches of Douglas fir (Gorissen and Van Veen, 1988; Smeulders et al., 1995) retention of recent photosynthate in a non-soluble fraction increased in needles following chronic exposure to ozone.

In other cases, carbohydrate concentrations declined. In Scots pine and Norway spruce (Peace et al., 1995) activities of sucrose phosphate synthase and sucrose-6-phosphatase, both active in sucrose synthesis, declined along with sugar contents following exposure to ozone. In aspen (Coleman et al., 1995), starch content declined following ozone-treatment. In a number of studies (soybean, Tingey et al., 1973, Miller et al., 1995; *Capsicum* spp., Bennett et al., 1979; upland cotton, Miller et al., 1989, Booker, 2000), sugar and often starch contents declined or were unchanged by ozone. In soybean, levels of soluble sugars and starch were reduced by ozone [65 or 95 parts per million hours (ppm-hr) sum of concentration times duration; Miller et al. 1995], while in cotton and bush bean, ozone-caused reductions in soluble protein were associated with increases in free amino acids (0.5 ppm-hr, Craker and Starbuck 1972, Ting and Mukerji 1971). Exposure to ozone reduced foliar starch and soluble sugar levels in red spruce (~550 ppm-hr, Amundson et al. 1991), starch in primary and secondary needles of loblolly pine (45 ppm-hr, Meier et al. 1990), soluble sugars in white pine and loblolly pine (Wilkinson and Barnes 1973), and total nonstructural carbohydrates and proteins in leaves, stems and roots of *Ulnus americana* (4.5 ppm-hr, Constantinidou and Kozlowski 1979). Carbohydrates in 1 year old needles of *Pinus ponderosa* declined with increasing ozone along a natural pollution gradient (Grulke et al., 2001). Monosaccharide and starch concentrations in fine roots also declined. Over shorter experimental exposures to ozone (9 days) (Smeulders et al., 1995) ozone increased retention of recent photosynthate within needles, though at the highest exposure (400 $\mu g/m^3$) needle starch declined. In these cases translocation could be substrate limited, as in brown rust-infected barley leaves (Tetlow and Farrar, 1993).

Reduced export of carbon from source leaves may be as effective as reduced assimilation in reducing source strength and decreasing carbohydrate allocation to sink tissues such as roots.

8.5 Crop Yield Loss Due to Ozone Damage

In 1987 (ARB 1987) a transition was occurring between evaluating ozone impacts on crops as visible injury (e.g., USEPA 1978), and attempts to assign specific and quantitative measures of damage associated with economic yield reduction (e.g., ARB 1987; USEPA 1986, 1996a). This change coincided with implementation of the studies of the National Crop Loss Assessment Network (NCLAN; summarized by Heck, 1988a) and resulting publications assessing quantitative exposure-yield loss relationships. These studies were conducted in open top field exposure chambers in multiple locations in the U.S., and used commercial cultivars and agronomic techniques appropriate to each location. The details of experimental design and statistical analytical protocols have been considered in detail (USEPA, 1986; Heck et al., 1988a). Crop loss was parameterized using mean ozone concentration (e.g., over 7 or 12 hour daylight periods, 7 hm, 12 hm), or in later studies, a Weibull function (a sigmoidal weighting scheme that emphasizes higher, peak, concentrations). The flexibility of Weibull functions facilitated use of common statistical models across multiple studies during retrospective analyses (Lesser et al., 1990).

The trend toward damage assessment rather than injury description has developed further with additional studies and resulting publications. Because it was concluded in the previous document (ARB, 1987) that many of the NCLAN response relationships may not be directly applicable to California, further studies funded by the ARB were conducted under California conditions.

NCLAN studies were limited to five sites, intended to represent, coarsely, the entire U.S. While one of these sites was in the southern San Joaquin Valley of California, four were in the humid Midwest and East (Heck et al., 1988a). The arid, irrigated, agricultural production practices of California were thus not emphasized in these national studies; composite yield loss equations are weighted toward the humid production areas. Those yield loss equations generated under California conditions, i.e., for alfalfa (*Medicago sativa*), cotton (*Gossypium hirsutum*), barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*) and tomato (*Lycopersicon esculentum*), are representative of only one location with its particular climate, soils, and other undefined production characteristics.

Much of the NCLAN research was devoted to crops not grown in California, particularly soybean (*Glycine max*), or to crops that dominate U.S. agriculture but not that of California, such as maize (corn, *Zea mays*) and wheat (*Triticum aestivium*). The highly diverse, and unique, production of California is necessarily poorly represented by any such national survey. The high value fruit and nut crops of the San Joaquin Valley were not represented at all. The more recent studies, including those reviewed by ARB (1987) attempted to represent yield losses for California agriculture, in a coarse survey fashion, much as the NCLAN studies represented U.S. agricultural losses.

It is clear that humidity and temperature influence plant growth and productivity (Mills et al., 2000; Benton et al., 2000). It is not well established how these impacts on growth scale to impacts on sensitivity orf yield to ozone exposure. As

it is not currently possible to predict ozone sensitivity from environmental conditions, and production conditions even within California are highly variable, there seems to be little scientific justification to exclude studies conducted elsewhere from consideration of yield loss in California. This is particularly so if the plant species, if not cultivar, is relevant to California agriculture. The current (and foreseeable) limitation of exposure studies to a small fraction of those agricultural genotypes grown in California can be expanded with these studies performed elsewhere.

The limited number of specific crops for which ozone-yield loss equations are available should not imply that conclusions cannot be drawn regarding ozone impacts on agricultural production. This was addressed in the context of standard development in Germany (Grunhage et al., 2001) through a meta-analysis of studies using a wide variety of crop species. The data assembled for this section (Tables 6-11) are restricted to crop species of relevance to California, but are drawn from exposure experiments conducted at many locations, generally in the U.S. and Europe.

A major limitation to the available exposure-response data base is its limitation to a very small number of plant species and genotypes. There is considerable genetic diversity among even closely related plants (Warwick and Taylor, 1995; Volin et al., 1998), and even between cultivars of the same domesticated crop species (Benton et al., 2000; Hormaza et al., 1996; Alscher and Wellburn, 1994; Pell et al., 1993; Bell and Treshow, 2002). This contributes to the observed large variation in plant exposure-response relationships, including crop yield loss, following controlled exposures to ozone. A multitude of plant response relationships is to be expected under these conditions, and has been observed. Extrapolation of these limited data, covering a few, largely domesticated, species to the plant kingdom in general has proven contentious. Nevertheless, a complete test of all possible genetic backgrounds in all possible environmental conditions is not feasible (Taylor et al., 1994) and generalization from limited data will be required for the indefinite future (Kickert and Krupa, 1991; Kickert et al., 1999). This is currently hindered by poorly characterized genetic determinants of ozone sensitivity (Pell et al., 1993, 1997). Extrapolation of controlled experiments to the larger ambient environment will be facilitated by greater physiological and genetic understanding, and by development of process models that adequately incorporate interactions between genetic characteristics such as antioxidant defenses, gas exchange behavior, environmental conditions, and ozone exposure dynamics. Unfortunately, existing models require further development to be applied in this way.

Recent efforts to model effective ozone fluxes (Grunhage et al., 2001; Massman et al., 2000) have the capability to integrate response relationships from ambient and a variety of experimental exposure technologies. But appropriate empirical parameterizations must still remain limited to a few genotypes due to experimental constraints of time and resources.

The NCLAN crop loss data judged appropriate to California conditions were summarized in the previous document (ARB, 1987). Some exposures led to

small yield increases, but no increases were statistically significant. It was concluded that ozone reduces crop yield (ARB, 1987) based largely on the studies of Thompson and Olszyk (1986).

Trees and vines (perennial crops) and annual crops each account for about 50% of California's primary agricultural crop production (2001 values). Among the top 50 agricultural commodities in California there are nearly twice the number of annual as perennial crop species. There are hundreds of crops grown commercially in California. From available yield loss vs. ozone relationships determined over prolonged periods under near commercial conditions, it appears that both perennial crops (trees and vines) and annual crops contain both sensitive and tolerant species among those that dominate California agriculture (cf. Tables 6,7,8,9).

In all classes of crops, only a small fraction of those grown in California have been investigated. Of these, yield loss equations vary widely (see Table 6, below; ARB, 1987; USEPA, 1986, 1996a). The interactions considered above suggest that it may never be possible to identify a single threshold ozone concentration that elicits yield reduction, even generalized between closely related species in a single location, or the same genotype grown in different locations.

A concern with all exposure technologies is specification of a control concentration of ozone against which relative loss can be calculated. In many cases charcoal filtered air mixes with incursion of ambient air in open top chambers (OTCs) to yield a concentration suitably near that of pristine sites (about 25 ppb). However, this concentration is not uniform spatially nor temporally, with estimates up to 40 ppb or higher under some conditions (Lefohn et al., 1990). In studies to be analyzed by regression analysis, a very low control concentration may anchor the relationship, and relative yield loss can be calculated between any two concentration points along the regression line.

The material above provides a general framework and rationale for ozone damage to crop plant species at ambient concentrations of ozone. The following considers the specific evidence for yield loss in specific crops grown in California. The studies considered here compute ozone damage to economic yield relative to various control concentrations of ozone. Given the uncertainties inherent in the crop loss functions themselves, this variability is not considered dominant, and is not explicitly considered unless uniform for a set of data (e.g., Tables 6,7).

8.5.1 Annual Crops

Many annual crops are grown for their reproductive parts (fruits, seeds). For example, time to reproductive development was decreased and pollen germination was inhibited in *Brassica campestris* (Stewart et al., 1996). It remains unclear whether ozone directly impacts reproductive development, or whether impacts on yield are secondary effects of primary lesions in the carbohydrate economy. These issues have been reviewed (Black et al., 2000; Stewart et al., 1996).

It has been considered that such crops may be particularly sensitive to ozone exposure during the period in which carbohydrate is actively allocated to fruit expansion or grain filling, whereas biomass crops such as leafy vegetables may be more evenly sensitive over the entire growing period (Lee et al., 1988; Younglove et al., 1994; Soja et al., 2000). However, such simplifying generalizations may be misleading, as biomass crops are vulnerable both in development of a robust root system, which is sensitive to ozone-inhibition throughout the growing period, and to biomass production, particularly during the brief periods between cuttings for multiply harvested crops, such as forages and alfalfa. Similarly, those species grown for reproductive structures may exhibit reduced yield capacity due to season-long exposure, if this results in impaired growth of vegetative portions of the shoot or root that must support (structurally and nutritionally) the yield components.

Using available ozone exposure/crop loss equations, with local ambient ozone concentration data and a GIS-based approach, crop loss due to ozone over a period of several years was estimated for California (Tables 6,7). The important yearly assessments of potential yield losses have been conducted in California by Mutters and colleagues (Mutters et al., 1993; Mutters and Soret, 1995, 1998). These studies have inserted a new ozone concentration data set into the same yield loss equations determined in California and elsewhere. This accounts for the similar estimates of yield loss predicted over the several years of the study (Tables 6,7) as ozone concentrations change only moderately from year to year.

These studies have advanced the science of crop yield reduction estimation by incorporating a geographic information system approach to merging land use (crop cover) with interpolated values of ambient ozone concentration. This finer grid analysis allows the specific crop and the actual ozone exposure to be mated for an accurate calculation of estimated yield loss. The subsequent summation of these calculated values over county or statewide areas improve the regional loss estimation. This improvement led in both annual and perennial crops, to a reduction in estimated yield suppression due to tropospheric ozone (Tables 6,7,8,9).

However, it remains unlikely, as noted by Adams et al. (1988) that either spatial or temporal variation in ambient ozone concentration is the largest contributor to uncertainty in crop yield sensitivity to ozone in the environment. A larger contributor is the actual yield loss equations, themselves, an area in which there has been an unfortunate lack of progress, in the USA and particularly in California, since the last such review of the subject (ARB, 1987). Progress in Europe over this time period has been considerably greater.

Among annual crops these data range from about no loss in grain sorghum to about a 30% loss in cantaloupe. Among perennial crops, alfalfa exhibited under 10% loss, while grapes exhibited about a 25% loss, statewide. The trends for most crops are for increasing losses. These equations, largely generated in OTC experiments, remain adequate at the present time. Thresholds for specific values of loss can be easily derived from the equations assembled by Mutters et al.

(1993) and Mutters and Soret (1995, 1998), from which the values in these tables were taken.

A number of crop loss studies have appeared since the last document (ARB, 1987). Some were considered in Tables 6 and 7. Many were performed in Europe. These studies are listed in comprehensive fashion in Table 8 for annual crops and Table 9 for perennial crops.

In addition to economic damage (yield loss) due to ozone, a variety of quality, aesthetic, or physiological endpoints are also degraded by ozone. These are considered in less comprehensive fashion in Table 10 for annual crops and Table 11 for perennial crops.

Only a few comments follow on several important California crops.

8.5.1.1 Cotton

Yield of upland cotton over two years was reduced in North Carolina by 21 and 22% when exposed season-long to 12 hm ozone concentrations of 71 and 51 ppb (Heagle et al., 1999). In the second year a larger reduction of 49% was induced by 78 ppb (Table 8).

ozone reduces yields of adapted upland cotton (*Gossypium hirsutum* L.) cultivars by up to 20% in the San Joaquin Valley of California (Grantz & McCool 1992; Olszyk et al. 1993; Oshima et al. 1979; Temple et al. 1988a), despite many cycles of yield selection with ozone-pressure. Yield and productivity of Pima (*G. barbadense* L.) cotton cultivars, selected in low-ozone environments and introduced into California, are even more sensitive (Grantz & McCool 1992; Olszyk et al. 1993). More recently developed cultivars of Pima cotton, selected in the San Joaquin Valley, appear to exhibit reduced ozone-sensitivity.

8.5.1.2 Rice

Nouchi et al., (1991) found no impact on yield at 50 ppb, though this was performed in hydroponic culture in growth chambers. Kats et al. (1985) found 12-21% at 200 ppb (5 h/day constant, a particularly high concentration relevant to California production in the San Joaquin Valley in the 1980s, but not relevant to the major production areas in the Sacramento Valley nor to current conditions in the San Joaquin Valley. In contrast, in Japan exposure to 7 hm concentrations of 40 ppb reduced yield by 3-10% (Kobayashi et al., 1994,1995a; Table 8). It is likely but unproven that the California cultivars may exhibit some level of enhanced ozone tolerance due to yield selection under ozone pressure.

8.5.1.3 Wheat

A number of cultivars of wheat have been investigated. All have some relevance to the California situation, though none is directly applicable to a specific production scenario in California. No effort is made here to distinguish between even widely divergent germplasm. Field exposures in OTCs of potted wheat plants (Fangmeier et al., 1994b) found that yield was suppressed by 35% in 7 hm = 71 ppb. Studies in Maryland found 20% reductions with 7 hm = 61-65 ppb (Mulchi et al., 1995; Rudorff et al., 1996a; Table 8). Similar exposures in North

Carolina to 12 hm exposures of 74 and 90 ppb over two years reduced yield but not significantly (Heagle et al., 2000). Combined data from multiple years of investigation in OTCs in Sweden (Danielsson et al., 2003) found a 23% loss in yield of wheat at AOT40 = 15 ppm-hr. These data were also examined for superior predictive performance of an ozone metric involving ozone flux (Emberson et al., 2000). In this case the flux approach improved the exposure (dose)-response relationship, but only slightly (r^2 = 0.34 to 0.39). A similar analysis of multiple experiments conducted under the ESPACE-WHEAT program (e.g., Bender et al., 1999) in Europe found a non-significant 13% reduction in yield at 12 hm = 51 ± 18 ppb and AOT40 = 28.2 ± 23.0 ppm-hr. Bender et al. (1999) did find a significant relationship with 12 hm. Exposure in OTCs in Finland (Ojanpera et al., 1998) reduced yield by 13% at 12 hm = 45 and 61 ppb over two seasons. A study from multiple sites in Europe (Fuhrer et al., 1997 found that an AOT40 = 2.8 ppm-hr caused a 5% yield loss, and AOT40 = 5.7 ppm-hr caused a 10% loss.

A free-air exposure of wheat (Ollerenshaw and Lyons, 1999) to AOT40 = 3.47 ppm-hr in the autumn and 6.18 in spring-summer (winter wheat) reduced yield by 13%.

Grain quality is also affected by exposure to ozone. The major effect seems to be a general inverse relationship between yield (which is suppressed by ozone) and grain protein content (e.g., Gelang et al., 2000).

8.5.2 Perennial Crops

As noted above for annual crops, many perennial crops are grown for their reproductive parts (mostly fruits). Thus the particular sensitivity of reproductive organs to ozone damage is also of considerable potential interest in these crops (e.g., Black et al., 2000). The same caveats apply, enhanced sensitivity to ozone exposure during fruit development may mask the season-long sensitivity as robust vegetative shoot scaffolding and root supporting structures are required for subsequent high yield potential (Lee et al., 1988; Younglove et al., 1994; Soja et al., 2000). Crops grown for biomass such as alfalfa, may be more sensitive to the entire growing season exposure, but the yield of a particular cutting may also respond to exposure during the brief period between cuttings in which currently displayed foliage is exposed to ambient ozone.

As observed in both perennial and annual crop species (Tables 8-11) the variability in ozone sensitivity varies within closely related species and even cultivars as much as it does across distantly related crop species.

8.5.2.1 Alfalfa

Averaged over two years of open top chamber exposures in the San Joaquin Valley, alfalfa yield was suppressed by about 10 % at ambient concentrations of ozone (Temple 1988b). The two years data were consistent despite modest inter-annual differences in ozone concentrations. Water deficit reduced yield in both years. In one year (1985, but not 1984) the sensitivity to ozone was reduced, though at a vastly reduced yield level in the absence of oxidant.

A relatively sensitive northern cultivar of alfalfa was reduced by 31% in a year with many peak exposures above 60 ppb and by 21% in a year with many fewer peak episodes. A more tolerant cultivar was reduced by 14% in the year with many peaks but not significantly in the year with fewer peak exposures (Renaud et al., 1997).

8.5.2.2 Stone Fruit

Stem growth (i.e., production of woody shoot material that does not contribute directly to reproduction or economic yield) of plums was increased by 14% in 12 hm = 91 ppb in Casselman plums grown in the San Joaquin Valley in large rectangular OTCs (Retzlaff et al., 1997). This growth may have come at the expense of the root systems during the 4 year exposure experiment (root biomass was not measured). Fruit yield was reduced by 42%. The ambient treatment of 12 hm = 48 ppb reduced fresh fruit yield by 16%.

Pollen germination was inhibited in a variety of deciduous fruit trees of horticultural importance or their ornamental relatives, including apple (*Malus domoestica*), apricot (*Prunus armeniaca*), almond (*Prunus dulcis=amygdalus*), peach (*Prunus persica*), and pear (*Pyrus communis*) (Hormaza et al., 1996). Ozone-induced changes in pollen tube extension were correlated with responses of photosynthetic carbon assimilation. It is not yet clear whether this indicates an indirect effect of reduced carbohydrate supply on the development of reproductive structures such as pollen tubes, or whether it is evidence of direct oxidant effects of ozone on these delicate structures. In either case, disruption of reproductive processes is likely to have deleterious consequences on economic yield.

8.5.2.3 Strawberry

Strawberry is a perennial plant grown as an annual in California. It has been found repeatedly to be relatively tolerant of ozone, particularly for total fruit yield, both in California and in Britain (Takemoto et al., 1988b; Drogoudi and Ashmore, 2000). Individual berry size, however, has variously been found to increase by 20% (California) and to decrease by 14% (Britain).

8.5.3 Summary and Economic Assessment

The national assessment of ozone-induced crop loss undertaken in USEPA (1996a) concluded that over half (58%) of crop species or cultivars would be expected to sustain a 10% yield reduction at ozone concentrations at or above 50 ppb (7 hm), with 34% between 40 and 50 ppb, and a much smaller fraction (11%) below 35 ppb. Various retrospective analyses of NCLAN data (Lesser et al., 1990; Lee et al., 1994) concluded that about 10% of major crops would exhibit yield suppression by ozone at 12 hm concentrations of 45 ppb, 7 hm of 49 ppb, or SUM06 values of 26.4 ppm-hr. The estimate that 40-50 ppb ambient ozone concentrations are damaging to production of about half of crops investigated has remained relatively conserved from USEPA (1978) through USEPA (1986) and USEPA (1996a). The most recently available data does little to challenge this conclusion. A consensus developed among ozone plant effects

specialists was that ozone concentrations greater than or equal to 0.06 ppm, summed over any 90-day period, during daylight hours (0800-2000 LST) should not exceed 15-20 ppm-hr (SUM06; Heck et al., 1998; Heck and Furiness, 2001) to avoid damage to a wide range of agricultural crops. A similar consensus in Europe led to development of AOT40 (Fuhrer et al. 1997), a similar summation of the amount by which ozone concentrations exceed 40 ppb, also for daylight hours over a 90-day period. Values in excess of 3 ppm-hr were considered damaging to sensitive agricultural crops (Karenlampi and Skarby, 1996).

Extremely sensitive cultivars (11% in the national assessment) would not be expected to be retained in California production areas characterized by high concentrations of ozone. In such areas an informal, ad hoc selection of ozone resistant cultivars has taken place, with those showing ozone symptoms or yielding poorly in polluted environments simply removed from the planting lists of growers and from the advanced breeding lines of cultivar development programs. In contrast, the 58% of crops exhibiting yield reductions below 7 hm concentrations of 50 ppb are unlikely to be removed consistently from California production, but are highly likely to exhibit yield reductions directly attributed to ambient ozone concentrations.

Of particular interest to California agricultural production systems, 18% of those cultivars tested nationwide (USEPA, 1996) were sufficiently resistant to ozone impacts that no yield reduction was likely below 80 ppb (7 hm). Grain crops have typically exhibited less sensitivity to ozone than many other crops, particularly those for which foliage is the marketable commodity.

It was considered by USEPA (1986) and reaffirmed by USEPA (1996a) that tropospheric ozone, at then current ambient concentrations, imposed a substantial economic cost to agricultural production on a national scale. The studies of Kopp et al., (1985) and Adams et al., (1986) and the welfare model studies reviewed by Adams et al. (1988) are considered to have incorporated as many relevant interactions within the economy as feasible. In the previous ARB (1987) document, benefits of reducing ozone concentrations in California to background levels (assumed to be 0.025 ppm) were found to be about 5-6% for both producers and consumers, relative to the economic benefit of the agricultural sector in total. However, this estimate only utilized yield losses on 15 of California's major crops, a small fraction of the total.

The recent review by Spash (1997) confirms the conclusion that current ambient ozone concentrations impose a substantial cost to both producers and consumers of agricultural products. The recent national study of Murphy et al. (1999) specifically of ozone derived from motor vehicle emissions in the U.S. also confirms these conclusions. This study suggested a total economic impact of ambient ozone (in 1990 dollars, relative to background of 0.025 ppm) of $2.8 – 5.8 billion USD. This study applied the same welfare modeling approach as Adams et al. (1986) but also underestimated the true impact, as only 8 major crops nationwide were considered. Various economic assessments have been considered by Mauzerall and Wang (2001).

The recent valuation of ozone reduction benefits in California's heavily agricultural San Joaquin Valley (Kim et al., 1998) found small effects of ozone on health endpoints, due to low population density, but large impacts on agricultural productivity. Costs of ozone control were also considered. A cost benefit analysis, based on the range of crop loss predictions from the previous document (ARB 1987) revealed substantial benefit to the agriculturally dominant central Valley counties of Tulare, Kings, Fresno and Madera. However, in other areas of the northern or southern Valley, which have lower production of crop value and larger production of ozone precursors, there were net costs of meeting any 24 h peak standard below about 150 ppb. This ozone concentration is well above those known to damage crop yield.

Table 8-1. Exposure technologies in increasing order of applicability to field systems and decreasing order of experimental control and reproducibility.

Exposure Technology	Types	Principal Characteristics
Controlled Systems	Controlled Environment Chambers;	High level of environmental and exposure control; Reproducible; Highly suitable for mechanistic studies; Difficult to extrapolate directly to field environment
Managed Systems	Greenhouse Chambers;	Intermediate level of environmental and exposure control; Intermediate reproducibility; Intermediate applicability to field environment
Seminatural Systems--Field Chamber Systems	Closed Top Chambers; Open Top Chambers	
Seminatural Systems--Field Chamberless Systems	Mechanical Field Exclusion; Chemical Exlusion; Open Field Exclusion	
Natural Systems	Gradients; Field surveys	Poor level of environmental and exposure control; Not reproducible; Directly applicable to field environment

Table 8-2 Open-top chamber effects on microclimate (after Heagle et al.,1988)

Variable	Effects in chamber plant-growth area	References
Air temperature	Increases of up to 3.7°C, but usually less than 2.0°C; greatest increase on calm, sunny, and hot days at midday; mean seasonal increase probably less than 1.0°C	Heagle et al., 1973 Heagle et al., 1979*b* Olszyk et al., 1980 Weinstock et al., 1982
Leaf temperature	Slight increase by amounts caused by chamber effects on air temperature.	Weinstock et al., 1982
Light (PAR)	Decreased by as much as 20%; greatest decrease with low sun angle on sunny days; mean seasonal decrease approximately 12%; in northern hemisphere, north chamber positions receive more light than southern positions: Normal vertical gradient (decreased light with increased height) in plant canopy at chamber periphery does not exist if border row plants are not adequate.	Heagle et al., 1979*b* Olszyk et al., 1980
Windspeed	Seasonal mean velocity decreased; but velocity never less than 2-3 km h^{-1}, in chambers; for plants taller than 120 cm, more air movement near base of plant canopy than near top during calm periods.	Heagle et al., 1979*b* Weinstock et al., 1982
Relative humidity	Up to 10% increase or decrease depending on ambient conditions, soil moisture, and type of plant canopy; usually less than 5% difference.	Heagle et al., 1973 Weinstock et al., 1982
Dew point	Up to 2.0°C higher when	Weinstock et al., 1982

	maximum differences in air temperatures occur; less than 0.5°C when cloudy or at night.	
Rainfall	Less direct rainfall at some chamber positions when rain is accompanied by wind; rain intercepted by panels is concentrated at base of chamber walls.	Weinstock et al., 1982

Table 8-3 Physiological and biochemical processes that are believed to be affected directly or indirectly by ozone exposure in crop plants. As the primary target of ozone impact has not yet been determined, these processes are those for which some consensus has emerged based on available data. Other processes, at other levels of biological organization, will likely be found to be affected in the future.

Process	**Impact**
Membrane Permeability	Increased with loss of selectivity
Chloroplast Ultrastructure	Degraded
Photosynthesis	Inhibited
Respiration	Enhanced or Inhibited
Protein Metabolism	Enhanced or Inhibited
Carbohydrate Metabolism	Enhanced or Inhibited
Carbohydrate Translocation	Inhibited
Lipid Metabolism	Degradation and Inhibited Synthesis
Ethylene Production	Increased

Table 8-4 The effect of ozone exposure on growth and allocation among plant groups. Shown are the number of total observations of ozone impacts surveyed in the literature, the number and percent of the total that led to significant results (whether positive or negative), the percentage of significant exposures that yielded negative changes in RGR[1] or k[2], the percentage of total exposures that yielded negative changes in RGR[1] or k[2], and the percentage of total exposures that yielded positive changes in RGR[1] or k[2].

Biological Endpoint	Plant Group	Total Ozone Exposures In Surveyed Literature	Significant Effects of Ozone on RGR or k (% Total Ozone Exposures)	% Significant Effects Yielding a Decrease in RGR or k	% Total Ozone Exposures Yielding a Decrease in RGR or k	% Total Ozone Exposures Yielding an Increase in RGR or k
RGR	Dicotyledonous	75	47 (63)	100	89	5
	Monocotyledonous	14	4 (28)	100	86	8
	Tree	8	0 (0)	na[3]	100	0
	All	97	51 (53)	100	90	5
k	Dicotyledonous	84	20 (24)	80	51	33

	Monocotyledonous	16	9 (56)	56	62	38
	Tree	8	2 (25)	100	75	12
	All	108	31 (29)	74	55	32

[1]RGR, Relative Growth Rate, defined as the difference in the dry weight of a plant or plant part over a time period, divided by the initial dry weight and the length of time.

[2]k, the allometric growth coefficient that describes the distribution of dry weight gain between competing plant parts, defined as the ratio of Relative Growth Rates of different plant parts.

[3]na=not available due to lack of significant effects.

Table 8-5 The effect of elevated ozone on mean whole plant relative growth rate (RGR) and root/shoot allometric coefficient (k). Values are the mean % change ± the standard error calculated from aggregated data without regard for the statistical significance of the individual original observation[a].

	RGR	k
Herbaceous dicotyledons	-7.8 % ± 0.7* n = 75	-2.4 % ± 1.0* n = 75
Herbaceous monocotyledons	-4.2% ± 0.8*** n = 14	-6.4 % ± 5.4 n = 14
Trees	-16.8 % ± 5.0* n = 8	-14.6% ± 16.3 n = 8

[a]From the publications summarized in Appendix Table 1.

*, **, *** = P < .05, .01, .001, respectively.

Table 8-6 An historical view of California statewide loss (%) estimation for selected annual crops, relative to 12 hm of 25 ppb or 7 h mean of 27.2 ppb.

Crop	Experimental Ozone Exposures Considered in ARB 1987[1]		Estimated Losses Sustained under Ambient Conditions					
	Range of Experimental Yield Losses[1]	Maximum Exposure[1]	1984 Yield Loss[2]	1989 Yield Loss[3]	1990 Yield Loss[4]	1991 Yield Loss[5]	1992 Yield Loss[6]	1993 Yield Loss[7]
Bean-Dry	46-100	0.35 ppm x 63 days	27.2	10.3	17.3	9.9	8.8	17.5
Cantaloupe	na[8]	na	na	29.8	30.2	31.5	32.3	32.8
Cotton	5-29	ambient x growing season	19.6	16.9	20.3	19.2	17.4	23.3
Lettuce	na	na	na	0.3	0.4	0.3	0.4	0.5
Maize-Field	<0-40	0.15 ppm x 88 days	1.7	na	na	na	na	1.2
Maize-Silage	na	na	3.5	2.6	1.8	1.8	7.5	na
Maize-Sweet	0-45	0.35 ppm x 71 days	6.1	4.5	4.8	5.7	5.8	na
Onion	na	na	23.2	17.4	10.4	11.3	8.9	10.6
Pepper	19-50	0.20 ppm x 77 days	na	na	na	na	na	na
Potato	25-42	0.20 ppm x 140 days	na	16.4	13.7	na	na	na
Rice	na	na	10.4	4.2	3.3	3.5	4.9	3.9
Sorghum-grain	na	na	na	0.7	0.6	0.6	0.5	na
Tomato	1-45	0.35 ppm x 99 days	na	0.9	0.5	0.5	0.6	0.6

Tomato-Processing	na	0.35 ppm x 99 days	4.5	3.9	6.5	3.0	3.4	6.8
Wheat	1-43	0.13 ppm x 53 days	1.7	5.3	6.9	5.3	5.1	6.7

[1]Table III-3 from ARB (1987) originally from USEPA 1986, describing the full range of experimental observations.

[2]Table III-26 from ARB (1987), based on measured average California ozone concentrations.

[3]Table 5; Mutters et al. (1993); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[4]Table 6; Mutters et al. (1993); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[5]Table 7; Mutters and Soret (1995); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[6]Table 8; Mutters and Soret (1995); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[7]Table 3, Mutters and Soret (1998); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[8]na, not available.

Table 8-7 An historical view of California statewide loss estimation for selected perennial crops.

Crop	Experimental Ozone Exposures Considered in ARB 1987[1]		Estimated Losses Sustained under Ambient Conditions relative to 12 h Mean of 25 ppb or 7 h Mean of 27.2 ppb					
	Range of Experimental Yield Losses[1]	Maximum Exposure[1]	1984 Yield Loss[2]	1989 Yield Loss[3]	1990 Yield Loss[4]	1991 Yield Loss[5]	1992 Yield Loss[6]	1993 Yield Loss[7]
Alfalfa	10-51	0.20 ppm x 70 days	7.6	4.8	7.3	8.5	8.5	9.5
Grape	12-61	0.25 x season	20.8	17.0	23.0	23.2	23.4	25.0
Grape-raisin	na[8]	na	na	20.3	26.1	27.2	26.6	26.2
Grape-table	na	na	na	22.4	27.4	27.2	24.4	29.9
Grape-wine	na	na	na	12.9	19.0	19.0	20.2	22.8
Lemon	32-52	0.1 x season	28.3	8.9	8.9	8.2	9.2	8.4
Orange	na	na	19.3	32.5	15.7	12.6	13.9	14.0

[1]Table III-3 from ARB TSD 1987 originally from USEPA CD (1986), describing the full range of experimental observations.

[2]Table III-26 from ARB TSD 1987, based on measured average California ozone concentrations.

[3]Table 5; Mutters et al. (1993); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[4]Table 6; Mutters et al. (1993); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[5]Table 7; Mutters and Soret (1995); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[6]Table 8; Mutters and Soret (1995); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[7]Table 3, Mutters and Soret (1998); 7 hm and 12 hm exposure models only, based on measured average California ozone concentrations.

[8]na, not available.

Table 8-8 Summary of studies published since publication of ARB (1987) demonstrating, under field conditions, ozone damage to economic yield of annual crops of relevance to California[1].

Crop Species	Exposure Technology	ozone Exposure	Summary of Ozone Impact (% loss)	Original Reference
Barley	OTC	7 hm=29 7 hm=45	No effect	Pleijel et al., 1992
Bean, fresh	OTC	7 hm = 63 ppb	>10	Eason and Reinert, 1991
Bean, fresh	OTC	12 hm = 45 ppb	15.5	Schenone et al., 1992
Bean, fresh	OTC	7 hm = 55-60 ppb	26 (sensitive cv.)	Heck et al., 1988b
Bean, fresh	OTC	8 hm = 80 ppb	20	Bender et al., 1990
Bean, fresh	OTC	9 hm = 44 ppb	29	Tonneijck and van Dijk, 1998
Beans, fresh[2]	OTC	AOT40=1600 AOT40=1700 AOT40=8212 ppb-hr=7 hm=40.4 ppb	5 10 21.4	Fumagalli et al. (2001a)
Bean, fresh[3]	OTC	7 hm = 26 ppb 7 hm = 50 ppb	+48[4] 42	Sanders et al., 1992a
Bean, fresh	OTC	7 hm = 45-50 ppb	18-31	Schenone et al., 1994
Bean, dry	OTC	12 hm = 72 ppb	55-75	Temple, 1991

Bean, dry[5]	OTC	7 hm = 38-39 ppb	10-11	Sanders et al., 1992b
Bean, dry	OTC	7 hm = 58 ppb	51	Sanders et al., 1992b
Beet	Closed Field Chambers	12 hm=25.0 ppb or SUM01=3860 ppb-hr 12 hm=62.6 ppb or SUM01=9650 ppb-hr	10 25	McCool et al., 1987
Celery	OTC	12 hm=66 ppb	12	Takemoto et al., 1988b
Cotton	OTC	12 hm = 44 ppb	19	Heagle et al., 1988b
Cotton	OTC	12 hm = 74 ppb	26.2	Temple et al., 1988a
Cotton	OTC	12 hm = 90 ppb	40-71	Temple, 1990c
Cotton	OTC	12 hm = 71 ppb	22	Heagle et al., 1999
Cotton	OTC	12 hm = 51 ppb 12 hm = 78 ppb	21 49	Heagle et al., 1999
Cotton	OTC multisite	7 hm = 23-53 ppb NF 7 hm = 31-56 ppb AA	0-20	Olszyk et al., 1993
Lettuce	OTC	12 hm=36 12 hm=63	No effect	Temple et al., 1990

Lettuce	Closed Field Chambers	SUM01=1930 ppb-hr SUM01=4820 ppb-hr	10 25	McCool et al., 1987
Maize, field	OTC	7 hm = 70 ppb	13	Mulchi et al., 1995 Rudorff et al., 1996a
Muskmelon	OTC	7 hm=30-40 over 2 years	21.3	Snyder et al., 1988
Onion	OTC	12 hm=36 12 hm=63	5	Temple et al., 1990
Onion	Closed Field Chambers	12 hm=12.6 ppb or SUM01=1680 ppb-hr 12 hm=31.5 ppb or SUM01=4190 ppb-hr	10 25	McCool et al., 1987
Pepper, green		12 hm=66 ppb	12	Takemoto et al., 1988b
Potato	OTC	8 hm 60 ppb	5.2	Craigon et al., 2002
Potato	OTC	8 hm 50 ppb	No effect	Lawson et al., 2001
Rice	OTC	5 hm = 200 ppb	12-21	Kats et al., 1985
Rice	OTC	7hm = 40 ppb	3-10	Kobayashi et al., 1995a
Tomato	OTC	12 hm=109 ppb	17-54	Temple 1990c
Turnip	Closed Field Chambers	12 hm=15.1 12 hm=37.9	10 25	McCool et al., 1987

Turnip	OTC	7 hm=40 ppb 7 hm=60 ppb	7 24	Heagle et al., 1985
Watermelon	OTC	7 hm= 27 ppb	20.8	Snyder et al., 1991
Watermelon[6]	OTC	S06= 295 ppb-hr S06= 4950 ppb-hr	19 39	Gimeno et al., 1999
Wheat	OTC	7 hm=42ppb 7 hm=54 ppb	33 22	Kohut et al., 1987
Wheat	OTC	24 hm = 40 ppb	13	Fuhrer et al., 1989
Wheat	OTC	AOT40=80-93.5 ppm-hr	48-54	Grandjean and Fuhrer, 1989
Wheat	OTC	7 hm=15-22 ppb	7	Pleijel and Skarby, 1991
Wheat	OTC	8 hm=17-23 ppb	10	Adaros et al., 1991
Wheat	OTC	8 hm=38 ppb	5	De Temmerman et al., 1992
Wheat	OTC	7 hm=37-45 ppb	9.5-11.6	Fuhrer et al., 1992
Wheat	OTC	AOT40=15 ppm-hr	23	Danielsson et al., 2003
Wheat	OTC	12 ht=32.6 ppm-hr 12 ht=33.4 ppm-hr 12 ht=34.0 ppm-hr	53 +17 17	Finnan et al. 1996a
Wheat	OTC	7hm=61 7hm=65	20 20	Mulchi et al., 1995 Rudorff et al., 1996c
Wheat	OTC	7h=73	35	Fangmeier et al., 1994b

Wheat	OTC	12h=61/45 ppb AOT40=54.6/40.6 ppm-hr	13/13	Ojanpera et al., 1998
Wheat	Chamberless	AOT40=6.18 ppm-hr Daily mean=80	13	Ollerenshaw and Lyons 1999

[1]Adapted from USEPA (1996a) and later publications.

[2]Mean of 4 cultivars x 2 locations x 3 years; interpolation from Mauzerall and Wang, 2001.

[3]Data indicate an apparent biphasic response, with mostly non-significant yield increases at moderate ozone concentrations, and yield suppression at higher ozone concentrations. Data shown are those concentrations that yield statistically significant differences, relative to CF air (7 hm = 10 ppb).

[4]+ indicates an increase due to ozone exposure.

[5]Similar yield responses were obtained at similar ozone exposures despite different experimental locations and ozone exposure dynamics.

[6]Mean of two years data.

Table 8-9 Summary of studies published since publication of ARB (1987) demonstrating under field conditions ozone damage to economic yield of perennial crops of relevance to California[1].

Crop Species	Exposure Technology	ozone Exposure Averaging Period/Concentration	Summary of Ozone Impact (% loss)	Original Reference
Alfalfa	OTC	12 hm=63 ppb 12 hm=78 ppb	15 19	Temple et al., 1988b
Alfalfa	OTC	12 hm=40 ppb 12 hm=66 ppb	2.4 18.3	Temple et al., 1987
Alfalfa	OTC	12 hm=53 ppb	22	Takemoto et al., 1988b
Alfalfa	OTC	12 hm=36 ppb	22	Takemoto et al., 1988b
Alfalfa	OTC	12 hm=39, 49, 110 ppb 12 hm=34, 42, 94 ppb	31 sens./14 tol.[4] 21 sens./2 ns tol.	Renaud et al., 1997
Alfalfa[2]	OTC	Filtered vs. Ambient (Southern California)	16.1	Olszyk et al., 1986b
Alfalfa[2]	Field Exclusion	Filtered vs. Ambient (Southern California)	14.7	Olszyk et al., 1986b
Alfalfa	Natural Gradient	SUM100=57,000 ppb-hr	31.4	Oshima et al., 1976

Orange, Valencia[3]	OTC	12 hm=75 ppb 12 hm=40 ppb	31 11	Olszyk et al., 1990b
Peach	OTC	AOT60=5398	No effect-yield Quality degraded	Badiani et al., 1996
plum	OTC	12 hm= 117 ppb	29 (fruit no.)	Retzlaff et al., 1992
Strawberry	OTC	12 hm=66 ppb	+20 (fruit weight)[5]	Takemoto et al., 1988b
Strawberry	OTC	8 hm=92 ppb AOT40=24.59 ppm-hr	14 (fruit wt.) ns (yield)	Drogoudi and Ashmore, 2000

[1]Adapted from USEPA (1996a) and later publications.

[2]Mean for shoot dry weight over 7 harvests in 2 consecutive years. Chamberless and OTC comparisons of ambient and charcoal filtered treatments were conducted simultaneously in the same environment.

[3]On year data. In off (non-bearing) year there was no effect.

[4]sens. = sensitive cultivar, tol. = tolerant cultivar. ns = no significant change.

[5]+ indicates an increase due to ozone exposure.

Table 8-10 Summary of studies published since publication of ARB (1987) demonstrating under field conditions injury to non-economic growth and physiology of annual crops of relevance to California[1].

Crop Species	Exposure Technology	ozone Exposure Averaging Period/Concentration	Summary of Ozone Impact (% loss)	Original Reference
Cotton	OTC	12 hm=111 ppb	42% shoot 61% root	Temple et al., 1988a
Potato	OTC	8 hm 60 ppb	9.2 shoot growth	Craigon et al., 2002
Potato	OTC	8 hm=60 ppb	29 (reducing sugars)	Vorne et al., 2002
Potato	OTC	8 hm 50 ppb	8.4 shoot dw	Lawson et al., 2001a
Wheat, spring	OTC	6 hm=125 ppb	35% shoot	Mortensen, 1990

[1]Adapted from USEPA (1996a) and later publications.

Table 8-11 Summary of studies published since publication of ARB (1987) demonstrating under field conditions injury to non-economic growth and physiology of perennial crops of relevance to California[1].

Crop Species	Exposure Technology	ozone Exposure Averaging Period/Concentration	Summary of Ozone Impact (% loss)	Original Reference
Almond	OTC	12 hm=51 ppb 12 hm=117 ppb	6.0 Trunk RGR 28.4 Trunk RGR	Retzlaff et al., 1991
Almond	OTC	12 hm=51 ppb 12 hm=117 ppb	1.7 C Assimilation 49.8 C Assimilation	Retzlaff et al., 1991
Almond	Closed field chambers	250 ppb x 4 h/week	8-36 growth	McCool and Musselman, 1990
Apple	OTC	12 hm=51 ppb 12 hm=117 ppb	20.5 Trunk RGR 33.3 Trunk RGR	Retzlaff et al., 1991
Apple	OTC	12 hm=51 ppb 12 hm=117 ppb	+0.3 C Assimilation[1] 36.5 C Assimilation	Retzlaff et al., 1991
Apricot	OTC	12 hm=51 ppb 12 hm=117 ppb	+25.5 Trunk RGR 52.9 Trunk RGR	Retzlaff et al., 1991
Apricot	OTC	12 hm=51 ppb 12 hm=117 ppb	8.0 C Assimilation 46.7 C Assimilation	Retzlaff et al., 1991
Apricot	Closed field chambers	250 ppb x 4 h/week	growth	McCool and Musselman, 1990
Avocado	OTC/pots	12 hm=86 ppb 12 hm=108 ppb	20 Leaf mass 61 Leaf mass	Eissenstat et al., 1991a

Avocado	OTC/pots	12 hm=86 ppb 12 hm=108 ppb	2° decrease in freeze tolerance (warmer)	Eissenstat et al., 1991a
Cherry	OTC	12 hm=51 ppb 12 hm=117 ppb	20.0 Trunk RGR 24.4 Trunk RGR	Retzlaff et al., 1991
Cherry	OTC	12 hm=51 ppb 12 hm=117 ppb	+4.2 C Assimilation +4.8 C Assimilation	Retzlaff et al., 1991
Grape	OTC		Gas exchange	Roper and Williams, 1989
Grapefruit	OTC/pots	12 hm=86 ppb 12 hm=108 ppb	1° decrease in freeze tolerance (warmer)	Eissenstat et al., 1991a
Grapefruit	OTC/pots	12 hm=86 ppb 12 hm=108 ppb	26 Leaf Mass No effect on Leaf Mass	Eissenstat et al., 1991a
Nectarine	OTC	12 hm=51 ppb 12 hm=117 ppb	+6.0 Trunk RGR +14.4 Trunk RGR	Retzlaff et al., 1991
Nectarine	OTC	12 hm=51 ppb 12 hm=117 ppb	4.2 C Assimilation 4.8 C Assimilation	Retzlaff et al., 1991
Orange	OTC/pots	12 hm=108	No effect leaf mass	Eissenstat et al., 1991a
Peach	Closed field chambers	250 ppb x 4 h/week	growth	McCool and Musselman, 1990
Peach	OTC	AOT60=5398	Shoot growth reduced	Badiani et al., 1996
Peach	OTC	12 hm=51 ppb 12 hm=117 ppb	8.9 Trunk RGR +5.6 Trunk RGR	Retzlaff et al., 1991

Peach	OTC	12 hm=51 ppb 12 hm=117 ppb	7.7 C Assimilation 5.1 C Assimilation	Retzlaff et al., 1991
Pear	OTC	12 hm=51 ppb 12 hm=117 ppb	8.1 Trunk RGR 59.4 Trunk RGR	Retzlaff et al., 1991
Pear	OTC	12 hm=51 ppb 12 hm=117 ppb	15.6 C Assimilation 57.3 C Assimilation	Retzlaff et al., 1991
Plum	OTC	12 hm=51 ppb 12 hm=117 ppb	18.4 Trunk area 42.8 Trunk area	Retzlaff et al., 1991
Plum	OTC	12 hm=51 ppb 12 hm=117 ppb	8.1 Trunk area 46.9 Trunk area	Retzlaff et al., 1991
Plum	OTC	12 hm= ppb	Postharvest water loss increased during storage	Crisosto et al., 1993
Prune	OTC	12 hm=51 ppb 12 hm=117 ppb	25.4 Trunk RGR 4.5 Trunk RGR	Retzlaff et al., 1991
Prune	OTC	12 hm=51 ppb 12 hm=117 ppb	18.4 C Assimilation 41.3 C Assimilation	Retzlaff et al., 1991

[1]+indicates an increase due to ozone exposure.

Table 8-12 Plant species and original references providing data for the growth and allometric analyses. (Data assembled by S. Gunn and D.A. Grantz).

	Binomial	**Reference**
Broadleaf Plants (herbaceous dicotyle-donous species)	*Anthyllis vulneraria*	Warwick & Taylor, 1995
	Brassica napus ssp oleifera	Ollerenshaw et al., 1999
	Chenopodium album	Reiling and Davison, 1992a
	Cirsium acaule	Warwick & Taylor, 1995
	Cerastium fontanum	Reiling and Davison, 1992a
	Citrullus lanatus	Fernandez–Bayon et al., 1993
	Cucumis melo	Fernandez–Bayon et al., 1993
	Calluna vulgaris (summer growth)	Foot et al., 1996
	Deschampsia flexuosa	Reiling and Davison, 1992a
	Epilobium hirsutum	Reiling and Davison, 1992a
	Lotus corniculatus	Warwick & Taylor, 1995
	Medicago sative	[a]Cooley & Manning, 1988
	Nicotiana tabacum Bel–W3	Reiling and Davison, 1992a
	Plantago coronopus	Reiling and Davison, 1992a
	Plantago lanceolata	Reiling and Davison, 1992a

	Plantago major	Reiling and Davison, 1992a [a]Lyons and Barnes, 1998 Reiling & Davison, 1992b Lyons et al., 1997
	Plantago maritima	Reiling and Davison, 1992a
	Plantago media	Reiling and Davison, 1992a
	Pilosella officinarum	Warwick & Taylor, 1995
	Pisum sativum	Reiling and Davison, 1992a
	Rumex acetosa	Reiling and Davison, 1992a
	Rumex acetosella	Reiling and Davison, 1992a
	Rumex obtusifolius	Reiling and Davison, 1992a
	Teucrium scorodonia	Reiling and Davison, 1992a
	Urtica dioica	Reiling and Davison, 1992a
Grass-like Plants (herbaceous monocotyl-	*Arrhenatherum elatius*	Reiling and Davison, 1992a
	Avena fatua	Reiling and Davison, 1992a
	Bromus erectus	Reiling and Davison, 1992a
	Brachypodium pinnatum	Reiling and Davison, 1992a
	Bromus sterilis	Reiling and Davison, 1992a
	Desmazeria rigida	Reiling and Davison, 1992a

edonous species)	*Festuca ovina*	Warwick & Taylor, 1995 Reiling and Davison, 1992a
	Holcus lanatus	Reiling and Davison, 1992a
	Hordeum marinum	Reiling and Davison, 1992a
	Koeleria macrantha	Reiling and Davison, 1992a
	Lolium perenne	Reiling and Davison, 1992a
	Poa annua	Reiling and Davison, 1992a
	Poa trivialis	Reiling and Davison, 1992a
	Triticum aestivum	Barnes et al., 1995 [a]Balaguer et al., 1995
Long-lived, woody Plants (tree species)	*Eucalyptus globules*	Pearson, 1995
	Fraxinus excelsior	Broadmeadow & Jackson, 2000
	Picea abies	[a]Karlsson et al., 1997
	Pinus sylvestris	Broadmeadow & Jackson, 2000
	Quercus petraea	Broadmeadow & Jackson, 2000
	Koeleria macrantha	
	Poa annua	
	Poa trivialis	

[a]Calculated values.

Table 8-13 Ozone Exposure Parameters and References to the Original Studies. (Data assembled by S. Gunn and D.A. Grantz).

Plants	**Stage of growth at initial exposure**	**Growth / exposure conditions**				**Reference**
		[ozone]		Exposure	Length of exposure[a]	
		Control	Elevated			
31 species	Cotyledon / first leaf stage	< 5 nl l^{-1}	70 nl l^{-1}	FC[a]	7 h d^{-1} for 2 weeks	Reiling and Davison, 1992a[b]
5 species	4 weeks	16 nl l^{-1}	71 nl l^{-1}	CEC	7 h d^{-1}, 5 d $week^{-1}$for 21 d	Warwick & Taylor, 1995[b]
Brassica napus ssp oleifera var biennis, (oilseed rape), 5 cv	Seedlings	CF[d]	CF + 75 nmol mol^{-1}	CEC	6.5 h d^{-1} for 16 d	Ollerenshaw et al., 1999[b]
Calluna vulgaris (summer growth)	Cuttings	CF	70 nl l^{-1}	OTC	8 h d^{-1}, 5 d $week^{-1}$ for 24 weeks	Foot et al., 1996[b]
Citrullus lanatus (watermelon) 2 cv *Cucumis melo* (muskmelon), 2 cv	22 d old	< 8 nl l^{-1}	70 nl l^{-1}	FC	6h d^{-1} for 21 d	Fernandez–Bayon et al., 1993[b]
Eucalyptus globulus	Seedlings	4.6 nl l^{-1}	52.3 nl l^{-1}	FC	7 h d^{-1} for 37 d	Pearson 1995[c]

Medicago sative (alfalfa)	4 weeks	CF	0.06 ppm	GH	6 h d^{-1}, 5 d week-1 for 56 d	Cooley & Manning, 1988[c]
Picea abies (Norway spruce), 2 clones	Seedlings	CF	NF	OTC	24 h d^{-1} for 106 d	Karlsson et al., 1997[c]
Plantago major Plants from 22 sites	6 d old seedlings	< 5 nmol mol^{-1}	70 nmol mol^{-1}	CEC	7 h d^{-1}, 14 d	Lyons et al., 1997[b]
Plantago major	6 d old seedlings	< 5 nmol mol^{-1}	70 nmol mol^{-1}	CEC	7 h d^{-1} for 14 d	Lyons and Barnes, 1998[2]
Plantago major, 28 British populations	Cotyledon	< 10 nl l^{-1}	70 nl l^{-1}	CEC	7h d^{-1} for 14 d	Reiling & Davison, 1992b[b]
Quercus petraea *Fraxinus excelsior* *Pinus sylvestris*	Seedlings	55 nl l^{-1}	100 nl l^{-1}	OTC	Max 4 h d^{-1}, 5 months $year^{-1}$ for 3 years	Broadmeadow & Jackson, 2000[b]
Triticum aestivum (spring wheat), 1 cv	8 d after emergence	< 5 nmol mol^{-1}	75 nmol mol^{-1}	CEC	7 h d^{-1}, 30 d	Balaguer et al., 1995[c]
Triticum aestivum 2 cv spring wheat; 3 cv winter wheat	2 leaf stage	< 5 nmol mol^{-1}	75 nmol mol^{-1}	CEC	Max 4 h d^{-1}, 41 d	Barnes et al., 1995[b]

[a]FC, Fumigation cabinets; CEC, Controlled environment cabinets; GH, Greenhouse.

[b]k obtained from tabulated data.

[c]k calculated from data presented.

[d]Charcoal filtered air.

8.6 Agricultural Crops – Summary

In California, forests cover 30-35-million acres of the landscape. Field studies conducted since the 1950s have found widespread damage from ambient ozone in conifer forests, but little, if any, damage in hardwood forests. Mixed conifer forests across California are exposed to elevated levels of ozone. In these forests, 12-hr average ozone levels across the growing season are 0.05-0.06 ppm, and harmful effects are well documented. The adverse effects of ozone culminate at the community-level, as a result of chronic effects on pine needle structure and physiological processes. Alterations in whole-tree biomass occur after several years of exposure due to higher carbon retention in the shoot for ozone detoxification, and lower carbon allocation to roots for maintenance and growth. As such, tree susceptibility to drought, windthrow, and root diseases could be exacerbated due to an imbalance in aboveground:belowground biomass. Changes in the mix of forest tree species may occur after one or more decades of ozone exposure due to the death of ozone-sensitive pines, and their replacement by faster-growing, ozone-tolerant cedar and fir species. It is postulated that ambient ozone will have a deleterious effect on long-term forest health in southern California and the southern Sierra Nevada at current growing season average concentrations.

8.7 Forest Trees – Introduction

Ozone is a gaseous air pollutant that damages plants after it reaches the leaf interior through surface pores called stomata. Within leaves, ozone causes cells to turn yellow or die, resulting in a loss of functional leaf area to carry out photosynthesis, the process by which plants manufacture their food. Depending on the extent of needle injury, the production of carbohydrates for tree growth and homeostasis is reduced. In addition to causing leaf damage, ozone also causes leaves on affected trees to drop off sooner than normal. This acceleration of leaf drop compounds ozone-caused losses in functional leaf area to carry out photosynthesis. After several successive years of exposure, insidious reductions in carbohydrate production lower the amount of resources that trees store to initiate bud break in the spring and to ward off the effects of other stresses (McLaughlin and Shriner 1980). As ozone exposures directly impact the aboveground portion of a tree, greater resources (e.g., energy and carbohydrates) are apportioned to the shoot for detoxification and repair, relative to other parts of the plant. Studies indicate that the increased allocations of carbon to the shoot are offset by reduced allocations to root growth (Grulke et al. 1998), starch storage, winter hardiness, and pest tolerance. On an annual-basis, the net effect of these compensatory reductions in carbon allocation on tree health are expected to be minor, but if they occur over 5-10 consecutive years, the potential for severe, growth-limiting tree damage could be high.

California spans over 100 million acres, of which 23% is occupied by conifer forests (CDF 1988). Of the 12-types of conifer forest in the state, mixed conifer forests cover ~9.3 million acres, and are variously located in the Northern Coast Ranges, Klamath Mountains, Southern Cascades, Sierra Nevada, Central Coast

Ranges, and Southern California Mountains. Reports of oxidant damage to ponderosa or Jeffrey pine in California's mixed conifer forests date back to the 1950s in the San Bernardino Mountains (Richards et al. 1968). Pine tree damage has also been found in the Los Padres National Forest (Williams and Williams 1986), and throughout the Sierra Nevada (Miller and Millecan 1971, Peterson et al. 1987, Peterson and Arbaugh 1988). In the 1990s, a research program by the USDA Forest Service, USDI National Park Service, University of California, Davis, and ARB was conducted to assess injury amounts in six National Forests and three National Parks in the Sierra Nevada and San Bernardino Mountains (Rocchio et al. 1993). The relationships between ozone exposure and injury to pines in the Sierra Nevada and San Bernardino Mountains were recently examined, and a strong relationship between injury scores and 4-yr cumulative summer ozone exposure was reported (Arbaugh et al. 1998). Figure 9.1 shows the locations of forest research sites in California.

In the San Bernardino Mountains, mixed conifer forests at Camp Paivika (1,600 m elevation) are exposed to high levels of ozone (24-hr average concentrations of 0.08-0.09 ppm) (Miller and McBride 1999). In forests of this kind, most of the ozone-sensitive trees are thought to have died as a result of the combined effects of elevated ozone exposure, drought, and insect attack since the 1950s. Of the trees that remain, many are ozone-tolerant, although visible injury is evident throughout forests on the western end of the mountain range. Resistance to drought is likely to have played a role in stand-level selection processes (Dale 1996).

In the San Gabriel Mountains, there are little data on ambient ozone levels in mixed conifer forests, but considerable data have been collected in the chaparral watersheds at Tanbark Flat at lower elevation (Bytnerowicz et al. 1987). At Tanbark Flat (800 m elevation), about 35 km north of Los Angeles, growing season 24-hr average ozone levels are 0.07-0.08 ppm (Bytnerowicz et al. 1989*b*). Relative to the western San Bernardino Mountains, ozone levels are slightly lower, and damage to forests less extensive, due in part to lower soil quality that may limit conifer growth.

Most of California's mixed conifer forest acreage exists on the western slope of the Sierra Nevada (CDF 1988). Cahill et al. (1989) reported that terrain-effect winds were capable of transporting ozone and particulates to 1,800 m elevation during daytime upslope flows, but pollutant transport to elevations at or above 3,000 m would be considerably less. Reports indicate that 24-hr average ozone levels during the growing season on the western slope of the Sierra Nevada are 0.05-0.07 ppm (Van Ooy and Carroll 1995). Currently, the risk posed by ozone in the Sierra Nevada is far less than in the San Bernardino or San Gabriel Mountains in the South Coast Air Basin (SoCAB). While ozone injury to trees has occurred in Sierra Nevada forests, decreases in growth have not been severe to our knowledge.

The principal effect of ozone on mixed conifer forests in California is expected to be reduced growth of ponderosa and Jeffrey pine. Of the western yellow pines, these two species are the most widespread, and documentation of their ozone-

sensitivity is extensive in the open literature. Throughout the state, ambient ozone concentrations are expected to decrease or remain level as a result of proposed control measures in the State Implementation Plan (SIP) (ARB 1994). In selected areas (e.g., San Joaquin Valley), increases in population are expected to be rapid, and ambient ozone levels could remain at current levels if projected emission reductions are offset by increases in emissions due to greater human activity (ARB 2005). While tree health would be impacted to a significant extent by ozone alone, the co-deposition of atmospheric N could exacerbate the deterioration of forest health at ozone-affected sites. This follows from the work of Aber et al. (1989) who advanced the nitrogen saturation hypothesis, in which the mix of tree species in a given forest is altered by excess levels of nitrogen in soil (i.e., above that needed for plant and microbial growth). As exposure to ozone may promote early needle drop and reduced root system growth in pines, a build-up in soil nitrogen levels could be accelerated (e.g., Takemoto et al. 2001). Over time, the leaching of nitrate ions from forest soils may alter nutrient balances and contaminate ground water.

8.7.1 Ambient Ozone Concentrations and Deposition

Relative to air pollution's effect on forest health in North America, ozone is the air pollutant of greatest current concern (Barnard et al. 1991, Shriner et al. 1991). Annual average ozone levels at clean, background sites range from ~0.015 to ~0.04 ppm (Lefohn et al. 1990, Table 1). In comparison, rural forest air quality in the U.S. is impacted by ozone and ozone-precursor transport from upwind urban centers, and an annual mean concentration of 0.030-0.045 ppm is considered to be a representative range for baseline, ambient ozone levels (Lefohn et al. 1990, Taylor and Hanson 1992). Forests that are subject to ozone transport commonly exhibit distinct diurnal profiles in which the difference between daily minima and maxima is 0.04-0.07 ppm (Carroll and Dixon 1993, 1995). Nighttime ozone levels decrease to a minima of 0.02-0.03 ppm at these sites due to the delivery of urban-generated nitric oxide (NO) emissions from roadways in nocturnal downslope air flows. In comparison, forests that receive limited amounts of urban-generated pollutants exhibit flat diurnal profiles, where daily fluctuations in ozone concentration are 0.010-0.015 ppm. Nighttime levels differ only slightly from daytime levels, remaining at 0.04-0.05 ppm, due to the lack of locally emitted NO to scavenge ambient ozone (Böhm et al. 1991, Peterson et al. 1991).

In the Sierra Nevada, growing season daytime 12-hr average ozone levels range from 0.05-0.07 ppm (Carroll 1991, 1992, Carroll and Dixon 1993, 1995). Both flat and distinct diurnal ozone profiles were observed at six mixed conifer forest sites (Figure 1), as well as varying amounts of ozone injury on pine trees growing in the vicinity of the monitoring stations (Arbaugh et al. 1998). Similarly, in the San Bernardino Mountains, growing season average ozone levels at Barton Flats were ~0.06 ppm (Miller et al. 1996a, 1996b), but needle injury amounts were more pronounced than in the Sierra Nevada. The average ozone concentration at Barton Flats in 1991-94 was similar to that reported for the mid-1970s (Miller et al. 1986). Growing season 24-hr average ozone levels of 0.09-0.10 ppm may still

occur at the highly polluted western end of the San Bernardino Mountains (e.g., Camp Paivika, Miller et al. 1986).

Dry deposition is defined as "all processes that deliver gases, vapors, large particles, and aerosols to the canopy in the interval between storms" (Parker 1990). For gaseous air pollutants like ozone, rates of pollutant deposition to the leaf interior are difficult to measure directly, and are commonly calculated by inferential methods, such as the Big Leaf Model (Hicks et al. 1987). Inferential models are used to calculate a deposition velocity (V_d), which is used to estimate pollutant deposition fluxes according to the following equation:

Flux (F) = Concentration (C) * V_d

Where the units of F may be kg/ha/yr, μg/m^3 for C, and cm/s for V_d. For ozone, V_d differs from site-to-site as a function of temporal, meteorological, and vegetation-related factors. For example, sites with elevated wind speeds (which create more turbulent down-mixing of air pollutants into plant canopies), and large amounts of physiologically active leaf area (capable of absorbing pollutants to the leaf interior) are factors that contribute to high values of V_d. Time of day is also important in that V_d tends to be lower at night when atmospheric turbulence is weaker, air temperatures are lower, and stomata are closed (Colbeck and Harrison 1985). A 24-hr average V_d for ozone for a range of vegetated systems (e.g., trees, crops, and grasses) is ~0.5 cm/s.

For a ponderosa pine plantation in the Sierra Nevada, Kurpius et al. (2002) estimated that over the course of a year, summer, fall, winter, and spring contributions to cumulative ozone flux were 37%, 18%, 15%, and 30%, respectively. Ozone flux to the pine plantation was highest 3-4 weeks after bud break, when the mean daytime flux was 70-80 μmol/m^2/hr (Bauer et al. 2000, Goldstein and Panek 2002). In comparison, ozone deposition to conifer forests in Tennessee was estimated to be ~80 μmol/m^2/hr during the day, and ~20 μmol/m^2/hr at night (Taylor and Hanson 1992). Massman et al. (2000) suggested that an ozone flux of 45 μmol/m^2/hr could be a threshold value for injury development in sensitive-crop species.

8.7.1.1 Levels of Ozone Exposure in California's Mixed Conifer Forests

Growing season levels of ambient ozone (24-hr average concentrations in May-October) in selected mixed conifer forests in California range from 0.05-0.06 ppm (Miller et al. 1986), 10-100% higher than the rural baseline level for U.S. forests (Table 9-14). As a consequence, ambient ozone may be causing site-specific phytotoxic effects (e.g., physiological impairment, leaf injury, or reduced growth) across the state. Theoretically, if pine trees growing in unmanaged California forests are exposed to an average ozone concentration of 0.05 ppm for 180-days, they would receive a cumulative growing season ozone exposure in excess of 200 ppm-hr (a.k.a. SUM0 – the sum of all hourly average ozone concentrations).

Controlled chamber studies have shown that ponderosa pine seedlings exposed to acute ozone levels (0.3-0.5 ppm) exhibited injury to current and one-year old

needles after 24-108 ppm-hr over a 2-3 week period (Miller 1969, Miller and Evans 1974, Miller et al. 1969). In field chamber studies conducted over 2- or 3-growing seasons, moderate amounts of ozone injury were observed in ponderosa pine seedlings grown at Whitaker's Forest (0.05-0.06 ppm, Temple et al. 1992), and at Shirley Meadow (~0.06 ppm, Takemoto et al. 1997). While the amount of ozone-caused needle injury is considerably less following chronic vs. acute exposure to ozone, multi-year exposures to ambient ozone in California pose a stress of similar magnitude for native pines to that applied in the two field studies mentioned above (Arbaugh et al. 1998). Physiologically, ozone-caused needle damage reduces the amount of photosynthetically active leaf area (Beyers et al. 1992). It also increases the need for energy and carbohydrates for maintenance respiration (Amthor and Cumming 1988, Barnes 1972, Barnes et al. 1990, Skärby et al. 1987), repairing injured tissues (Evans and Ting 1973, McLaughlin and McConathy 1983, McLaughlin and Shriner 1980, Ting and Mukerji 1971), and detoxifying reactive oxygen species produced by the dissolution of ozone (Lee and Bennett 1982, Mehlhorn et al. 1986).

Currently, the occurrence of acute ozone episodes, comparable to those that occurred in the 1950-60s, are rare, making the impacts from chronic exposures of greater concern. At present concentrations, chronic ozone exposure requires trees to allocate greater amounts of energy and carbohydrates for repair and maintenance, which limits the amount available for growth and storage. Over time, decreased carbohydrate reserves may limit tree resiliency in terms of responding to tree-killing stresses (e.g., drought, insects, and severe winds). As such, chronic ozone stress has a cumulative impact on native pines in that successive years of exposure can increase the amount and severity of needle injury, and rates of starch accumulation that are key to the initiation of bud break in the spring.

8.8 Effects of Ozone on Photosynthesis

Photosynthesis is the process by which plants capture the energy in sunlight, and use the energy to produce carbohydrates (e.g., starch; cf. Wallace et al. 1981). In clean air, photosynthesis occurs at rates that allow plants to produce carbohydrates in amounts sufficient to sustain healthy rates of growth. However, in the presence of ozone, photosynthetic rates in plants may be negatively affected if ozone impedes the plant's ability to absorb sunlight and/or processes involved with carbohydrate production (e.g., Kozlowski et al. 1981).

Concerning the harmful effects of ozone on conifer photosynthesis (e.g., Reich and Amundson 1985), studies have primarily been conducted using ponderosa pine *(Pinus ponderosa)* in the western U.S. (Beyers et al. 1992, Grulke 1999, Takemoto et al. 1997), loblolly pine *(Pinus taeda)* in the southeastern U.S. (Adams et al. 1990, Kelly et al. 1993, Richardson et al. 1992), red spruce *(Picea rubens)* in the northeastern U.S. (Laurence et al. 1989, Rebbeck et al. 1993, Taylor et al. 1986), and Norway spruce *(Picea abies)* and Scots pine (*Pinus sylvestris*) in Europe (Barnes et al. 1990, Benner and Wild 1987, Schulze 1989, Skärby et al. 1987, Zimmerman et al. 1988). As economically important, long-lived conifers that grow in regions with vastly different aerometric and edaphic

(soil-related) characteristics, ambient ozone variously affects photosynthesis in these five-species. In this regard, plant growth is fundamentally linked to rates of photosynthesis, in that nearly all of the carbon used to build cellular components and other biomolecules is a by-product of photosynthesis. To date, ambient ozone has not had a pronounced effect on growth in most cases, but selected studies have found either decreases (Benner and Wild 1987, Richardson et al. 1992, Takemoto et al., 1997) or increases (Kelly et al. 1993) in process rates (Table 2).

Physiologically, conifers exhibit inherently low gas exchange process rates (Larcher 1975), which limits ozone uptake and the magnitude of photosynthetic depression (Reich 1987). As such, it is more difficult to detect statistically significant ozone-caused alterations in conifer photosynthesis than it is for agricultural crop or hardwood species, which have inherently higher gas exchange rates (Reich 1987). While significant decreases in conifer photosynthesis have been observed following acute ozone exposures, adverse effects following exposure to ozone at near-ambient/ambient concentrations have in some cases been observed only after two or more years of exposure. For loblolly pine, however, seedling photosynthesis was significantly decreased by 10% after 100-150 ppm-hr ozone in one-growing season (Table 9-15, Richardson et al. 1992). The lack of consistent impacts on photosynthesis may be due to the relatively low-level of stress imposed by ambient concentrations of ozone (vs. acute), and to the detection limits of existing infrared gas analyzers and the designs of leaf cuvettes (cf. Beyers et al. 1992). With respect to the former, ambient ozone may pose a stress that adversely impacts a subset of genetically related, ozone-sensitive trees, but may not affect those that are tolerant to ozone. In comparison, exposure to a high concentration of ozone would likely damage both sensitive and tolerant trees. As such, studies that used a small number of trees of non-uniform parental stock may yield inconsistent results.

In an acute exposure study, ponderosa pine seedlings were exposed to 81 ppm-hr ozone over a 30- or 60-day period (Table 9-15, Miller et al. 1969). Seedlings exposed to 0.15 ppm ozone for 60-days, exhibited 35% lower rates of photosynthesis than pines grown in charcoal-filtered air, while in pines exposed to 0.30 or 0.45 ppm ozone for 30-days, rates were reduced by 70% or more. The degree of photosynthetic inhibition caused by acute ozone exposure is much greater than that caused by near-ambient/ambient levels of ozone for multi-year periods, where decreases may be ~10% or less. During an acute exposure, large amounts of ozone are deposited to the leaf interior in a short period of time, and the amount of ozone in the leaf interior greatly exceeds plant capacities of ozone detoxification. As such, extensive damage to cellular components occurs before the plant closes its stomata to prevent further ozone uptake. Visible leaf damage may be present as large bands of brown necrotic flecks (Reference). On the other hand, when plants are exposed to ozone at ambient levels, ozone uptake occurs at a much slower rate, and ozone can be metabolized by the plants inherent antioxidant-based defense systems. Visible damage to leaves may be evident as yellow spotting due to the oxidation of chlorophyll. However, under

ambient conditions, plants may not close their stomata to completely prevent ozone uptake (cf. Musselman and Minnick 2000). By keeping their stomata partially open, low-level ozone uptake continues, but concomitant CO_2 uptake serves to maintain photosynthesis at rates that may generate energy and carbon skeletons to mitigate the toxic effects of ozone.

The effect of ambient ozone on photosynthesis in ponderosa pine seedlings was investigated in three field chamber studies. No significant effects were found in two studies, and periodic inhibition was observed during the second growing season in the other. No significant effects were reported after exposure to 140-340 ppm-hr ozone in five- or six-months at Tanbark Flat in the San Gabriel Mountains (Bytnerowicz et al. 1989b, Bytnerowicz and Takemoto 1989), or after 200-600 ppm-hr ozone over three-growing seasons at Whitaker's Forest (Beyers et al. 1992). In comparison, rates in one-year-old needles were inhibited on selected dates after exposure to ~500 ppm-hr ozone in their second growing season at Shirley Meadow in the southern Sierra Nevada (Bytnerowicz and Temple 1993, Takemoto et al. 1997). In many instances, the depression of photosynthesis in ponderosa or Jeffrey pine by ambient ozone has not been strongly correlated with stomatal closure (cf. Weber et al. 1993). Instead, ozone impacts on carboxylation processes (e.g., initial activation of Ribulose Bisphosphate Carboxylase Oxygenase [RuBisCO], regeneration of ribulose bisphosphate [RuBP]) or the light reactions (chlorophyll fluorescence or damage to photosystem II) have been suggested as the site of physiological disruption (Barnes and Davison 1988, Benner and Wild 1987, Coyne and Bingham 1982, Grulke 1999, Patterson and Rundel 1989).

In terms of whole-tree photosynthesis, rates in ozone-stressed trees would be further reduced by decreased levels of foliar chlorophyll (Benner and Wild 1987) and higher rates of needle abscission/senescence (Coyne and Bingham 1981, Richardson et al. 1992). Studies have shown that ozone can oxidize chlorophyll molecules, rendering them unable to trap sunlight to energize the photosynthetic process. Moreover, ozone can oxidize proteins in chloroplast membranes as well as enzymes that catalyze carbohydrate production (Reference). Assuming that trees in central and southern California receive growing-season ozone exposures of 150-250 ppm-hr per year, a cumulative exposure of 600-1,000 ppm-hr would be achieved within 4-years. Upon reaching this level of ozone accumulation, adverse effects on photosynthesis may become evident in needles that are retained for several seasons (Coyne and Bingham 1981), possibly due to the loss of normal stomatal function (Grulke 1999). In highly sensitive ozone-stressed trees, needle retention may be limited to only two or three annual whorls in some cases. For these plants, photosynthetic process rates in two- and three-year-old needles may not be significantly affected during the current year until a threshold level of chlorotic mottle, evident as lower foliar chlorophyll concentration, is achieved (Benner and Wild 1987). If the injury threshold is reached, the needle will likely abscise from the tree during fall or winter, which could result in a net loss in photosynthetic capacity in the following growing season. By dropping ozone-injured needles at the end of the growing season,

some amount of nutrient reabsorption would occur, and fewer resources would be expended in maintenance respiration over winter.

8.8.1 Effects of Ozone on Carbon (C) Allocation and Partitioning

While rates of photosynthesis are a useful indicator of the amount of carbohydrates that can be produced by plants, unless the carbohydrates are distributed and used in the proper proportions, healthy growth may not occur. In clean air, plants typically utilize carbohydrates to avoid or repair injuries caused by insects, disease, and weather-related stresses. In polluted air, plants may also need to utilize carbohydrates to avoid or repair injury caused by ozone, thus reducing the amount that is available for warding off other stresses. Depending on how much injury ozone causes, the amount of carbohydrates available for growth processes may be significantly reduced, thereby limiting the amount of plant growth that can occur during the growing season.

Carbon (C) skeletons produced by photosynthesis (photosynthates) are distributed within plants to sustain growth, homeostasis (Amthor and Cumming 1988), defense (i.e., stress avoidance and/or tolerance, Lechowicz 1987), and repair (i.e., restoration of damaged organs and tissues, McLaughlin and Shriner 1980). The flux of carbon from one plant organ to another, in any chemical form, is referred to as C-allocation (Dickson and Isebrands 1993), which also includes the incorporation of newly produced photosynthates, and carbon mobilized from storage compounds or recycled materials into new leaf tissue. For the most part, carbon is allocated from leaves (photosynthate “sources”) to other plant organs, which are commonly referred to as photosynthate “sinks.” However, sinks may act as storage organs for excess photosynthates produced during periods of high photosynthetic activity, thereby serving as sources to other sinks under certain circumstances. Studies show that when plants are subject to moderate levels of stress, most plants will utilize their carbon reserves in lieu of altering their normal patterns of C-allocation to maintain a balanced [root:shoot] biomass ratio. Changes in C-allocation generally occur only if a stress is severe enough to deplete the stored carbon reserves of a plant (e.g., starch). Under these situations, plant growth may be curtailed due to increased C-allocation to defense and repair, or affected plant parts shed, in order to maintain whole-plant integrity (Laurence et al. 1994).

Numerous workers have postulated that the primary effect of ozone on C-allocation is to reduce the [root:shoot] ratio of a plant: C-allocation to roots is reduced due to greater resource needs in leaves for ozone-related defense and repair (McLaughlin and McConathy 1983). Over time, the change in C-allocation leads to a lower [root:shoot] ratio in ozone-exposed plants relative to control plants (Andersen et al. 2001, Grulke et al. 1998). Since ozone is principally deposited to leaves, ozone molecules that reach the leaf interior may directly affect photosynthesis in mesophyll cells (Spence et al. 1990), and sucrose translocation in phloem sieve cells. In their review of ozone effects on C-allocation and C-partitioning, Cooley and Manning (1987) reported that while ozone often reduces whole plant dry matter content, in most cases, the mass of plant storage organs is most affected by ambient ozone. In loblolly pine, Kelly et

al. (1993) and Spence et al. (1990) both observed greater C-retention in the shoot, and lower C-allocation to roots in seedlings exposed to ozone (Table 2). Sustained reductions in C-allocation to roots were projected to adversely affect water and nutrient acquisition, especially on water-stressed sites (Kelly et al. 1993). Root associations with *Pisolithus tinctorius* could significantly increase root sink strength (Chapin et al. 1987), as mycorrhizal trees are often less sensitive to ozone effects on C-allocation than non-mycorrhizal trees (Cooley and Manning 1987).

In comparison, C-partitioning refers to the transformation of carbon from one chemical form to another within a plant organ (Dickson and Isebrands 1993). With respect to ozone, the depletion of starch reserves in leaves, to produce antioxidants, activate enzymes, and/or to repair ozone damage to cell membranes, is the physiological response most commonly reported. In studies on tobacco and cotton, plant susceptibility to ozone injury was correlated with foliar carbohydrate level at the time of exposure -- plants sustained greater amounts of ozone-caused leaf injury when carbohydrate levels were lowest. Lee (1965) reported that high levels of sucrose were associated with stomatal closure, which limited ozone uptake and damage expression in tobacco (~5 ppm-hr). In cotton, Ting and Mukerji (1971) suggested that greater amounts of damage resulted from the lack of soluble reserves for repair processes at the time of ozone exposure (~1 ppm-hr). Other crop studies have shown that exposure to ozone reduces both carbohydrate and protein levels relative to levels in plants grown in clean air. In soybean, levels of soluble sugars and starch were reduced by ozone (65 or 95 ppm-hr, Miller et al. 1995), while in cotton and bush bean, ozone-caused reductions in soluble protein were associated with increases in free amino acids (0.5 ppm-hr, Craker and Starbuck 1972, Ting and Mukerji 1971). Exposure to ozone reduced foliar starch and soluble sugar levels in red spruce (~550 ppm-hr, Amundson et al. 1991), starch in primary and secondary needles of loblolly pine (45 ppm-hr, Meier et al. 1990), soluble sugars in white pine and loblolly pine (Wilkinson and Barnes 1973), and total nonstructural carbohydrates and proteins in leaves, stems and roots of *Ulmus americana* (4.5 ppm-hr, Constantinidou and Kozlowski 1979).

8.8.2 Effects of Ozone on Tree Growth

In consideration of the mechanistic relationship between photosynthesis and crop yield (Zelitch 1982), if exposure to ozone causes a decrease in tree photosynthesis (Reich and Amundson 1985), it may also adversely affect on tree growth. Pye (1988) reviewed the responses of 43-tree species to ozone, and postulated that reductions in seedling growth and photosynthesis may be occurring in many parts of the U.S. However, in order to estimate the potential for ozone-caused effects on mature trees and whole-stands from seedling data, several key uncertainties needed to be addressed. For example, seedling growth and physiology is more rapid in seedlings than in mature, and ozone effects tend to be more severe in seedlings due in part to higher rates of ozone uptake. As responses in seedlings are often used to estimate impacts to trees of other age

classes, challenges lie in avoiding major errors in over- or underestimating true impacts on tree growth, wildlife habitat, and forest structure and composition.

In the eastern U.S., studies have focused on the effects of ozone on loblolly pine (e.g., Kelly et al. 1993). Adverse ozone effects in red spruce (400-530 ppm-hr, Amundson et al. 1991; Alscher et al. 1989) and slash pine (~122 ppm-hr, Hogsett et al. 1985) have also been reported, but not in white pine (44 ppm-hr, Reich et al. 1987). For loblolly pine, reductions in aboveground biomass (stem, branches, and leaves) have been found to result from ozone effects on foliage and stem growth. Stem volume reductions of similar magnitude have been reported in two 12-week studies; a 14% reduction was observed after exposure to ~70 ppm-hr (McLaughlin et al. 1994), and a 15% decrease after 45 ppm-hr (Meier et al. 1990). With respect to foliage effects, Adams et al. (1988) found that secondary foliage (new needles) was the most ozone-sensitive biomass component (~190 ppm-hr), which largely accounted for observed reductions in aboveground and whole-plant biomass in five half-sib families. Kress et al. (1992) observed a significant reduction in fascicle retention following exposure to ambient ozone for two-growing seasons (~290 ppm-hr). These workers reported that ambient ozone accelerated the abscission of foliage, such that most foliage abscised before the end of the second growing season. Collectively, the decreases observed on measures of stem and foliage biomass are consistent with the results of Shafer et al. (1987), who found that ambient ozone (~120 ppm-hr) inhibited the growth of three full-sib families by as much as 10% in one growing season. After two growing seasons of exposure to ambient ozone (~240 ppm-hr), seedlings from four full-sib families exhibited about 20% foliar injury and mean growth reductions ranging from 0-19% (Shafer and Heagle 1989). In his review of the loblolly pine growth responses to ozone, Taylor (1994) suggested 12-hr mean threshold concentrations for growth effects on average and sensitive seedlings were in the range of 0.045 and 0.025 ppm, respectively. As such, extant ozone levels in the Southeast are adversely affecting average pines intermittently, and sensitive cohorts frequently. On a regional scale, effects on whole-plant biomass are apparent at exposure levels of ~150 ppm-hr, and a general pattern of biomass suppression with increasing exposure was indicated. Incipient effects were estimated to occur at 97 ppm-hr, and 3% declines in whole-tree biomass were projected for every 100 ppm-hr increase in cumulative exposure.

In the western U.S., studies have primarily focused on the responses of ponderosa and Jeffrey pine to ozone in the San Bernardino Mountains (Arbaugh et al. 1999) and Sierra Nevada (Peterson et al. 1991). In addition, growth responses in big-cone Douglas fir *(Pseudotsuga macrocarpa)* on the western end of the San Bernardino Mountains (12-hr daytime mean = 0.09 ppm), were also found to be inhibited by ambient ozone (Peterson et al. 1995). In the 1980s, studies conducted in the Sequoia National Forest showed that radial growth in mature ponderosa pine trees, which exhibited ozone injury (symptomatic trees) was 14% lower than in trees without injury (asymptomatic trees) (Williams and Williams 1986). Ozone injury refers to the yellow spotting or “chlorotic mottle” to needles of pine trees exposed to elevated ozone concentrations (Miller and Evans, 1974). In subsequent studies, Peterson and co-workers (1987) in

Sequoia National Park observed a similar level of growth inhibition in symptomatic Jeffrey pine trees (11% lower than asymptomatic trees), which tended to be more pronounced in larger, older trees. However, these workers did not find significant growth reductions in ponderosa pine trees from 1956-1984, which exhibited chlorotic mottle and premature needle senescence (Peterson and Arbaugh 1988, Peterson et al. 1991). The lack of a significant ozone effect may have been due to the presence of ozone-tolerant and -sensitive trees in native forests, and the need to examine a larger number of trees.

At Whitaker's Forest in Kings Canyon National Park, Temple et al. (1993) found that after three-years, well-watered ponderosa pine seedlings exposed to ozone (~900 ppm-hr over three-seasons) exhibited 70% and 48% decreases in two-year-old and one-year-old needle biomass, respectively. While the biomass of current year needles was not reduced, significant decreases were observed in stem diameter (6%) and coarse root dry weight (14%). Total plant dry weight was 14% lower in ozone-exposed plants relative to plants grown in charcoal-filtered air. No significant effects were observed in drought-stressed plants exposed to ozone after three-seasons. This may in part be due to the need to conserve water during periods of drought. Under water-limited conditions, plants may close leaf surface pores that regulate the flow of atmospheric gases into and out of the leaf interior. In doing this, plants balance their need for carbon dioxide for photosynthesis against limiting the amount of cell damage from ozone. Photosynthetic rates in current year needles of well-watered seedlings exposed to 350 ppm-hr ozone in 1990 (the third season) were 40% higher than in plants exposed to charcoal-filtered air. This compensatory response was suggested to result from a higher tissue N level and increased inorganic phosphate cycling as a consequence of the abscission of previous year needles. Temple and Miller (1994) later reported that the reduction in radial growth was correlated with foliar injury in well-watered trees; needles with > 30% injury tended to abscise before the end of the next growing season.

In the San Bernardino Mountains, radial growth responses in ponderosa pine have varied over the period from 1925-1991 (Arbaugh et al. 1999). From 1925-1949, radial increment growth rates exhibited an increasing trend, reaching a maximum in about 1945. A decreasing trend was observed from 1950-1974, due in part to the combined effects of drought and high ambient ozone. Impacts were most severe in trees > 100-years old. From 1975-1991, radial growth responses appear to have recovered to levels similar to those in the 1930s, which coincides with an increase in precipitation amount, but only slight declines in ambient ozone.

8.8.3 Effects of Ozone on Winter Hardiness

Over centuries, extremes in regional climate have largely defined the conditions that forest trees must adapt to, in order to thrive in a particular location. In California, ponderosa pine trees, which retain their foliage year-round, have developed strategies to minimize the potentially growth-limiting impacts of low-soil moisture in the summer and cold air temperature in the winter. A key factor in a tree's ability to limit the impacts of freezing temperatures in winter is the

accumulation of carbohydrates that minimize cell damage due to ice formation (e.g., Alscher et al. 1989). In the presence of ozone, negative impacts on photosynthesis and carbon allocation could hinder efforts to produce and store the carbohydrates involved with avoiding injury from exposure to cold temperatures.

As tree species that retain their foliage year-round, conifers undergo a suite of morphological and physiological activities to increase frost hardiness each autumn. These activities include decreasing rates of photosynthesis, accumulating polyunsaturated fatty acids (e.g., 18:4 fatty acids, Wolfenden and Wellburn 1991), increasing levels of antioxidants (e.g., glutathione (GSH), Hausladen et al. 1990) and soluble sugars (e.g., raffinose and sucrose, Alscher et al. 1989, Amundson et al. 1991, Barnes et al. 1990). At the physiological level, it has been suggested that the effects of cold temperature are comparable to the impacts of ozone, since both cause increases in reactive oxygen species. The increase in reactive oxygen species caused by cold temperatures occurs as a result of the freezing of extracellular water during the first autumn frosts. In periods of prolonged chilling, the temperature of extracellular water continues to decrease, with a concomitant increase in oxygen solubility. Ultimately, oxygen concentrations equilibrate across plant cell membranes, and at higher oxygen tensions, the rate of intracellular superoxide formation may be enhanced. For ozone, reactive oxygen species are formed upon dissolution to the substomatal cavity (Skärby et al. 1998).

Ozone-caused decreases in frost hardiness have been suggested to result from cell membrane damage during the growing season in high-elevation Norway spruce forests in Europe (Barnes and Davison 1988). Trees, which were damaged by ozone in summer, may be predisposed to greater amounts of freezing injury and winter desiccation than trees that were not affected by ozone. Additionally, ozone may also increase susceptibility to winter chilling by increasing rates of dark respiration, leading to decreased levels of ethanol-soluble sugars (e.g., raffinose, Barnes et al. 1990). With respect to lipids, Wolfenden and Wellburn (1991) reported that ozone may interfere with the biosynthesis of 18:4 fatty acids during frost hardening by inhibiting the Δ^5 desaturation of oleate. In cold temperatures, a high proportion of polyunsaturated fatty acids allows membranes to maintain fluidity and to remain semi-permeable. Thus, if trees are unable to achieve a prescribed level of lipid unsaturation in autumn, membrane dysfunctions may occur in subsequent winter months, leading to more extensive damage to cell membranes and loss of cell integrity.

8.8.4 Overview of Potential Ozone Effects on Western Pines in California

8.8.4.1 Ozone's Role in Forest Health/Productivity

Reports of oxidant-caused injury to pines in California forests date back to the 1950s in southern California (Richards et al. 1968), and the 1970s in the Sierra Nevada (Miller and Millecan 1971). At the cell/tissue level, the development of chlorotic mottle and/or necrotic flecks constitutes a loss of photosynthetically active leaf area, and in some cases, a net decrease in whole-plant carbon

assimilation during the growing season. Depending on the extent of needle injury, energy and carbohydrate allocations to maintenance respiration during winter could be considerable if injured needles are retained by the plant as sources for initiating new needle growth in the spring. Since ozone is known to accelerate leaf senescence, it may also be a factor that influences the number of needle whorls retained by a tree after several successive years of exposure. At the tissue/organ level, the stress imposed by ozone on the plant shoot requires greater resource allocations to defense, repair, and homeostasis during the growing season to sustain plant productivity (Grulke 1999, McLaughlin and Shriner 1980). Greater energy and carbohydrate allocations to the shoot for detoxification and repair are balanced against reduced allocations to root growth, starch storage, winter hardiness, and pest tolerance. On an annual-basis, the net effect of these compensatory reductions in C-allocation on tree health are expected to be minor, but if they occur over 5-10 consecutive years, the potential for severe, growth-limiting tree damage could be high. Adverse impacts are expected to be greater for western pines, as opposed to cedar and fir species, which may emerge as the dominant species in mixed conifer forests over the next century (Peterson et al. 1991).

Rosenbaum et al. (1994) examined the extent to which critical levels for ozone, nitrogen dioxide and sulfur dioxide, developed by the United Nations-Economic Commission for Europe (UN-ECE), were exceeded in the U.S. Average ozone concentrations at 99% of monitored sites exceeded the 7-hr (0.025 ppm) and 8-hr (0.03 ppm) critical levels, and the 1-hr level (0.075 ppm) was exceeded at 50% of sites. As noted above, forest lands in California are among the sites exceeding the UN-ECE critical levels for ozone, and these results are consistent with the findings of Olson et al. (1992), who concluded that ozone is the primary pollutant of concern in western U.S. forests. Over the next 50-years, forest exposures to ozone and atmospheric N in California may continue to occur at levels that can cause adverse plant and soil responses, based on current knowledge. Although the results from tree surveys suggest that exposure to ozone is a contributing factor to reduced pine growth in mixed conifer forests across the state, the interactive effect of other possible contributing factors (e.g., rising rates of atmospheric N deposition and increasing levels of carbon dioxide) are not known.

8.8.4.2 Conclusions

Throughout California, ambient ozone levels are expected to decrease through 2010 as a result of proposed hydrocarbon and NO_X control measures in the California State Implementation Plan. In consideration of Beyers et al. (1992) and Bytnerowicz et al. (1989a), mixed conifer forests in the Sierra Nevada and SoCAB are exposed to ~250 or ~350 ppm-hr ozone, respectively, during the growing season. In postulating a worse case scenario, sustained annual exposures to ozone in the range of 250-350 ppm-hr would accelerate leaf senescence, and symptomatic trees may only retain 2-3 whorls of needles. While tree health would be impacted to a significant extent, the effects may not be as great as those projected for loblolly pine in the Southeast (Taylor 1994). A key uncertainty is the co-deposition of atmospheric N-containing compounds, which

may exacerbate the deterioration of forest health at ozone-affected sites if soil N-saturation occurs within the next 50-100 years (Takemoto et al. 2001).

Table 8-14 Ambient Ozone Concentrations at Background and Rural U.S. Forest Sites

Concentration (ppm)	Site Location	Type of Value	Reference
~0.015	American Samoa	Annual average	Lefohn et al., 1990
~0.04	Apache National Forest, AZ	Annual average	Lefohn et al., 1990
0.03-to-0.045	"Rural U.S. forests"	Growing season, 12-hr mean	Lefohn et al., 1990; Taylor and Hanson, 1992
0.05-to-0.07	Mixed conifer forests, western Sierra Nevada	May-to-October, 12-hr mean (1991-1994)	Carroll, 1991; 1992; Carroll and Dixon, 1993; 1995
0.05-to-0.06	Whitaker's Forest, Sierra Nevada	Growing season, 12-hr mean	Temple et al., 1992
~0.06	Shirley Meadow, Sierra Nevada	Growing season, 12-hr mean (1989-1990)	Takemoto et al., 1997
~0.06	Barton Flats, San Bernardino Mountains	May-to-October, 12-hr mean (1991-1994)	Miller et al., 1996a
~0.09	Camp Paivika, San Bernardino Mountains	May-to-October, 12-hr mean (early 1980s)	Miller et al., 1986

Table 8-15 Comparison of Ozone Exposures (ppm-hr) Used in Plant Effect Studies

Tree Species	Selected Response(s)	Ozone Exposure	Reference
Loblolly pine	Reductions in foliar starch and stem volume	45 ppm-hr; 0.15 ppm, 5-hr/day, 5-day/wk, 12-wks	Meier et al., 1990
Loblolly pine	Greater C-retention in the shoot	50 ppm-hr; 0.12 ppm, 7-hr/day, 5-day/wk, 12-wks	Spence et al., 1990
Loblolly pine	Reduction in stem volume	~70 ppm-hr; 0.04 ppm (24-hr mean), 90 days	McLaughlin et al., 1994
Loblolly pine	Decrease in photosynthesis	100-150 ppm-hr; 0.034 ppm (24-hr mean), 37 wks	Richardson et al., 1992
Loblolly pine	Reduction in growth	~120 ppm-hr; 0.05 ppm, 12-hr/day, 150-days	Shafer et al., 1987
Loblolly pine	Decrease in secondary foliage mass	~190 ppm-hr; 0.08 ppm (24-hr mean), 14 wks	Adams et al., 1988
Loblolly pine	Reduced fascicle retention	~290 ppm-hr; 0.03 ppm (24-hr mean), 6-mo, 2-seasons	Kress et al., 1992
Loblolly pine	Greater C-retention in shoot	~800 ppm-hr; 0.06 ppm (24-hr mean), 3-seasons	Kelly et al., 1993

Ponderosa pine	Decrease in photosynthesis	81 ppm-hr; 0.15 ppm , 9-hr/day, 60-days	Miller et al., 1969
Ponderosa pine	Decrease in photosynthesis	81 ppm-hr; 0.30 ppm, 9-hr/day, 30-days	Miller et al., 1969
Ponderosa pine	No significant effect on photosynthesis	140-340 ppm-hr; 0.06 ppm (24-hr mean), 6-7 months	Bytnerowicz and Takemoto, 1989
Ponderosa pine	Intermittent de-creases in photo-synthesis in 2nd growing season	~500 ppm-hr; 0.06 ppm (24-hr mean), 2-seasons	Takemoto et al., 1997
Ponderosa pine	No significant effect on photosynthesis	~600 ppm-hr; 0.06 ppm (24-hr mean), 3-seasons	Beyers et al., 1992
Red spruce	Reductions in foliar carbohydrates	~400-530 ppm-hr; 0.06 ppm (24-hr mean), 2-seasons	Amundson et al., 1991
Slash pine	Decrease in stem growth	~122 ppm-hr; 0.08 ppm (7-hr mean), 111-days	Hogsett et al., 1985
White pine	No significant growth effect	44 ppm-hr; 0.14 ppm, 7-hr/day, 3-day/wk, 15 wks	Reich et al., 1987

Figure 8-1 Location of forest/ozone research sites in California.

8.9 Effects on Materials

The very great chemical reactivity of ozone results in significant damage to a wide range of materials, including rubber, plastics, paint, and metals. In areas experiencing high ambient concentrations, ozone can shorten material lifespans, increase maintenance costs, and progressively damage functional or cosmetic aspects of materials. Based on results from chamber and field studies, such damage includes increased rates of loss of physical integrity of rubber and other elastomers, corrosion of metals and electrical components, erosion and discoloration of paint, fading and reduction of tensile strength of fabrics, and soiling and spalling of nonmetallic building materials. Ozone damage to these materials leads to costs for producers and consumers in two general classes:

1) accelerated replacement and repair costs, as when the service life of a material is impaired of appearance is degraded; and

2) increased avoidance costs, as when industries (e.g., tires, plastics, dyes) substitute processes and materials, or use additives and reformulation, in order to avoid or minimize losses.

Materials that can be adversely affected by ozone include elastomers, paints, fabrics, dyes, artists' pigments, and various types of plastics such as polyethylene. The discussion that follows briefly describes ozone damage for each of these types of materials.

8.9.1 Elastomers

A number of commercially important rubber-based products are fabricated from unsaturated elastomers with well-known sensitivities to ozone damage. These materials include tires, rubber seals, pharmaceutical goods, and many others. Of the materials listed, vehicle tires have been identified as the single major user of elastomers with associated significant economic costs resulting from effects of ozone.

Oxidation affects tires most significantly by causing cracks to develop in the tire sidewalls. Some types of flexible unsaturated rubber are placed over the strength-bearing cords in a tire's sidewall. However, such unsaturated rubber can be susceptible to ozone damage. In order to minimize such damage, tire manufacturers add anti-oxidant materials to such rubber formulations. Benefits from reduced concentrations of ozone can be expected both by tire manufacturers and by tire retreading firms. The manufacturers would benefit by decreasing the quantity of anti-oxidant required to protect vehicle tires through predicted use lifetimes, and tire retreaders would benefit through increased numbers of usable tire carcasses (Rowe, et al., 1986).

8.9.2 Paints

Past studies have shown that of various paints only vinyl and acrylic coil coatings are affected (Haynie, et al., 1976), and that this impact has a negligible effect on the useful life of the material coated. Another more recent study has found that the costs associated with premature replacement of acrylic and vinyl coil coatings were minimal and could not be attributed to pollutants alone (McCarthy, et al., 1981). The results of an EPA study indicated that the possible effect of ozone on latex paint was masked by the effects of other pollutants and environmental factors (Haynie and Spence, 1984).

8.9.3 Fabrics

Ozone has an adverse effect on cotton, nylon, and acrylic fibers and reduces breaking strength of these fibers. The degree of reduction in strength depends upon the moisture present. The effect of ozone on the economic life of fabric material is extremely uncertain. However, it is believed to be quite small (Rowe, et al., 1986).

8.9.4 Dye Fading

Oxidants cause certain sensitive dyes to fade. Research suggests that the magnitude of oxidant-caused dye fabric fading varies significantly with temperature, relative humidity, location of exposure, type and area of exposure, and variation in seasons. A nationwide field exposure study of dye fabric samples showed that one of the highest rates of fabric fading occurred in Los Angeles (Ajax, et al., 1967). Another nationwide field study conducted at the same sites indicated that two-thirds of exposed fabrics showed evidence of dye fading (Beloin. 1972, 1973).

8.9.5 Artists' Pigments

Pigments in artists' paints have been tested under controlled conditions. A recent laboratory study exposed seventeen pigment samples and two Japanese woodblock prints to 0.40 ppm of ozone in a controlled test chamber for three months. The study concluded that several artists' pigments when applied on paper will fade if exposed to an atmosphere containing ozone at the concentrations found in photochemical smog (Shaver, et al., 1983). Indoor-outdoor ozone monitoring in a Pasadena art gallery confirmed that ozone concentrations half as high as those outdoors can be found in art galleries that lack a chemically protected air conditioning system. Under those conditions, it would take roughly six years to accumulate an ozone exposure equivalent to the study's chamber experiment. A more recent study was conducted to further assess the risk of color fading hazard for works of art. Twenty-seven artists' pigments were exposed to ozone for three months. This study found that many types of pigments were susceptible to fading when exposed to ozone. Several ozone-resistant pigments were also identified in the study (Drisko, et al., 1984). Because art work often requires protection for hundreds of years, ozone concentrations in unprotected buildings may pose a significant risk of damage to many art collections.

8.10 Conclusions

Elevated concentrations of ozone can cause adverse effects on agricultural crops, forest trees and materials at current ambient levels. The proposed health-based ozone standards will also provide protection to crops, forests and materials.

8.11 References

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Air Resources Board

State of California

Governor Arnold Schwarzenegger

October 2005 Revision

Review of the California Ambient Air Quality Standard For Ozone

Volume III of IV
Chapters 9-11

Staff Report
Initial Statement of Reasons for Proposed Rulemaking

October 27, 2005

California Environmental Protection Agency

Air Resources Board

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

Alan C. Lloyd, Ph.D., Secretary

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Abbreviations and Definitions

abscission	the normal separation, involving a layer of specialized cells, of flowers, fruits and leaves of plants
AOT40	accumulated exposure over threshold of 40 ppb ozone
AQDA	air quality data action
ARB	Air Resources Board
AVG	aminoethoxyvinyl glycine
BSA	Broader Sacramento Area
Ca^{2+}	calcium ion
canopy	a cover of foliage that forms when the leaves on the branches trees in a forest overlap during the growing season
CEC	controlled environment chamber
CFR	Code of Federal Regulations
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
d	day
edaphic	the physical, chemical, and biological characteristics of soil
ESPACE	European Stress Physiology and Climate Experiment
FACE	Free Air Carbon Enrichment system, a chamber-free, open-air fumigation design
FEF25-75%	forced expiratory flow rate between 25 and 75% of forced vital capacity
FEM	federal equivalent method (for air monitoring)
FEV1	forced expiratory volume in one second
fine roots	roots with a diameter between 0.5 to 3 mm
foliar	of or referring to a plant leaf
FRM	federal reference method (for air monitoring)
full-sib	seedlings that have the same parents, but not necessarily from seed produced in the same year
FVC	forced vital capacity
g	gram
GBVAB	Great Basin Valleys Air Basin
gdw	gram dry weight
GIS	geographic information system
gfw	gram fresh weight

hr	hour
ha	hectare (= 10,000 m^2; an area that is 100 m x 100 m)
half-sib	seedlings that have one parent in common
hm	hourly mean
HNO_3	nitric acid
homeostasis	the tendency toward maintaining physiological stability within an organism (plant or animal)
H&SC	Health and Safety Code
IPM	Integrated Pest Management.
Jeffrey pine	*Pinus jeffreyi* Grev. and Balf.
k	allometric growth coefficient describing the distribution of dry weight gain between competing plant parts, defined as the ratio of the relative growth rates of the competing plant parts
K^+	potassium ion
kg	kilogram (= 1,000 g = 2.205 pounds)
km	kilometer (= 1,000 m = 0.6214 miles)
L	liter
LCAB	Lake County Air Basin
LST	local standard time
LTAB	Lake Tahoe Air Basin
m	meter (= 3.28 feet)
m^2	square meter, an area that is 1 m x 1 m
MCAB	Mountain Counties Air Basin
MDAB	Mojave Desert Air Basin
mesophyll cells	the internal cells of a leaf, distinct from cells at the leaf surface or from cell layers immediately adjacent to the leaf surface
mixed conifer	forests with a tree-layer dominated by a mixture of conifer species
montane	of or relating to a mountain or mountainous area
mRNA	messenger RNA (ribonucleic acid)
mycorrhizae	a biological association of a fungus (e.g., *Pisolithus tinctorius*) with the root cells of a plant (e.g., ponderosa pine tree)
mycorrhizal trees	trees with roots associated a mycorrhizae fungus
n	sample size
NARSTO	a public/private partnership to coordinate research in Canada, Mexico and the United States on tropospheric air pollution (formerly the North American Research Strategy for Tropospheric Ozone)

NCAB	North Coast Air Basin
NCCAB	North Central Coast Air Basin
NCLAN	National Crop Loss Assessment Network, a national study of ozone impacts on crops, undertaken during the 1980s
NEPAB	Northeast Plateau Air Basin
ng	nanogram (= 0.000000001 g = 10^{-9} g)
NH_4N_3	ammonium nitrate
nL	nanoliter (10^{-9} L)
nm	nanometer, or one billionth of a meter
NO	nitric oxide, the primary nitrogen-containing by-product of combustion
NO_2	nitrogen dioxide
NO_X	nitrogen oxides (or oxides of nitrogen)
ns	not statistically significant at p =0.05
O_3	ozone; triatomic oxygen
OII	ozone injury index
OTC	open top field exposure chamber
PAR	photosynthetically active radiation (400 – 700 nm)
phloem	the plant tissue through which sugars and other organic materials are transferred to different parts of the plant
photosynthesis	the production by green plants of organic compounds from water and carbon dioxide using energy absorbed from sunlight
Pisolithus tinctorius	a mycorrhizae-forming fungus that forms root-associations with a wide variety of pine and other tree species
ppb	parts per billion by volume
ppb-hr	parts per billion hours (i.e., sum of concentration times duration), a measure of exposure to ozone
ppm	parts per million by volume
ppm-hr	parts per million hours (i.e., sum of concentration times duration), a measure of exposure to ozone
process rates	the degree or amount at which specific actions or activities occur (e.g., water vapor loss from leaves of plants)
QAS	Quality Assurance Section (of ARB)
R:S	ratio of root biomass (dry weight) to shoot biomass
RGR	relative growth rate, defined as the difference in the dry weight of a plant or plant part over a time period, divided by the initial dry weight and the length of the time period

RH	relative humidity
RuBisCO	ribulose bisphosphate carboxylase-oxygenase
RuBP	ribulose bisphosphate
SCCAB	South Central Coast Air Basin
SCOIAS	Sierra Cooperative Ozone Impact Assessment Study
SDAB	San Diego Air Basin
senescence	the onset of aging -- a phase in plant development from maturity to the complete loss of organization and function in plants
SFBAAB	San Francisco Bay Area Air Basin
shoot	the aboveground portion of the plant (e.g., leaves, stems, flowers, and fruits)
sieve cells	the primary type of cell found in the phloem of plants
SIP	State Implementation Plan
SJVAB	San Joaquin Valley Air Basin
SoCAB	South Coast Air Basin
SSAB	Salton Sea Air Basin
sucrose	a disaccharide (with 12 carbon atoms) commonly found in plants
(sucrose) translocation	the movement of sucrose (or other soluble organic food materials) through plant tissues – most commonly from leaves to stems/roots
SUM06	an ozone exposure metric involving concentration weighting, defined as the sum of all hourly mean ozone concentrations equal to or greater than 70 ppb
terrain-effect winds	air currents influenced by the geographic features of the land that it passes over
TREEGRO	a physiologically based computer simulation model of tree growth and development
Ulmus americana	the scientific name for “American Elm”
UN-ECE	United Nations Economic Commission for Europe
USD	United States dollars
USDA	United States Department of Agriculture
USDI	United States Department of the Interior
USEPA	United States Environmental Protection Agency
USV	Upper Sacramento Valley
V_d	deposition velocity, defined as deposition flux of ozone divided by its concentration in air (usually in cm/s or m/s)

VPD	vapor pressure deficit, a measure of evaporative demand of air
whorl	the arrangement of leaves, petals, etc., at about the same place on a stem
wk	week
yr	year
ZAP	zonal application system, a chamber-free, open-air exposure system
µg	microgram (= 0.000001 g = 10^{-6} g)
µm	micrometer or micron (= 0.000001 m = 10^{-6} m)

9 Controlled Ozone Exposure Studies

9.1 Introduction

This chapter describes the results of studies of humans and animals exposed to controlled concentrations of ozone. Although not an exhaustive review, the chapter includes most of the available studies, particularly those using human subjects. The number of controlled ozone exposure studies published since 1998 is very small because research priorities have shifted toward examination of the health impacts of particulate matter. Consequently, the fact that relatively few recent papers are cited in this chapter is a function of the available literature.

9.2 Ozone Dosimetry

9.2.1 Uptake

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

9.2.2 Human Studies

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

However, two studies have reported no differences in ozone-induced changes in pulmonary function or respiratory symptoms among volunteers breathing orally, oronasally, or nasally, when ozone was administered via a facemask (Adams et al. 1989; Hynes et al. 1988). These reports suggest that ozone is likely to be scrubbed more or less equally by the nose and mouth. Ozone removal efficiency increases directly with increasing concentration and inversely with breathing rate (Gerrity et al. 1988). Continuous ozone inhalation decreases the efficiency of ozone absorption in the central airways, facilitating increased delivery of ozone to the deep lung (Asplund et al. 1996). Presumably this occurs because of the

reduction of reactive mucus substrates in the airway. Reduction of tidal volume, a common functional response to ozone exposure, results in a significant decline in lower respiratory tract uptake of ozone (Gerrity et al. 1988). Increasing ventilation rates decrease absorption by the upper and lower airways, enhancing ozone penetration to the deep lung (Hu et al. 1992a; Hu et al. 1992b). Bush et al. (1996) reported that, on average, women absorb ozone somewhat higher in the respiratory tract than men (lower penetration volume). However, due to women's smaller dead space volume, if penetration volume is normalized to dead space volume, the absorption distribution throughout the upper airways of men and women is similar. The authors hypothesized that differences between ozone dosimetry of men and women could be correlated with the difference in anatomic dead space.

9.2.3 Dosimetry in Children

There is little literature that has investigated ozone dosimetry in children or compared children to adults. Particular attention has recently been focused on assessing the adverse effects of ozone exposure in infants and children, particularly because the young may inhale a greater relative dose of ozone as a result of their increased ventilation rate per unit body weight compared to adults. Overton and Graham (1989) were the first to estimate regional and local ozone uptake in the lower respiratory tract of children compared to adults. They constructed a model that was used to estimate ozone dosimetry to the lower respiratory tract from birth to adulthood. The model was based on several data sets on age-dependent airway dimensions and volumes, and a model of the adult acinus. The results indicated that the lower respiratory tract distribution of absorbed ozone and the ozone dose to the centriacinar tissue are not particularly sensitive to age during quiet breathing. During maximal exercise, lower respiratory tract ozone uptake increased regardless of age, although regional percent uptakes were more dependent on age than during quiet breathing. The results also showed that regardless of age and manner of breathing, the largest tissue does of ozone was predicted to occur in the centriacinar region, in agreement with animal morphology studies (see Section 9.4).

Kleinman (1991) performed an analysis based on the concept of internal thoracic dose. This represents the dose of inhaled pollutant reaching and affecting target sites in the lower respiratory tract, based on a mathematical model derived in part from theory and in part from human and animal exposure data. As a function of age, the results of the model suggest that children under the age of 6 years receive greater doses to respiratory tract tissues than older children or adults under equivalent exposure conditions. Table 9.1 below shows internal thoracic dose of ozone per kilogram body mass for infants through adults during several activities.

Table 9-1: Internal Thoracic Dose of Ozone (µg ozone/kg body mass)

Age (yrs)	Sleep	Awake (Rest)	Light Exercise	Moderate Exercise	Heavy Exercise
0-1	1.26	1.47			
1-5	1.01	1.24	2.80	5.57	8.24
6-17	0.63	0.95	2.13	4.35	6.34
18 +	0.59	0.82	1.84	3.84	5.64

Derived from Kleinman (1991)

Physiologically based pharmacokinetic (PBPK) modeling estimates show that the regional extraction of ozone is relatively insensitive with age, but the extraction per unit surface area is two- to eightfold higher in infants (≤1 yr of age) compared to adults (Sarangapani et al., 2003). Extraction per unit surface area differences between adults and infants were greatest for the pulmonary region, suggesting that up to eight times the amount of ozone reaches and reacts with target regions of the deep lung in infants compared to adults. Additionally, lung development occurs over the entire perinatal period. Thus, exposure effects can have significant consequences whether they occur during the pre- or postnatal period and can result in long-term effects persisting into adult life.

9.2.4 Animal Studies and Animal to Human Extrapolation

Several experimental and theoretical dosimetry studies have estimated the amount or rate of ozone absorbed by target sites within the respiratory tract. An understanding of the dosimetry of ozone can assist in extrapolation of animal data to estimate human responses. Mathematical models have incorporated species differences in airway anatomy, regional airway differences in ozone dose, and physicochemical interactions within the liquid lining layer of the upper and lower respiratory tracts (Miller et al. 1985; Overton et al. 1987). These models support experimental animal studies that suggest that the primary site of lung damage due to ozone inhalation is in the centriacinar region. Experimental dosimetry studies with ^{18}O-labeled ozone show that exercising humans had four- to five-fold greater ^{18}O concentration in their BAL fluid constituents than rats exposed at rest to an identical ozone concentration (Hatch et al. 1994). While this finding supports the conclusion that experimental rodent species are more resistant than humans to ozone-induced injury at a given dose, differences in exertion level show that ventilatory parameters, such as tidal volume (V_T) and respiratory frequency (f_R) are also important determinants of ozone dose to target tissues. Nevertheless, theoretical models have predicted greater sensitivity of humans compared to rodents, in that a given exposure concentration of ozone may result in a local dose to the lower lung of rats roughly half that predicted for humans (Cheek et al. 1994; Overton et al. 1987; Gerrity et al. 1988). This comparative dosimetry is consistent with greater effects of ozone on lung function in humans than in animals (Costa et al. 1989; Overton et al. 1987). While knowledge of dosimetry has allowed

quantitative animal-to-human extrapolation for effective ozone doses, species sensitivity issues, such as antioxidant status, metabolic rates, and repair/defense mechanisms, are also important determinants of effective ozone dose and are not as well defined.

9.2.5 Effective Dose Concept

The degree of response to ozone is related to three factors: 1) the ozone exposure concentration, 2) V_E, the ventilation rate, and 3) T, the duration of exposure. The consensus of available research is that ozone concentration is the most important of the three factors (Adams 2003a; Adams et al. 1981; Folinsbee et al. 1978).

Adams (2003a) provides an illustration of the significance of ozone concentration, compared to V_E and V_T. The subjects in this study completed 6.6-hour exposures to 0.08 ppm ozone (protocol described in Section 9.6.3.2), and 2-hour exposures to 0.30 ppm with alternating 15 min periods of rest and exercise. Although the inhaled ozone dose in the 2-hour protocol was only 1.44 times greater than that for the 6.6-hour protocol, the decrements in FEV1 averaged 3.51% following the 6.6-hour protocol (0.08 ppm), and 12.36% following the 2-hour protocol (0.30 ppm).

The importance of the dose-rate is evidenced by the results of Hazucha et al. (1992) who used an 8-hour exposure protocol with two different ozone concentration profiles: a constant ozone concentration of 0.12 ppm, and a variable concentration profile (linear increase from 0 to 0.24 ppm over four hours, followed by linear decrease from 0.24 to 0 ppm over 4 hours). The total inhaled effective dose of ozone was equivalent for the two exposures (difference $<$ 1%). Exposure to the constant ozone concentration induced a group mean decrement in FEV1 of approximately 5% by the fifth hour of exposure, which did not change over the remainder of the exposure, indicating a response plateau, consistent with Horstman et al. (1990). In contrast, during the first three hours of the variable concentration protocol response was minimal, followed by a mean decrease in FEV1 over hours 4 through 6. The FEV1 decrement peaked at approximately 10% followed by improvement during the last two hours of the exposure. By the end of the variable concentration exposure experiment the FEV1 decrement was nearly identical to that following the constant concentration exposure.

Adams (2003a) also compared responses of healthy young adults to two ozone concentration profiles: (1) a constant ozone concentration of 0.08 ppm, and (2) a triangular ozone profile where the ozone concentration increased from 0.03 ppm to 0.15 ppm over four hours, and then decreased to 0.03 over the next 2.6 hours (mean ozone concentration = 0.08 ppm). The total inhaled dose of ozone was equivalent for both protocols. The group mean decrement in FEV1 was smaller, at least partly due to the lower ozone concentration, compared to Hazucha et al. (1992), the maximal decrement occurred at the time of the peak ozone concentration with the triangular profile, but after six hours in the constant concentration exposure.

The results of Hazucha et al. (1992) and Adams (2003) illustrate that the FEV1 response is dependent on the dose rate as well as the cumulative dose of ozone inhaled, and point to the need to consider multiple exposure scenarios when

evaluating the health effects of inhaled ozone. Further, they illustrate that a shorter exposure to a higher concentration of ozone, even one that delivers a smaller total inhaled dose, can lead to larger effects than a longer, lower concentration exposure that delivers a larger total dose.

Normal, healthy people exposed to ozone concentrations ≥ 0.12 ppm (the federal one-hour standard) typically develop significant, transient reversible decrements in pulmonary function if V_E or T are increased sufficiently.

9.3 Mechanisms of Ozone Toxicity

9.3.1 Introduction

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

Several lines of evidence point to involvement of more than one biological mechanism in mediating responses to ozone exposure. For example, research has shown that the onset of increased airway resistance (R_{aw}) with ozone exposure is rapid onset (Beckett et al. 1985) compared with the gradual onset of decrements in forced expiratory endpoints (Kulle et al. 1985). There is also evidence that while pulmonary function is substantially recovered by 12 to 20 hours after ozone exposure (Foster et al. 2000; Hiltermann et al. 1995) after ozone exposure, methacholine responsiveness remains increased. Folinsbee and Hazucha (2000) reported that young adult females exposed for 75 min to 0.35 ppm ozone still demonstrated reduced pulmonary function and increased airway hyperresponsiveness at 18-hour post exposure, although all endpoints had returned to baseline by 42 hour after ozone exposure.

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

There is also evidence for a relatively weak association between the degree of nonspecific airway responsiveness and individual-specific symptomatic and lung function responses to ozone (Aris et al. 1995). McDonnell et al. (1987) and

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

The available literature suggests that airway inflammation peaks several hours after exposure ends, at a time when pulmonary function decrements and symptoms have largely resolved (Foster et al. 2000; Hiltermann et al. 1995; Schelegle et al. 1991). There is also evidence for a relatively weak association between degree of nonspecific airway responsiveness and individual-specific symptomatic and lung function responses to ozone (Aris et al. 1995). Several studies have reported that changes in pulmonary function and symptoms are not significantly associated with airway inflammation (Balmes et al. 1996; Schelegle et al. 1991; Frampton et al. 1997; Torres et al. 1997; Jorres et al. 2000; Holz et al. 1999), substantiating that multiple mechanisms are involved in mediating the various responses observed in response to ozone exposure.

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

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

9.3.2 Neural Mechanisms

9.3.2.1 Vagal

Parasympathetic innervation of the lungs is via the vagus nerves. These are large neurons that contain several different types of cholinergic nerve fibers whose primary mediator is the neurotransmitter acetylcholine. Stimulation of vagal fibers that innervate airway smooth muscle fibers results in bronchoconstriction and increased airway resistance; activity of the parasympathetic nervous system is the

principal determinant of airway tone. Studies by Beckett et al. (1985) and Adams (1986) support the involvement of a vagal reflex mechanism for ozone-induced bronchoconstriction. Studies in dogs by Gertner et al. (1983a,b) also showed that ozone-induced changes in airway resistance were initially mediated through the parasympathetic system, while later phase responses were partially mediated by histamine.

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

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

9.3.2.2 C-Fibers

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

1998; Schelegle et al. 1993; Coleridge et al. 1993) point to this mechanism as mediating involuntary inhibition of full inspiration, which leads to reduction in forced vital capacity (FVC), and a concomitant decrease in maximal expiratory flow rates. The study by Passannante et al. (1998) also supports the notion that bronchial C-fibers contribute to ozone-induced symptoms of respiratory discomfort and irritation.

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

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

9.3.2.3 Rapidly Adapting Stretch Receptors

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

9.3.2.4 Summary

Available data suggest that ozone stimulation of vagal afferents, including C-fibers and rapidly adapting receptors, resulting in vagal reflexes is the principal mechanism for responses measured during and shortly after ozone exposure. These responses include reduced pulmonary function, increased airway

resistance, increased frequency of respiration (f_R), reduced tidal volume (V_T), and increased symptoms of respiratory irritation.

9.3.3 Biochemical and Inflammatory Mechanisms

9.3.3.1 Introduction

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

9.3.3.2 Mechanisms of Inflammation – Human Studies

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

Ozone does not penetrate cell membranes, although it appears to initiate a cascade mechanism that begins with reaction with unsaturated fatty acids at the air-tissue barrier interface. There is considerable evidence that ozone exposure results in airway inflammation of the lung tissues (see Sections 9.6.3.4; Tables 9-7, 9-8, 9-10). Given the chemical reactivity of ozone, it is unlikely that it penetrates far into tissues, or that it can pass unreacted into cells, much less penetrate into lung tissue (Pryor 1992). It is more likely that ozone reacts with the fluid lining the respiratory tract (epithelial lining fluid, or ELF) or in areas where the ELF is thin or absent with the epithelial cell membranes. Further, it is likely that ozone primarily reacts with lipids. The fluid lining the respiratory tract ELF and the epithelial cell membranes are constituted of about 90% lipid, with a high concentration of unsaturated fatty acids. Ozonation of lipids results in small, diffusible products that are structurally similar to known lipid-derived signal transduction species. If

ozonation occurs in an area with significant water content, such as the lungs, ozonation produces aldehydes, hydroperoxides and small amounts of the Criegee ozonides (Pryor et al. 1995; Frampton et al. 1999; Leikauf et al. 1993). These ozonation products stimulate airway epithelial cells to respond with release of a variety of pro-inflammatory lipid mediators, for example eicosanoids (Schelegle et al. 1989; Leikauf et al. 1995), platelet activating factor (PAF), reactive oxygen species, and cytokines (Pryor et al. 1995).

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

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

There is evidence that some of the mediators released through this cascade mechanism contribute to the immediate responses to ozone exposure, as well as to late-phase responses. For example, prostaglandins and cyclooxygenase products of arachidonic acid appear to play a role in pulmonary function responses to ozone exposure (Coleridge et al. 1976; Coleridge et al. 1978; Schelegle et al. 1987; Eschenbacher et al. 1989; Ying et al. 1990) in the short-term. Koren et al. (1991) reported a positive correlation between ozone-induced pulmonary function decrements and the level of prostaglandin E_2 in bronchoalveolar lavage fluid collected within 1-hour of the end of exposure in human subjects who varied greatly in ozone responsiveness. Hazucha et al. (1996) concluded that the changes in inflammatory markers including PGE_2, thromboxane B_2 (TBX_2), IL-6 and percentage of neutrophils in BALF one-hour following ozone exposure were not significantly related to the magnitude of responses in forced vital capacity (FVC), forced expiratory volume in one second (FEV1) or specific airway resistance (SR_{AW}) in subjects who were pretreated with placebo or the cyclooxygenase inhibitor ibuprofen.

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

9.3.3.3 Mechanisms of Inflammation - Animal Studies

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

9.3.3.4 Genetic Factors in Ozone Sensitivity of Human Subpopulations

Recent studies suggest that wide genetic variability among humans likely contributes to individual sensitivity to inhaled oxidants such as ozone, and also may explain the wide variability in responsiveness in the population as a whole. A number of recent studies have shown that the variation in response to ozone exposure can be genetically influenced.

The inflammatory response to ozone is associated with induction of a number of enzymes, including nicotinamide adenine dinucleotide phosphate

(NAD(P)):quinine oxidoreductase (NQO1 or DT-diaphorase) and glutathione-S-transferases (GSTs). Polymorphisms in the genes coding for these enzymes can influence responses to or protection from epithelial oxidative damage to the airway epithelium. This is thought to play a role in the inter-individual variability in the responsiveness to ozone. NQO1, which plays a detoxifying role, catalyzes direct conversion of quinones to hydroquinones without generation of semiquinones. This limits redox cycling of these compounds and the associated oxidative stress. This is important because hydroquinones are a target molecule for ozone, and because they can be converted to semiquinones and hydroxyl radicals, which cause subsequent cellular damage. GSTs and glutathione (GSH) peroxidase protect cells against the toxic effects of reactive oxygen species generated by ozone, by influencing both the rate of GSH conjugation of hydroquinones and scavenging hydroxyl radicals, respectively. People carrying the *NQO1* wild type (*NQO1wt*) genotype, but lacking class μ-1 GST (*GSTM1null*), are less able to conjugate hydroquinones, a condition which favors responsiveness to ozone. Approximately 30% of Caucasian subjects carry both genotypes.

Post-ride lung function parameters showed significant decrements mainly accounted for by a subgroup of subjects (n = 8) bearing both *NQO1wt* and *GSTM1null* genotypes (Bergamaschi et al., 2001) in subjects who completed a two hour bike ride while exposed to ambient air containing ozone concentrations between 0.032 and 0.103 ppm. Prior to the ride FEV1 in this subgroup was decreased compared to baseline measured several weeks before the test ride. The post-ride decrement in FEV1 for the subgroup noted above was greater and had lower variability than observed in subjects bearing other combinations of genotypes. The *NQO1wt* plus *GSTM1null* subgroup also had greater serum concentrations of the lung Clara cell secretory protein (CCSP), a biomarker for increased lung permeability, and greater formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG). These observations provide indirect evidence for ozone induced oxidative stress and increased formation of hydroxyl radicals.

In a related study, differences in inflammatory biomarkers were measured following chamber exposure to 0.1 ppm ozone for 2 hour while performing moderate intermittent exercise (Corradi et al., 2002). Individuals bearing the *NQO1wt* and *GSTM1null* genotypes had increased levels of 8-isoprostane, thiobarbituric reactive substances, and leukotriene B-4 in exhaled breath condensate compared to subjects with other genotype combinations, suggesting that having this combination of genotypes increased lung inflammation and oxidative stress. Formation of 8-OHdG from blood of this sensitive subgroup of individuals was also significantly elevated.

Romieu et al., (2004) investigated the effect of genetic polymorphism of in *GSTM1* and antioxidant supplementation in 158 asthmatic children living in an area of Mexico City with high ozone concentrations. The *GSTMnull* genotype was present in 39% of the children, although more of the children with moderate and severe asthma had the *GSTMnull* genotype (p = 0.03). In the placebo group, which did not receive supplementation with vitamins C and E, ozone levels were significantly and inversely associated with forced expiratory flow (FEF25-75) in children with

the *GSTMnull* genotype (2.9% fall in FEF25-75 per 50 ppb of ozone (95% CI –5.2 to –0.6), p = 0.01). No significant decrement was observed in *GSTM* positive children. Children receiving antioxidant supplementation had no statistically significant ozone related decrement in FEF25-75, regardless of genotype. . A related epidemiologic study by these researchers found that carriage of the inactive Ser allele for *NQO1* with the *GSTM1null* genotype conferred a reduced risk of asthma in Mexico City children exposed to high levels of both diesel exhaust and ozone (David et al., 2003). Although often detoxifying quinines, NQO1 can catalyze the reduction of some quinones found in diesel exhaust to hydroquinones, which are more reactive and which can autooxidize to reactive oxygen species. The assumption is that reduced quinone activation in *NQO1* (Pro 187Ser) individuals reduces activation of diesel exhaust particulates and decreases interaction with ozone causing resulting in oxidative stress in the lung. In a study of nasal biopsies, *GSTM1null* individuals had significant increases in the superoxide dismutase activity in their biopsy tissue with ozone exposure, possibly as a result of accumulation of products of lipid peroxidation (Otto-Knapp et al., 2003).

Genetic markers such as tumor necrosis factor-alpha (TNF-α) and other polymorphisms may also identify individuals who are at higher risk of change in lung function consequent to ozone inhalation. TNF-α is a proinflammatory cytokine that has a central role in inducing neutrophil chemoattractants such as interleukin-8 and intercellular adhesion molecule-1 (ICAM-1). In individuals exposed to ozone (0.20 to 0.40 ppm) for up to 4 hour with intermittent exercise, the TNF–308 locus was found to be associated with change in lung function with ozone challenge (Yang et al., 2005). The mean change in FEV1 was –3% in TNF-308G/A or A/A individuals, compared with –9% in G/G individuals (p = 0.024), suggesting TNF-α as a genetic factor contributing to susceptibility to ozone exposure in humans.

Chen et al. (2004) investigated whether 1-hour exposure to 0.2 ppm ozone (with exercise) enhanced the late airway inflammatory response, as well as the early bronchoconstrictor response, to inhaled house dust mite allergen in sensitized asthmatic subjects. No significant differences in cellular and/or biochemical markers were found between the ozone and air exposures. Although genetic factors contributing to individual ozone sensitivity was not explored in this study, the results suggest that a subgroup of asthmatics may acquire increased sensitivity to aeroallergens after ozone exposure. Studies with larger group sizes and better genetic characterization would assist in determining the mechanism(s) responsible for the increased sensitivity to aeroallergen after ozone exposure.

Although there is little literature on genetic contributions to ozone sensitivity, several recent studies support the role of genetic factors in variability in inter-individual sensitivity to ozone exposure in humans. Polymorphisms in the antioxidant enzymes NQO1 and GSTs, and in proinflammatory cytokine TNF-α appear to have a biological role in the pulmonary response to inhaled ozone and explain much of the inter-individual variability.

9.3.4 Mutagenic and Carcinogenic Potential of Ozone

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

9.3.5 Summary

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

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

9.4 Morphological Effects

9.4.1 Introduction

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

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

which themselves show morphological changes. The loss of ciliated epithelium is likely to affect mucocillary clearance, and the hyperplasia of nonciliated bronchiolar (Clara) cells, which are rich in mixed function oxidases, is likely to impact lung xenobiotic metabolism. In the centriacinar region, type 1 cells (the most sensitive), across which gas-exchange occurs, are destroyed and replaced by the thicker type 2 cells, which thickens the interaveolar septa. Inflammatory changes (e.g., increased PMNs and AMs and edema) also occur in this region.

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

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

There are many investigations of the effects of ozone on lung cellular organization and architecture, and several include correlated measurements of pulmonary function or lung biochemistry. The presentation of this morphology section will begin with short-term exposure effects (<1 week). The subsequent discussion of long-term effects (>1 week) is based on the fact that repair has already been initiated at this time; the chronic studies that included evaluation of various

durations of exposures are presented totally in this subsection to illustrate the effects of exposure duration. The impact of differences in interexposure period is also addressed.

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

9.4.2 Morphological Effects Associated with Short-Term Exposure

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

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

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

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

The effects of exposure duration are complex and are likely responsible for the similar patterns of biochemical responses. Ciliated and alveolar type 1 cells became necrotic and were sloughed as soon as 2 to 4 hour into an exposure of rats to around 980 μg/m^3 (0.5 ppm) (Stephens et al. 1974; Stephens et al. 1974b), with the damage no greater after 48-hour of exposure (Stephens et al., 1974a). Repair of the damage, as indicated by increased DNA synthesis by nonciliated bronchiolar (Clara) and alveolar type 2 cells, begins by 18 to 24 in both rodents and monkeys (Evans et al. 1976; Stephens et al., 1974a; Castleman et al. 1980; Castleman et al. 1980). While cell damage, including necrosis, continued throughout short (Castleman et al., 1980) and long (Barr et al. 1990; Schelegle et al. 2003) exposures in rodents and primates, the morphological lesion was fully developed after about 3 days of continuous exposure. After that time, the rate of damage was exceeded by the rate of repair and the animal entered the "reparative-adaptive" period. Adaptation may be due in part to the greater resistance to ozone of the newly formed nonciliated bronchiolar (Clara) cells and alveolar type 2 cells. For example, Evans et al. (1976) observed that alveolar type 2 cell hyperplasia in rats peaked around Day 2 of an exposure to 686 to 1,960 μg/m^3 (0.35 to 1.0 ppm) ozone and decreased to near control by Day 4, although exposure continued through Day 8. In the centriacinar region of nonhuman primates, alveolar type 1 cell necrosis was maximal after 4-hour exposure to 1,568 μg/m^3 (0.8 ppm) ozone, but continued at a reduced rate

throughout a 50-hour exposure (Castleman et al., 1980). In that study, DNA labeling was maximal at the end of the 50-hour exposure. The same was true for both midlevel bronchi and centriacinar bronchioles and alveolar ducts in adult rats (Schelegle et al. 2003). In the tracheas of monkeys, ciliated cell lesions consequent to exposure to 1,254 μg/m^3 (0.64 ppm) ozone were more severe after 3 days of exposure; by 7 days of exposure, the epithelium had returned towards normal (Wilson et al. 1984). As another indicator of the complexity of the response, Schwartz et al. (1976) found that there was very little difference in the intensity of lung lesions in the terminal bronchioles or centriacinar regions of rats exposed for 8-hour/day versus 24 hour/day to 392 to 1,568 μg/m^3 (0.2 to 0.8 ppm) for 7 days.

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

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

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

responses in mice (Paquette et al. 1996), while elevation in taurine levels served to protect against acute injury and reduced inflammation (Schuller-Levis et al. 1995; Gordon et al. 1998).

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

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

Table 9-2: Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.) Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
250, 1000, 1500	0.12, 0.5, 0.75	Continuous for 1-7 d	Rat, M (WSTR) 8 wks	LM: Increased AMs in CAR and parenchyma. CAR increase persisted 5 days PE. TEM and SEM: BAL AMs had microvilli and blebs in addition to ruffles characteristic of AMs from controls.	Dormans et al. 1990
294, 980	0.15, 0.5	Continuous for 3 or 7 d	Rat, M (WSTR) 8 wks	Elastase-induced emphysema and saline control rats. LM histopathology and morphometry for alveolar size. Incidence and severity of CAR LM lesions was the same in elastase- or saline-treated rats exposed to ozone. No change in alveolar size due to ozone.	Dormans et al. 1989
392,784	0.2, 0.4	3, 7, 28, 56 d	Rat, M, (Wistar), 7wk	All three species same centriacinar inflammatory response and cell injury up to 7 days.-all changes concentration dependant- mouse bronchiolar hyperplasia at 3 days	Dormans et al. 1999
392, 686, 980, 1568	0.2, 0.35, 0.5, 0.8	8-hour/d, 7 d	Monkey (rhesus and bonnet)	Respiratory bronchiolitis 392 µg/m^3. Increased number of AMs. Bronchiolar epithelium both hyperplastic and hypertrophic. Increase in Type 2 cells. Random foci of short, blunt cilia or absence of cilia. (LM, EM)	Dungworth et al. 1975
980	0.5	8-hour	Rat, Male, (F344, WSTR, SD), 14 mon	Strain differences in injury based on lavage LDH, protein, fibronectin, PGE2 and IL6 levels and increased PMN and macrophages. Wistar>SD or F344. In vitro epithelial response is reflective of lavage	Dye et al. 1999
686, 980, 1372, 1470, 1960	0.35, 0.5, 0.7, 0.75, 1.00	1, 2, 4, 5, 6, or 8 d	Rat (SD)	All tritiated thymidine labeled cells increased and then decreased to near control levels by D 4. Type 2 cells showed largest changes in labeling index. (LM, autoradiography)	Evans et al. 1976
784 1568	0.4 0.8	3 hour	Mice, F 8-16 wks	Mice with inducible nitric oxide synthase knocked out had reduced inflammation and injury, based on lavage protein and cells, and lower macrophage number, peroxynitrite and PGE2 product.	Fakhrzadeh et al. 2002

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
3920	2	3 hour	Rat, F, (SD)	Taurine pretreatement (50% in drinking H_20) reduced mild inflammation and injury shortly (6 hour) post exposure and reduced disruption of tight junctions in epithelium	Gordon et al. 1998
235, 1568, 2940	0.12, 0.8, 1.5	6 hour	Rat, M (F344) 12-18 wks	LM histopathology of lungs and LM morphometry of lavaged AMs: No LM histologic effect detected at 0.12 ppm. No LM histologic effect at 0.8 and 1.5 ppm immediately or 3 hour PE. At later PE times, there was mild, patchy CAR bronchiolitis and alveolitis. Increase in AMs and PMNs from 18-66 hour PE. Progressive thickening of TB walls and CAR AD septa at 18, 42, and 66 hour PE.	Hotchkiss et al. 1989a
235, 1568, 2940	0.12, 0.8, 1.5	6 hour	Rat, M (F344) 12-18 wks	LM histopathology of CAR is the same; new morphometry of PMNs in CAR and nasal mucosa. Emphasis on PMNs in nasal mucosa and nasal lavage compared with PMNs in CAR tissues and BAL at exposure end and at 3-66 hour PE.	Hotchkiss et al. 1989b
1568	0.8	6 hour/d, 3 or 7 d	Rat	Nasal nonciliated cuboidal epithelium studied. Epithelial hyperplasia and secretory metaplasia in rats exposed for 7 days and examined 18-hour PE and exposed for 3 days and examined 4 days PE, but not in rats exposed for 3 days and examined 18-hour PE.	Hotchkiss et al. 1991
980 - 5880	0.5 -3.0	3 hour	Rat, M, (SD)	Experimental hyperthyroidism (thyroxine injection, 0.1-1.0 mg/kg) increased acute injury based on elevated lavage protein, LDH, albumin, PMN levels. Not completely dependant on high metabolic rate	Huffman et al. 2001
1568	0.0, 0.8	8-hour	Primate, M, (Rhesus), 3yrs	Inhibition of PMN migration with specific anti-CD18 antibody increased the abundance of necrotic cells in distal airways. Promoting migration of PMN into airways by lavage of C5a promoted removal of necrotic cells from injured sites	Hyde et al. 1999

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980	0.5	6 hour	Primate (Rhesus)	*In vitro* exposure of bronchial epithelial cells at air/liquid interface alters apical and basolateral expression of β-1 integrins without increased mRNA production	Jabbour et al. 1998
1960	1.0	90 min	Rat, (SD)	Using isolated perfused lung, showed that distribution of airway epithelial injury, based on exclusion of cell impermeant dye, is altered by breathing pattern and that injury is high in many sites besides the central acinus	Joad et al. 2000
235, 529, 1568	0.12, 0.27, 0.8	6 hour/d, 7 d	Rat (F344)	Effects in nasal epithelium only at 1,568 μg/m_3. At Days 3 and 7, increased DNA replication in nonciliated cuboidal/transitional epithelium. At Day 3 only, increased DNA replication in respiratory and olfactory epithelia. Seven days PE, decreased DNA replication in squamous epithelium. (LM, EM)	Johnson et al. 1990
2940	1.5	48-hour	Mice	Mice without the gene for extracellular superoxide dismutase had increases in inflammation and injury, based on lavage IL6 and histopathology, without differences in lavage protein, cells, or LDH	Jonsson et al. 2002
490,3920	0.25, 2.0	3 hour	Mice, M, (C3H/HeJ), 8-9wk	HNE based protein adduct formation, is produced in lung cells obtained by lavage	Kirichenko et al. 1996

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	4, 6, 8, 24 hour	Rat, M (SD) 63 days old	TEM morphometry. Volume of necrotic cells per area basal lamina (Vs) in the TB larger than controls at the end of 4- and 24-hour exposure, but not at other exposure or PE times. With increasing exposure time, there was a shift from necrotic cells on the basal lamina to necrotic cells free in the TB lumen. The Vs of necrotic alveolar cells was increased after 4, 6, and 24 hour of exposure. Viable undifferentiated cell Vs in TBs was increased after 6-hour exposure followed by 18-hour PE, 8-hour exposure followed by 16 hour PE, and after a 24-hour exposure. In alveoli, viable Type 1 cell Vs was increased after a 24-hour exposure. Total connective tissue Vs changes only increased in TBs after 8-hour exposure followed by 4 hour PE and in alveoli at the end of 8-hour exposure. The Vs of migratory cells in TB interstitium was only increased 4 hour after a 6-hour exposure. In alveoli, the Vs of capillaries was increased after 8-hour exposure.	Pino et al. 1992
784-1960	0.4-1.0	2 hour	Primate, M, (Rhesus), 3 yr	Local dose, based on excess $^{18}0_2$, varied within airway tree with respiratory bronchiole highest and parenchyma lowest. Degree of epithelial injury and inflammatory cell infiltration closely correlated with local dose and loss of reduced glutathione in a concentration dependant manner	Plopper et al. 1998
490, 980, 1960	0.0, 0.25, 0.5, 1.0	20-90 min	Rat, M, (SD)	Exposure of isolated perfused lungs produced acute epithelial injury, based on permeability of cell-impermeant dye (Ethidium homodimer-1): The greatest injury was in minor daughter side branches of proximal bronchi and the least injury in terminal bronchioles	Postlethwait et al. 2000
1568	0.8	3 hour	Rat, M, (SD)	Elevation of anti-inflammatory cytokine IL-10 prior to exposure reduces lung inflammation based on lavage protein, albumin, PMN and fibronectin content	Reinhart et al. 1999

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1960	1.0	8-hour	Rat, (WSTR)	Elimination of lung C-fibers by neonatal capsaicin treatment produced marked increases in the degree and distribution of lung injury and inflammation, including coagulate necrosis in all airway epithelium	Sterner-Kock et al. 1996
1960	1.0	8-hour	Rat, Primate (rhesus), Ferret	Compared to rats, the acute epithelial injury and inflammation in lungs of ferrets is much greater and very comparable to the response in rhesus monkeys	Sterner-Kock et al. 2000
686, 1568	0.35, 0.8	72 hour	Rat (SD)	Typical ozone effects. Older rats (444-days old) had smaller lung volumes after 1,568 µg/m^3, but young rats (60-days old) were not affected. No impact of age on TB responses or volume fraction of parenchyma. Younger rats had more of an increase in AMs, but older rats had a greater accumulation of mucus. Younger rats had a larger volume fraction of centriacinar lesions. Older rats had a decrease in body weight; young rats did not.	Stiles and Tyler 1988
784	0.4	Continuous up to 14 days	Rat, M (WSTR) 5 wks	SEM morphometry, immunocytochemistry. Number of Clara cells/µm2 increased at 14 days, but not earlier. The length of the Clara cell apical projection was increased after 6 hour, decreased at 1 day, and not different at other periods. Cytochrome P-450 was localized to agranular endoplasmic reticulum of Clara cells.	Suzuki et al. 1992
1568, 784	0.8, 0.4	Continuous for 7 days	Rat, M (WSTR) 8 wks	LM: Clara cell numbers/mm of TB basement membrane unchanged. Cell isolation: Although the number of isolated Clara cells was increased, the percent Clara cells in the isolate was not changed. The percent Type 2 cells in the isolate was increased. No morphologic observations at 0.4 ppm.	VanBree et al. 1989

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

Table 9-2 (cont.): Effects of Ozone on Lung Structure: Short-Term Exposures

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

See Glossary for abbreviations

9.4.3 Morphological Effects From Longer-Term Exposures

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

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

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

Barry and Crapo (1985) studied the effects of ozone on the proximal alveolar region (i.e., centriacinar region) of the rats discussed above (Barry et al. 1988). In the animals exposed to 490 $\mu g/m^3$ (0.25 ppm) ozone from one day of age, the number of Type 1 cells doubled, their mean surface area decreased by 38%, and their mean thickness increased by 24%. The number of alveolar Type 2 epithelial cells increased, and the number of macrophages doubled. Adult animals exposed to 490 $\mu g/m^3$ (0.25 ppm) ozone showed similar patterns of changes and also exhibited a doubling of interstitial macrophages. Adult animals exposed to 235 $\mu g/m^3$ (0.12 ppm) ozone showed smaller, still statistically significant changes in Type 1 cells. In both the terminal bronchioles and proximal alveolar region, no statistically significant age-dependent effects were found. However, as shown by Stephens et al. (1978), rats were not sensitive to ozone until they were at least 20 days old. Others who used image analysis to compare newborn to adult mice

exposed intermittently for 6 weeks to 588 μg/m^3 (0.3 ppm) ozone also found that the neonates were more resistant (Sherwin and Richters 1985). Therefore, the neonates in the Barry et al. (Barry and Crapo 1985; Barry et al. 1988) studies may have received effective exposures only 22 of the 42 days of exposure, whereas the 42 day old "adults" received effective exposures each of the 42 days. As stated by the investigators, this raises the possibility that the neonates may have been more sensitive because they developed as severe a lesion after 22 days of exposure as the "adults" did after 42 days of exposure.

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

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

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

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

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

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

Table 9-3: Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1862	0.95	8-hour/d, 90 d; 7 units of 8-hour/d for 5 d then 9 d air for 89 d total	Rat (SD)	Centriacinar lesion from episodic exposure similar but less severe than in daily exposure group, but more than predicted on C-T basis. (LM, EM)	Barr et al. 1990
1862	0.95	8-hour/d for 90 d	Rat, M (SD) 61 d	LM and TEM morphology. RB: Increased volume of total RB and of RB wall and lumen. RB walls thickened by interstitial inflammation with edema, hyperemia, fibrosis, and hypertrophied smooth muscle and by interstitial mononuclear cells, granulocytes, and plasma cells. Epithelial and vascular basal lamina fused. TB: Smaller internal diameter and smaller luminal volume, but no change in total TB volume or in wall volume. Proximal AD: Most severe cell damage and inflammation at alveolar septal tips (alveolar entrance rings). Epithelium at these tips was frequently necrotic or missing, leaving bare basement membrane. Duct walls thicker due mainly to increased interstitial edema, fibrosis, and cellular infiltrates. Basal lamina thickened, split or duplicated, and had granular deposits. Site of most severe injury shifted progressively distally as new segments of RB were formed.	Barr et al. 1988

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

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

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
118 base, spike rising to 490	0.06 base, spike rising to 0.25	13 hour/d, 7 d/wk base; ramped spike, 9 hour/d, 5 d/wk; 1, 3, 13, and 78 wks	Rat (SD)	Shift from acute inflammatory phase to more chronic character. Changes in TBs: Decreased surface areas of ciliated and Clara cells, and domes of Clara cells reduced after 78 weeks; recovery PE. Changes in proximal alveolar region. Type 1 cells: increased in number at 1 week and remained changed to about the same degree at 3, 13, and 78 weeks; still altered 6 weeks after the 13-week exposure, but not 17 weeks after end of 78-week exposure. Type 2 cells: biphasic response, increased at 1 and 78 weeks; recovery PE. Interstitial volume: cells increased at 1 week only; matrix increased at 1, 13, and 78 weeks. Changes did not return to normal 17 weeks after the 78-week exposure. Increased collagen observed after 78-week exposure; partial recovery 17 weeks PE, but basement membrane still thickened. AMs: Increased at 1 week only. (LM, EM)	Chang et al. 1992
686	0.35	4.5 hour/d, 5 d/wk for 4 wks, 380 mmHg (5,400 m) or sea level P_B	Mouse, M (Swiss-Webster) 32 g	Automated LM morphometry of stainable elastin in alveolar walls. Simulated high altitude (5,400 m) with ozone (SHA-X) or without ozone (SHA-C) resulted in larger lung volumes than sea-level controls (SL-C), but not different from each other. Unlike most studies, sea-level, ozone-exposed mice had the smallest lung volumes. Alveolar wall areas, after adjustment to SL-C lung volumes, were increased only in the SHA-X group. Alveolar wall elastin area, adjusted to the SL-C lung volumes, increased in both high-altitude groups compared to SL-C and also were different from each other with the largest amount of elastin area in the SHA-X group. However, if the elastin areas were not adjusted for differences in lung volumes, there were no differences between the groups.	Damji and Sherwin 1989

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

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

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980	0.5	20 hour/d, 7 d/wk for 52 wks	Rat (F344)	LM histopathology. 6-mo exposure: Inflammation, mononuclear cells, and fibroblasts in AD walls and walls of adjacent CAR alveoli. TB not involved. 12-mo exposure: Similar to 6 mo with possibly a slight increase in AMs, some increased thickening of centriacinar AD and alveolar walls, and a few foci of bronchiolization (CAR remodeling). 12-mo exposure + 6 mo PE: Slight dilation of ADs, minimal inflammatory reaction, slight thickening of AD and CAR alveolar walls, and a few foci of bronchiolization.	Gross and White 1987
294, 588	0.15, 0.3	8-hour/d, 6 or 90 d	Monkey (bonnet)	Effects in nasal epithelium. At 6 or 90 days, 294 mg/m^3, ciliated cell necrosis, shortened cilia, and secretory cell hyperplasia. Inflammation at Day 6. Morphometric changes in epithelium, especially in goblet cells, at Day 90. (LM, EM)	Harkema et al. 1987b
294, 588	0.15, 0.3	8-hour/d, 6 or 90 d	Macaca radiata, F, M 2-6 yrs	TEM, SEM, and LM morphometry. First generation RBs had epithelial hyperplasia, and alveoli opening into these RBs had increased AMs. RB epithelium thickened, but no difference due to either exposure time or concentration. RB interstitium was thickened in all exposed monkeys, but both cellular and acellular compartments were individually thickened only after 90-days exposure to 0.3 ppm. No differences due to age or gender. No evidence of epithelial cell necrosis nor of inflammatory cell infiltration other than the increased AMs.	Harkema et al. 1993
980	0.5	Continuous for 120 days	Mice, F (Swiss) 20-23 g	LM morphometry and histopathology. More tissue, primarily inflammatory cells, at Day 9. Little change from Days 10 to 120. Thickened airway walls with increased collagen. Increased collagen in alveolar walls along the ADs.	Jakab and Bassett 1990
510	0.26	8-hour/d, 5 d/wk, 1-90 d	Mice, M, 6-8 wk	Exposure produced fewer lavagable macrophages, epithelial cells, and PMN and reduced cell proliferation in mast cell deficient mice than in wildtype controls	Kleeberger et al. 2001

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
980, 1568, 2156, 2940	0.5, 0.8, 1.1, 1.5	24 hour/d, 7, 14, 21 d	Rat, M (SD)	Increased collagen synthesis rate at all exposures; correlated with fibrosis (histological) of alveolar duct walls after 14 or 21 days. Effects were concentration dependent; for duration, 7 <14 = 21 days.	Last et al. 1979
784, 1254	0.4, 0.64	8-hour/d, 90 d	Monkey (bonnet) adult	Changes in RBs: (1) thicker walls, narrower lumens; (2) more cuboidal cells, fewer Type 1 cells; (3) thicker interstitium; and (4) more cellular organelles associated with protein synthesis. Many of the changes were not concentration dependent. (LM, EM)	Moffatt et al. 1987
980	0.5	24 hour/d, 3, 50, 90, 180 d	Rat, M (SD)	Inflammatory cell response of centriacinar region peaked at Day 50, but was still elevated at Day 180 and 62-days PE. Total lung volume increased at Day 180 and 62-days PE. Formation of respiratory bronchioles in centriacinar region at Day 180 only partially reversed PE. (LM, EM)	Moore and Schwartz 1981
1882	0.96	8-hour/night, 7 d/wk for 3 or 60 nights	Rat, M (SD) 234-263 g	LM morphometry, histochemistry, autoradiography, and SEM, and TEM morphometry. Neither 3 nor 60 days of exposure altered the cell density of ciliated, serous, basal, brush, migratory, or unidentified cells in tracheal epithelium. 3 days: Damage to cilia and ciliated cells, including necrosis. Thymidine labeling index increased. Serous cell histochemistry unchanged. 60 days: Less evidence of injury than at 3 days, but more damaged ciliated cells than in controls. Complete recovery of the epithelial changes by 42 days PE.	Nikula et al. 1988
1568	0.8	8h/d, 90 days	Rat, M, (SD)	P450 2B activity and protein elevated in distal bronchioles after 90-d exposure. Protein localized to Clara cells in all airway levels	Paige et al. 2000

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1117, 2156	0.57, 1.1	19 hour/d, 11 d	Rat	At Day 12, concentration-dependent inflammation and Type 2 cell hyperplasia. Increased elastolytic/collagenolytic activities. At 60-days PE, increase in total collagen and alveolar ductal fibrosis. (EM)	Pickrell et al. 1987
235, 980, 1960	0.12, 0.25, 0.5	6 hour/d, 5 d/wk 20 mo	Rat, M (F344) 6-8 wks	LM morphometry of CAR remodeling. Thickened tips of alveolar septa lining ADs (alveolar entrance rings) 0.2 mm from TB in rats exposed to 0.12 ppm and to 0.6 mm in rats exposed to 1.0 ppm. At 0.5 and 1.0 ppm increased volume of interstitium and epithelium along alveolar ducts due to epithelial metaplasia. Bronchiolar epithelial hyperplasia. At 1.0 ppm mild fibrotic response (increase in interstitial matrix and cellular interstitium; the latter due to increase in volume in interstitial fibroblasts). More effects in PAR than in terminal bronchioles.	Pinkerton et al. 1995 Chang et al. 1995
1921	0.98	8-hour/d, 7 d/wk for 90 d	Rat, M (SD) 65 d	LM morphometry and SEM of CAR. Remodeling of CAR. Increased thickness of septal edge (tips) of alveoli, which form the walls of ADs (alveolar entrance rings) up to 0.6 mm from TB. Alveolar septa thickened by replacement of Type 1 cells by Type 2 and bronchiolar cells to 0.6 mm from TB.	Pinkerton et al. 1992

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
235, 980, 1960	0.12, 0.5, 1.0	6 hour/d, 5 d/wk for 20 mo	Rat, M (F344) 6-8 wks	LM morphometry and histochemistry of "short-" and "long-path" conducting airways and CAR. Trachea: No changes in epithelial thickness, cell populations or stored glycoconjugate, but a dose-dependent loss of stored glycoconjugate was found. Bronchi: No changes in epithelial thickness or cell populations. Rats exposed to 1.0 ppm had increased stored gylcoconjugates in cranial (short-path) and caudal (long-path) bronchi, but not in central (short-path) bronchi. Bronchioles: TB of rats exposed to 1.0 ppm had thicker epithelium with increased volume fraction (Vv) of nonciliated bronchiolar cells. Vv also increased in caudal (long-path) TB of 0.5-ppm exposed group. Mass ($\mu m^3/\mu m^2$) (Vs) of nonciliated cells increased in caudal (long-path) TBs of all exposed rats, but not in short-path TBs. CAR: Increased Vs of bronchiolar epithelium in former ADs in cranial and caudal CARs of rats exposed to 1.0 ppm and in cranial CAR of rats exposed to 0.5 ppm.	Plopper et al. 1994a
1960	1.0	8-hour, 5 d	Rat, M, (SD), 70 d	Alternation of acute inflammatory response and cellular injury with 2nd to 4th 5-day exposure periods. Hyperplasia and macrophage accumulations increase with each reexposure and recovery period	(Schelegle et al. 2003)
196	0.1	2 hour/d, 5 d/wk for 1 yr	Rabbit, M (NZW) 3-3.5 kg	LM morphology and morphometry of intrapulmonary conducting airways. No difference in number of airways/area or in distribution of airway size. ESCs in the smallest conducting airways (< 0.30 mm) increased at 4, 6, and 12 mo of exposure to ozone and decreased at 6 mo PE. ESCs in the next larger airways (0.31-0.49 mm) only increased after 4 mo of exposure and decreased at 6 mo PE. No effect on airways >0.50 mm.	Schlesinger et al. 1992a
588	0.3	7 hour/d, 5 d/wk, 6 wk	Mouse (Swiss Webster)	Newborns had increase in Type 2 cell area, but no effect on cell number or alveolar wall areas or linear intercepts. Adults had increase in all these parameters. (Image analysis)	Sherwin and Richters 1985

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
235, 490, 980	0.12, 0.25, 0.5	20 hour/wk, 7 d/wk for 2 yrs (Examined at 4, 12, 26, 52, 78, and 104 wks)	Rat, M (F344) 42 days	Rats exposed to 0.5 ppm had smaller BW after 7-weeks exposure. LM histopathology. Nose: At 0.25 ppm, mucous cell respiratory epithelium hyperplasia; no lesions in mainstem or large bronchi. CAR: 0.25 ppm, TB epithelium hyperplastic and hypertrophic; bronchiolarization and airway remodeling. No changes in 0.12-ppm group after 26 weeks of exposure. Peribronchiolar tissue and AD walls thickened by eosinophilic material after 12 weeks at 0.5 ppm and after 26-weeks at 0.25 ppm. Collagen found in these areas using special stains. Increased AMs at 0.25 ppm.	Smiler et al. 1988 Wright et al. 1990 Wright et al. 1989
1254, 1882	0.64, 0.96	8-hour/night, 42 nights	Rat (SD)	Rats 28-days old at start of exposure. At 1,254 µg/m^3, increase in volume of RBs immediately (both ozone levels) and 42 days post-exposure (high ozone level). At 1,882 µg/m^3, increase in lung volume and volumes of parenchyma and alveoli immediately after exposure; latter two also increased PE. (LM, EM)	Tyler et al. 1987
490	0.25	8-hour/d, 18 mo "daily" and "seasonal" (1 mo ozone, 1 mo air repeated for 18 mo)	Monkey, M, (Rhesus) Rat, M, (SD)	Monkey: Both groups: respiratory bronchiolitis, increased number of RBs, alterations in lung growth. (LM, EM). Daily group: Increased number of inflammatory cells (mostly macrophages) in lumen and interstitium. Seasonal group: Increase in total lung collagen, chest wall compliance (suggestive of delay in lung maturation), and inspiratory capacity. Rat: Daily and seasonal groups had equivalent increase in number of bronchiole/alveolar duct junctions, as total number and number/unit area.	Tyler et al. 1988

Table 9-3 (cont.): Effects of Ozone on Lung Structure: Long-Term Exposures

Concentration (ug/m$^{3)}$	Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1254	0.64	8-hour/d for 12 mo	Macaca faciscularis, M, 6-7 mo	LM morphometry of CAR airway remodeling. Both Vv and V of RBs, and their walls and lumens, increased at end of exposure and 6 mo PE. RB internal diameters were smaller at exposure end, but not at 6 mo PE. Vv free cells, mostly AMs, increased only at exposure end. No differences in BW or fixed lung volumes.	Tyler et al. 1991
980	0.5	24 hour/d, 7, 21, or 35 d	Mouse (Swiss Webster)	Most severe damage at TB/alveolar duct junction. TBs had focal hyperplastic nodules of nonciliated cells. Proximal alveoli had accumulations of AMs and thickening of IAS by mononuclear cells at Day 7. At Day 35, changes much less evident, but numbers of Type 2 cells increased.	Zitnik et al. 1978
392 980	0.2 0.5	7 d 8-hour/d	Rat	Focal areas of missing or damaged cilia in trachea and bronchi. TB nonciliated (Clara) cells were shorter and had increased surface granularity and less smooth endoplasmic reticulum. Ciliated cells of TB had fewer cilia and focal blebs. Centriacinus had clusters of AMs and PMNs. Type 1 cells swollen and fragmented and type 2 cells frequently in pairs or clusters. Proximal IAS were minimally thickened. Lesions in 0.2 ppm rats were mild.	Schwartz et al., 1976

See Glossary for abbreviations

9.4.4 Interexposure Interval

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

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

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

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

day exposure period, bronchiolarization of the central acinus persisted. By the end of the fourth 5-day exposure, the acute inflammatory response was attenuated, macrophage/ monocyte counts were increased, epithelial hyperplasia was elevated, remodeling of the centriacinar region has increased and cell proliferation was decreased. The remodeling and macrophage infiltration were even further elevated by the end of the 9-day recovery period.

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

9.5 Pre- and Post-Natal Effects of Ozone

9.5.1 Introduction

Particular attention has recently been focused on assessing the adverse effects of ozone exposure in infants and children, particularly because the young may inhale a greater relative dose of ozone as a result of their increased ventilation rate per unit body weight compared to adults. Physiologically based pharmacokinetic (PBPK) modeling estimates show that the regional extraction of ozone is relatively insensitive with age, but the extraction per unit surface area is two- to eightfold higher in infants (≤1 yr of age) compared to adults (Sarangapani et al., 2003). Extraction per unit surface area differences between adults and infants were greatest for the pulmonary region, suggesting that up to eight times the amount of ozone reaches and reacts with target regions of the deep lung in infants compared to adults. Additionally, lung development occurs over the entire perinatal period. Thus, exposure effects can have significant consequences whether they occur during the pre- or postnatal period and can result in long-term effects persisting into adult life.

9.5.2 Effects of Prenatal Exposure to Ozone

Maternal exposure of rats to 1.0 ppm ozone continuously during mid- or late gestation (Days 9-12 or 17-20) resulted in reduced neonatal growth rates, with late gestational exposure resulting in retardation of early reflex development and in open field behavior (Kavlock et al., 1980). However, maternal toxicity was not discussed. Lower maternal exposures of mice or rats to ozone during gestation had little or no impact on developmental measures of the offspring. Intermittent and continuous maternal exposure to ozone concentrations between 0.2 and 0.8 ppm during gestation produced occasional transient decreases in maternal body weight and food and water consumption, but physical developmental effects and major neurobehavioral developmental effects of the pups were not apparent (Bignami et al., 1994; Petruzzi et al., 1995; Kavlock et al., 1979; Sorace et al., 2001). Recently, a wide-ranging battery of neurobehavioral tests was conducted in mice exposed continuously to 0.3 or 0.6 ppm ozone prenatally up to day 17 of gestation (Sorace et al., 2001). Results from the low concentration prenatal exposure condition (0.3 ppm) suggested long-term neurobehavioral impairment when the animals were tested at adulthood, but the data failed to show a concentration-dependent effect. Impaired reversal learning in the Morris water maze test, longer latency to step-through on the passive avoidance test, and a decrease in wall rearing in the hot-plate test were recorded at 0.3 ppm but not at 0.6 ppm. Petruzzi et al. (1995) also reported possible neurobehavioral findings only at low ozone concentrations, suggesting that low exposure levels do not yield to adequate compensatory mechanisms for protection from oxidant injury compared to high concentrations of ozone.

In other studies using high ozone concentrations, exposure of pregnant female rats to 1.0 ppm for 12 hour/day during gestation resulted in morphological anomalies of the cerebellum of the offspring, including damaged Purkinje cells, a decrease in total area and number of Purkinje cells, abnormal fibrillar structures in the molecular layer, and a diminished folding pattern over the surface of the anterior lobe (Rivas-Manzano and Paz, 1999; Romero-Velazquez et al., 2002). 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 sleep cycle. It was theorized that ozone reaction products permeated the circulatory system and reached the brain to produce these effects. However, it was also acknowledged that ozone-induced maternal effects and subsequent decreased body weights of pups might also be responsible for the CNS changes.

9.5.3 Effects of Both Pre- and Postnatal Exposure of Ozone

A few studies examined the effects of pre- and postnatal ozone exposure in mice. Continuous exposure to 0.6 ppm ozone from several days before start of pregnancy up to 26 days after birth produced transient depressed dam body weight and long-lasting depressed body weight in pups. There was also attenuation of sex differences in some activities that suggest persistent neural and endocrine changes similar to early stress effects (Bignami, 1996; Dell'Omo et al., 1995a; Dell'Omo et al., 1995b). Exposed offspring subjected to swimming

navigation tests did not show consistent developmental effects with the exception of left-turning preference. Swimming navigation tests are reported to be a sensitive indicator for hippocampal damage. Subsequent tests for handedness of mice 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 did not produce clear differences in paw preferences for delivery of food pellets (Petruzzi et al., 1999). Offspring tested for morphine reactivity to a hot plate also did not produce consistent results across exposure concentrations, though there was general tendency towards reduced drug sensitivity at the highest concentration (0.9 ppm).

9.5.4 Effects of Postnatal Exposure of Ozone

Shore et al. (2002) investigated age-related pulmonary function responses to acute ozone exposure (0.3, 0.5, 1.0 ppm or greater for 3 hour) in immature and adult mice. Pulmonary function tests showed that ozone concentrations >0.3 ppm caused a concentration-related decrease in minute ventilation in mice of all ages, but the response was significantly less in 2-week-old mice than in mice 4 to 12 weeks of age. This change resulted in a two- to threefold increase in the inhaled dose of ozone normalized for body weight in the immature mice. Subsequent tests noted greater protein content in BAL fluid in exposed immature mice compared to exposed adult mice, but exposures were conducted only at ozone concentrations greater than 1 ppm.

The earliest studies investigating age-related differences in ozone susceptibility reported conflicting findings. A qualitative morphological examination by Stephens et al. (1978) reported that postnatal rats are resistant to ozone-induced pulmonary injury until weaning. In groups of 1 to 40-day-old rats exposed to 0.85 ppm ozone for 1 to 3 days, the appearance of “tissue nodules” (denser toluidine blue-staining areas), hypertrophy of epithelial lining, loss of cilia from ciliated cells, and type I cell injury in the centriacinar regions did not occur until rats were 20 days of age or older. The tissue nodules were reported to be due to proliferation of nonciliated and type II cells following ozone injury. In another study, 5 to 15 day-old neonatal rats exposed to 0.9 ppm ozone continuously for 3 days had reduced body and lung weights, while exposed weanling rats 21 to 41 days of age displayed reduced body weights and increased lung or lung/body weights (Tyson et al., 1982). Nursing mothers also had increased lung/body weight (suggestive of reduced body weights) and protein/DNA ratios, indicating slight ozone-induced pulmonary edema compared to neonatal rats. GSH-shuttle enzymes in lungs were unchanged or decreased in neonatal rats, but increased in lungs of weanlings 21 days old or older. Elsayed et al. (1982) conducted a similar exposure study (0.8 ppm ozone for 3 days), but reported that the effects seen were indicative of increased susceptibility in neonatal rats. Effects included increased death (7- and 12-day old), decreased body weight, lung weight, and enzyme activities in lung homogenates of neonatal rats (7- day old) compared to rats 18 days old or older.

Further support for the notion that immature rats are more sensitive to the acute inflammatory effects of ozone comes from a study of neonatal rats (13-days of

age) exposed to 1 ppm ozone for 2 to 6 hour. The results from the neonatal rats showed a considerably greater peak concentration of lavaged prostaglandin E_2, a greater percentage of lavaged leukocytes that were non-viable, and larger numbers of lavaged dead epithelial cells compared to rats that were 18 or more days old or older when exposed (Gunnison et al., 1992). The concentration of protein in lavage fluid following exposure did not show age-dependence, possibly as a result of lavaging before protein increases can be measured in lung airways. Juvenile rodents have also shown increased sensitivity to the acute inflammatory effects of ozone compared to older rodents. Dormans et al. (1996) observed that 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, with lesser increases occurring in 3, 9, and 18-month-old rats. A decrease in the net percentage of PMN influx in BAL fluid was also observed in older rats. Semiquantitative morphological evaluation following acute or 7-day exposure showed that the extent of centriacinar lesions was significantly lower as age increased.

Many of the enzymes that play a critical role in lung metabolism are not fully developed at birth (Pinkerton and Joad, 2000). A number of these enzymes, including antioxidant enzymes, are responsible for both the activation and detoxification of xenobiotic compounds. The effect of age on changes in antioxidant enzyme activities in homogenized rat lungs was assessed following 72-hour continuous exposure to 0.9 ppm ozone (Tyson et al., 1982). GSH-shuttle enzyme activity was elevated in young adult and, to a lesser degree, in weanlings. Enzyme levels in exposed neonates 5 to 15 days old remained unchanged or were lower than weanling rats. Elsayed et al. (1982) noted similar age-related differences in enzyme activities resulting from acute ozone exposure (0.8 ppm for 3 days). Dormans et al. (1996) observed increased GSH-shuttle enzyme activities in both juvenile and adult rats (1 to 18 mo of age) following 12 hour or 7 day exposure to 0.8 ppm ozone. However, no overall age-related change in enzyme activities was apparent, suggesting that ozone-related pulmonary induction of GSH antioxidant activities are near, or at, adult capacities by 1 month of age. Other age-related effects of ozone on biochemical indicators of inflammation investigated changes in chemokine and cytokine expression. Adult mice showed early increases in mRNAs encoding antioxidants, chemokines, and cytokines after acute ozone exposure (1 ppm) compared to newborn mice, indicating more extensive epithelial cell injury in adult mice (Johnston et al., 2000). In contrast to the ozone findings, the researchers observed similar responses of newborn and adult mice in response to an agent not causing epithelial injury (endotoxin), suggesting the altered inflammatory control observed between newborn and adult mice following ozone exposure is secondary to epithelial cell injury. The relationship of age to rat lung collagen synthesis has also been investigated. Three-day exposure of 24-365 day-old rats to 0.8 ppm ozone resulted in increased collagen synthesis, indicated by greater incorporation of ^{14}C into ^{14}C-hydroxyproline in all age groups (Hacker et al., 1986). However, there was a relatively greater increase in older rats roughly starting at 60 days of age.

Studies investigating age-related susceptibility to infection following ozone exposure are also conflicting. Exposure to 0.4 and 0.8 ppm ozone followed by infection with *S. zooepidemicus* produced greater death in 5-week old mice compared to 9-week old mice (Gilmour et al., 1993). Ingestion and intrapulmonary killing of the bacteria by alveolar macrophages (AM) were reduced in all ozone-exposed mice, but the apparent reduction of AM phagocytosis in younger mice was more marked. In contrast, Dormans et al. (1996) observed no effect of age on the reduced pulmonary clearance of *Listeria* bacteria among 1, 3, 9, and 18 month-old rats exposed to 0.8 ppm ozone for 12 hour, or 12 hour/day for 7 days.

A few studies carried out investigated the effects of 6-week ozone exposures in young or neonatal rodents. Pulmonary function tests on rats exposed to ozone (0.08-0.25 ppm, 12 hour/day, 7 days/week) beginning at birth for 6 weeks and compared to adult rats exposed to the same ozone regimen indicated greater sensitivity in the neonates (Raub et al., 1983). Exposed neonates, but not the exposed adults, showed evidence of increased lung distensibility of the lungs (i.e., increased inspiratory reserve volume, reduced peak inspiratory flow, and increased inspiratory reserve volume, inspiratory capacity, vital capacity at high distending pressures). Image-analysis quantitation of lungs of mice exposed to 0.3 ppm ozone 7 hour/day for the first 6 weeks of life showed small but statistically non-significant increases in alveolar wall area and mean type II cell area immediately after end of exposure (Sherwin and Richters, 1985). Similar trends were noted in an earlier exposure study of adult mice, though the newborn mouse findings suggested a greater propensity for type II cell aggregation than in adults. Morphometric studies by Barry et al. (1985;1988) observed altered centriacinar epithelium in both one-day-old and 6-week-old rats exposed to 0.25 ppm ozone (12 hour/day) for 6 weeks, but did not find age-related differences in lung structure or lung maturation in this lung region. It was speculated that the 3 weeks of exposure following weaning might have resulted in the overall changes, which were not substantially different from those occurring in the adult rats. In another morphometric study, exposure of rats to 0.64 or 0.96 ppm ozone for 6 weeks (8-hour/night) beginning at 28 days of age resulted in larger lungs and greater volumes of parenchyma, alveoli and respiratory bronchioles (Tyler et al., 1987). Exposed rats also had reduced body weights and lengths compared to rats fed *ad libitum*, but not compared to pair-fed rats. At the end of a 6-week post-exposure period, body weights of both ozone groups were reduced compared to both control groups, and lung volume and centriacinar changes had not fully recovered in high exposure rats.

Age-related differences in ozone susceptibility have also been carried out in higher mammals. Phalen et al. (1986) exposed 6-week old beagle dogs to 1 ppm ozone, 4 hour/day for 5 days to investigate the effects of ozone on postnatal lung maturation. Beagle dogs were selected because postnatal lung development is similar to humans and alveolar development occurs over a period of a few months. Six weeks after exposure, morphometric analysis of mean linear intercept changes showed a small but statistically significant decrease in lung surface area of about 4%, indicating an anatomical retardation in formation of

new alveoli. However, no other gross or histologically observable defects in lung morphology were observed. 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 hour/day for 5 days) during the first week of life retarded the normal development of the tracheal mucociliary system by decreasing epithelial cell density, retarding the normal developmental decrease in the number of mucus cells, altering the lectin detectable carbohydrate composition of mucus in these cells, reducing tracheal ciliated and basal cell populations, increasing total mucus load, and reducing mucus velocity (Mariassy et al., 1990; Mariassy et al., 1989). 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. In comparison, 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).

Monkeys provide an ideal model for developmental effects of ozone exposure in children because of similarities in postnatal lung and immune system development. Juvenile, 7-month old male cynomuolgus monkeys exposed to 0.25 ppm ozone 8-hour/day either daily or daily only during alternate months for 18 months showed abnormal lung growth, with increased volume fraction of respiratory bronchioles and their lumens (Tyler et al., 1988). Both groups exhibited respiratory bronchiolitis but the seasonal model of exposure had significantly increased total lung collagen content, chest wall compliance, and inspiratory capacity indicating alterations in growth of pulmonary functions and delay in maturation. A follow-up study in male juvenile cynomuolgus monkeys exposed to 0.64 ppm ozone 8-hour/day for 12 months investigated distal lung remodeling changes at end of exposure and after a 6-month post-exposure period (Tyler et al., 1991). As with the previous study, ozone exposure resulted in greater volume fractions and volumes of respiratory bronchioles, but had progressively worsened after a 6-month clean air post-exposure period. No changes in volume fractions of the alveolar compartment were observed.

Structural remodeling and airway immune changes in the developing Rhesus monkey lung was investigated in a series of studies using a cyclic regimen of ozone exposure and allergen inhalation in sensitized animals (Schelegle et al., 2003; Larson et al., 2004; Evans et al., 2003). Groups of 30-day old monkeys were exposed to ozone (0.5 ppm, 8-hour/day) and house dust mite allergen (HDMA), or ozone and HDMA alone for 11 cycles (5 days exposure followed by 9 days of clean air). Cyclic exposure of nonsensitized monkeys to ozone alone had only mild, non-significant effects on most immune, structural, and functional end points examined. However, combined inhalation exposure to ozone and HDMA in sensitized monkeys acted synergistically to produce an allergic-reactive airway phenotype characterized by increased serum histamine, increased airways eosinophilia, altered structural development resulting in longer, narrower tracheobronchial airways, elevated airway resistance, and non-specific airway hyperresponsiveness to histamine challenge. In addition, highest levels of serum

IgE and mucous cell content in terminal bronchioles were observed in the ozone + HDMA group. Altered development of neural innervation within the epithelium was also observed, including fewer airway generations with abundant nerve plexuses in allergen + ozone groups, and the appearance of a new population of undefined neuroendocrine or neuroendocrine-like cells in both ozone-exposed groups (Larson et al., 2004). Abnormal development of the basement membrane zone of lung airways was also observed in both groups of ozone-exposed monkeys, with irregular and thin areas of collagen in the zone and altered levels of cytokines and molecules important for responses to lung injury (Evans et al., 2003).

Prenatal or combined pre- and postnatal exposure to episodic, low ambient ozone concentrations has not been performed in animals. At higher concentrations, prenatal or combined pre- and postnatal exposure to ozone in rodents produced suggestive evidence of subtle neurobehavioral effects, cerebellum changes and long-term body weight reductions. However, considering the high ozone concentrations (0.8 to 1.0 ppm) and extended exposure durations needed to produce these affects, maternal toxicity likely had a significant impact on development of the offspring. The earliest studies investigating age-dependent susceptibility to ozone were not detailed enough to provide definitive evidence for increased sensitivity of newborn rodents. More recent studies in rodents and higher mammals have shown that indicators for pulmonary inflammation (e.g., increased protein and cell count in BAL fluid and morphological evidence of changes in tracheal and centriacinar epithelium) increase in neonates and juveniles relative to adults upon exposure to ozone. However, these effects were noted after relatively high ozone concentrations. Other postnatal toxicity data suggest that differences in age-related susceptibility to ozone depend on the outcome indicator examined. For example, anti-oxidants and proinflammatory cytokines and chemokines respond quicker to acute ozone exposure in adult rodents than in newborns, suggesting increased lung injury in the adults. Six-week ozone exposures in young versus adult rodents have suggested decreased pulmonary function in the young, but morphological comparisons have not shown age-related differences at the level of the centriacinar unit. The best evidence for ozone injury in the young at low, episodic ozone exposures is in monkeys, particularly when combined with house dust mite allergen (HDMA). While strict comparisons with similar adult exposures were not performed, ozone clearly enhanced the effects of allergen sensitization and altered the development of airway structural and immune system components.

Table 9-4 Effects of Prenatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.4, 0.8	24 hour/d 10 d (GD 7-17)	Mice (CD-1) prenatal	Transient depression of maternal food and water intake (0.4, 0.8 ppm) and maternal weight (0.8 ppm). Offspring cross-fostered at birth with dams non-exposed during pregnancy. No somatic and neurobehavioral effects on offspring.	Bignami et al. 1994
1	12 hour/night about 21d for entire gestation	Rats prenatal	No description of maternal effects Offspring: reduced weight at birth to 90 days of age; decreased duration of paradoxical sleep and inversion of sleep-wake pattern mainly in waking and slow wave sleep at up to 90 days of age.	Haro et al., 1993
1.04 0.64, 0.93,1.00 0.44	24 hour/d GD 6-9, or 24hr/d GD 9-12, or 8hr/d GD 6-15	Rats (Long-Evans) prenatal	Maternal effects included decreased food and water intake and reduced maternal weight change in all exposure groups. Offspring: GD 6-9: fewer implantation sites. GD 9-12 and GD 6-15: no fetotoxicity or teratagenic effects	Kavlock et al., 1979
1	24 hour/d 3 d (GD 9-12 or GD 17-20)	Rats (Long-Evans) prenatal	No description of maternal effects Offspring: GD 9-12 - reduced weight to day 6 of age; GD 17-20 – reduced weight to day 60 (males) of age, delayed righting reflex, eye opening, horizontal movement, and open field behavior.	Kavlock et al., 1980

Table 9.4 (cont.): Effects of Prenatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.2, 0.4, 0.6	24 hour/d 7-10 d prior to start of pregnancy to GD 17	Mice (CD-1) prenatal	Maternal effects: initial concentration-dependent reduction in food and water intake up to day 6 of exposure. Offspring: no postnatal somatic and neurobehavioral development (as assessed by a Fox test battery), adult motor activity, or social response endpoint changes. Increased frequency of self-grooming and decreased duration of exploring that was concentration-dependent. Inconsistent water maze results.	Petruzzi et al. 1995
1.0	12 hour/d entire gestation - approx. 21d	Rats (Wistar) prenatal	No statistical description of maternal effects. Cerebellar effects: Offspring: Cerebellar necrotic signs at PND 0, diminished area of the molecular area with Purkinje cells with pale nucleoli and perinuclear bodies at PND 12, and Purkinje cell nuclei with unusual clumps of chromatin in the periphery at PND 60.	Rivas-Manzano and Paz, 1999
1.0	12 hour/d entire gestation - approx. 21d	Rats (Wistar) prenatal	No description of maternal effects. Cerebellar effects: Offspring: at PND 90, decreased total area and number of Purkinje cells, areas of degenerating Purkinje cells, molecular layer contained abnormal circular fibrillar bodies, incomplete folding pattern of some lobes.	Romero-Velazquez et al. 2002
0.3, 0.6	24 hour/d From 30 d prior to formation of breeding pairs to GD 17.	Mice (CD-1) prenatal	No description of maternal effects, no reproductive effects. Offspring: no somatic or neurobehavioral developmental (by modified Fox test) changes. At 0.3 ppm: impaired reversal learning in Morris water maze test, longer latency to step-through in first trial of the passive avoidance test, decreased wall rearing in the hot-plate test. No effects at 0.6 ppm.	Sorace et al. 2001

Table 9.4 (cont.): Effects of Prenatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.6	24 hour/d 6 d prior to formation of breeding pairs to PND 22 or 26	Mice (CD-1) prenatal	No description of maternal toxicity, no reproductive effects. Offspring: reduced body weight to PND 120, attenuation of sex differences in rearing and sniffing in open field test (PND 29) and overall activity in final conditional place preference (CCP) test (PND 59-60), a change in response choices in the final CCP test (in absence of a main effect on conditioning), reduction of grooming in the activity test (PND 29), impairment of passive avoidance acquisition during initial training (PND 59-60).	Dell'Omo et al. 1995a
0.6	24 hour/d 6 d prior to formation of breeding pairs to PND 21	Mice (CD-1) prenatal	No description of maternal toxicity. Swimming navigation tests (Morris water maze)on offspring on PND 84-91: decreased body weight of females, no impairment of swimming navigation during acquisition phase, but increased swimming paths during reversal phase, higher swimming speed and strong tendency for left turns.	Dell'Omo et al. 1995b
0.3, 0.6, 0.9	24 hour/d 6 d prior to formation of breeding pairs to PND 26	Mice (CD-1) prenatal	No reproductive effects, no description of maternal toxicity. Offspring: decreased body weight (0.9 ppm) from PND 19-100, left-handed paw preferences for delivery of food pellets in females compared to males at 0.6 ppm only (PND 70), attenuation of morphine effect in hot plate test at 0.9 ppm (PND 100).	Petruzzi et al. 1999

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.25	12 hour/d, 6 wk	Rats (F344) 1 d, 6 wk	Morphometry of proximal alveolar region: no age-dependent effects. For both age groups: Increased volume of tissue. Increased number of type I and II cells. Increased thickness and decreased mean surface area of type I cells. Increased number of alveolar and interstitial macrophages.	Barry et al. 1985
0.25	12 hour/d, 6 wk	Rats (F344) 1 d, 6 wk	Morphometry of terminal bronchioles: no age-dependent effects found. For both age groups: decreased surface area contributed by cilia and luminal surface of Clara cells, and decreased number of brush cells in basement membrane.	Barry et al. 1988
0.8	12 hour, or 12 hour/d for 7 days during dark period	Rats (Wistar) 1, 3, 9, 18 mo	BAL analysis (1 d exposure): Highest protein and albumin in BAL fluid of 1 mo old rats, highest % PMNs in 1 and 3 mo old rats. Pathology and morphometry (1 d and 7 d exposure): age-related effect for centriacinar inflammatory cells (1 d exposure, highest in 1 mo old rats; 7 d exposure, highest in 1 and 3 mo old rats). Lung lesions slightly greater in 1 mo old rats at 1 d exposure, more pronounced in 3 mo old rats with 7 d exposure. Biochemical parameters (7 d exposure): ozone increased antioxidant enzyme activities at some ages, and enzyme activities were generally greater in males, but no overall interaction of ozone x age. Host resistance to lung infection (7 d exposure): Ozone decreased *Listeria* clearance in lungs, but there was no age-related effect.	Dormans et al. 1996

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.8	24 hour/d for 3 d	Rats (SD) M, F, 7, 12, 18 d M, 24, 30, 45, 60, 90 d	Increased death in 7- and 12 d old rats and decreased body and lung weight in 7 d old rats. Enzyme activities in lung homogenates: Decreased mg protein/lung, succinate oxidase, and glucose-6-phosphate dehydrogenase in 7 d olds, but increased in 18 d old rats. Enzyme activities related to production of NADPH in rats 24- to 90-d old increased with exposure, but were more pronounced in 60- to 90-d old rats.	Elsayed et al. 1982
0.5 ± House Dust Mite Allergen (HDMA)	8-hour/d, 5 d each followed by 9 d of fresh air for 11 episodes. HDMA 2 hour/d, on d 3-5	Monkey (Rhesus) 30 d	Effect on tracheal basement membrane zone (BMZ) development in sensitized monkeys. Both ozone-exposed groups: Immunoreactivity expression: Decreased perlecan and fibroblast growth factor (FGF)-2 in BMZ compared to other 2 groups. Increased syndecan-4 and FGF-2, and decreased fibroblast growth factor receptor (FGFR)-1 in basal cells compared to fresh air controls. Increased attenuated fibroblast compartments compared to fresh air controls. Ozone + HDMA: Synergistic increase in percent of BMZ <2.0 µm in width. HDMA alone: Immunoreactivity expression: Decreased FGFR-1 in basal cells compared to fresh air controls.	Evans et al. 2003
1	2, 4, or 6 hour	Rats (SD) 13 d, 18 d, 27 d, 8 wk, 16 wk	BAL analysis: 2 hour exposure: PGE_2 highest in 13 d old rats compared to other groups, percentage of non-viable leukocytes showed age-related inverse correlation with 4 and 6 hour exposure, qualitative finding of increased clumps of dead cells in 13 d old rats.	Gunnison et al. 1992

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.8	24 hour/d for 3 d	Rats, M (SD) 24-365 d	Lung collagen synthesis: Exposure increased lung dry weight, ^{14}C-hydroxyproline, and ^{14}C-proline incorporation at all ages. Total protein in lung increased at age 90, 182, and 365. Relatively greater increase in older rats for incorporation of ^{14}C-proline into ^{14}C-hydroxyproline.	Hacker et al. 1986
1.0	4, 20 hour (newborn) 4, 24 hour (adult)	Mice (C57B1/6J) 36 hour, 8 wk	Effects on chemokine and cytokine mRNA expression in lung. Newborn: 4, 20 hour: increased metallothionein (Mt). 20 hour only: increased eotaxin, macrophage inflammatory protein (MIP)-1alpha, MIP-2, and monocyte chemoattractant protein (MCP)-1. Adult: 4 hour only: interleukin (IL)-6. 4, 24 hour: increased Mt, eotaxin, MIP-1alpha, MIP-2.	Johnston et al. 2000
0.5 ± House Dust Mite Allergen (HDMA)	8-hour/d, 5 d each followed by 9 d of fresh air for 11 episodes. HDMA 2 hour/d, on d 3-5	Monkey (Rhesus) 30 d	Effect on neural component development in proximal airways of sensitized monkeys. HDMA, ozone, and ozone + HDMA groups: Decreased protein gene product 9.5 (PGP 9.5) positive nerves per surface area of epithelium compared to fresh air controls. Both ozone groups: Increased PGP 9.5 positive neuroendocrine cells per surface area of epithelium compared to other groups.	Larson et al. 2004
1.0	4 hour/d 5 d	Sheep First wk of life	Effects on tracheal mucosa. At 2 weeks of age: decreased ciliated and basal cell populations, and epithelial density. Retarded natural decline of mucus cell population, and retarded natural decline or increased lectin detectable carbohydrate composition of mucus in epithelium.	Mariassy et al. 1989

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
1.0	4 hour/d 5 d	Sheep First wk of life	Effects on tracheal epithelium. Tracheal mucus velocity decreased up to 24 wk of age. At 2 weeks of age: prevented age-dependent decrease of basal secretion rates of macromolecule-bound sulfate and mucus cell populations, and prevented age-dependent increase of ciliated cell population. Increased tissue conductance.	Mariassy et al. 1990
1	4 hour/d, 5 d	Dogs (Beagle) 6 wk	Effect on body, gross lung, and lung morphometry. Increased mean linear intercept (inversely related to lung surface area) of the lung.	Phalen et al. 1986
0.08, 0.12, 0.25	12 hour/d, 7 d/wk, 3, 6 wk (neonates), 1, 3, 6 wk (young adults)	Rats, M (F344) 1 d, or young adults	Effects on pulmonary function. Young adults: increased end-expiratory volume (0.25 ppm). Neonatal: 3-wk exposure: increased inspiratory reserve (0.25 ppm). 6-wk exposure: reduced peak inspiratory reserve (0.12, 0.25 ppm). At high distending pressures, increased inspiratory reserve volume, inspiratory capacity, vital capacity, and total lung capacity (0.25 ppm).	Raub et al. 1983

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.5 ± House Dust Mite Allergen (HDMA)	8-hour/d, 5 d each followed by 9 d of fresh air for 11 episodes. HDMA 2 hour/d, on d 3-5	Monkey (Rhesus) 30 d	Effects on airway immune and structural development in sensitized monkeys. Ozone alone: No significant changes towards development of reactive airway disease. HDMA alone: Increased eosinophilia in terminal bronchioles compared to other 3 groups, and in proximal airway compared to fresh air controls and ozone exposed. Increased mucous cell content compared to ozone exposed. Ozone + HDMA: Synergistic increase in serum IgE, airway resistance, hyperresponsiveness, airway eosinophilia, and longer, narrower tracheobronchial airways. Increased mucous cell content in terminal bronchioles and total lobe volume/body wt., and decreased volume fraction of blood vessels compared to fresh air controls. Increased volume fraction of parenchyma, mucous cell content in 5th intrapulmonary airway, and proximal airway eosinophilia compared to fresh air controls and ozone exposed.	Schelegle et al. 2003
0.3	7 hour/d, 5 d/wk, 6 wk	Mice, M (Swiss-Webster) Newborn	Image-analysis of type II cells and alveolar walls. Increased type II cell area and type II cell linear intercept. Possible increased type II cell clustering in exposed neonates compared to a similar adult exposure study in adult mice.	Sherwin and Richters, 1985

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.3, 0.5, 1.0	3 hour	Mice (A/J) 2, 4, 8, 12 wk	Ozone-induced reduction in minute ventilation (0.5 and 1.0 ppm) less in 2-week old mice compared to other age groups. Estimated 2- to 3-fold increase in the inhaled dose of ozone normalized for body weight in the immature mice (2- and 4-wk-old mice compared to 12-wk–old mice). No exposure or age-related effect on airway hyperresponsiveness at or below 1.0 ppm.	Shore et al. 2002
0.85	24 hour/d 1, 2, or 3 d	Rats (SD) 1, 5, 10, 15, 20, 25, 30, 35, 40 d	Morphological light microscopic examination of the central acini: "tissue nodules" appeared and increased in size and intensity with increasing age after weaning (20 d). Loss of cilia on ciliated cells and slight hypertrophy of epithelium of terminal bronchioles, and evidence of type I cell injury or sloughing in proximal alveoli with exposure at 20 d of age or older.	Stephens et al. 1978
0.64, 0.96	8-hour/night 6 wk Pair fed and ad libitum controls	Rats, M (SD) 28 d	Lung morphometric examination: Increased volume fraction of respiratory bronchioles or their components, the wall and lumen, at 0.64 and 0.96 ppm. Increased fixed and saline lung volume, increased total volume of parenchyma and volume of parenchyma/unit body weight at 0.96 ppm. Following 6-week post-exposure period, body weights of exposed rats were lower compared to both control groups, and some morphometric changes at 0.96 ppm had not fully recovered.	Tyler et al. 1987
0.25	8-hour/d 7 d/wk (daily) or 7 d/wk on alternate mo (seasonal), for 18 mo	Monkeys, M (Macaca fascicularis) 7 mo	Seasonal exposure model: increased total lung collagen content, chest wall compliance, and inspiratory capacity. Morphological effects in alveolar and centriacinar regions: respiratory bronchiolitis, increased volume fraction of respiratory bronchioles and their lumens (both exposure groups) and walls (daily exposure only).	Tyler et al. 1988

Table 9.4 (cont.): Effects of Postnatal Exposure to Ozone (≤ 1 ppm) in Animals

Ozone Concentration (ppm)	Exposure Protocol	Species, Sex, (Strain), Age	Observed Effects	Reference
0.64	8-hour/d 7 d/wk, 12 mo	Monkeys, M (Macaca fascicularis) 6-7 mo	Morphological effects in alveolar and centriacinar regions: increased volume fractions and volumes of respiratory bronchioles, and decreased internal diameter of respiratory bronchioles. Following a 6-mo post-exposure period in clean air: increased volume and volume fraction of respiratory bronchioles still present, and greater as a percent change from control values.	Tyler et al. 1991
0.9	24 hour/d 3 d	Rats (SD) 5, 10, 15, 25, 35, d Nursing mothers, 60-90 d, 180+ d	Decreased body weights in neonates and weanlings. Mean lung weights: decreased at 5 and 10 d of age, increased at 35 d of age and in nursing mothers (180+ d). Increased lung/body weight ratio in weanlings and nursing mothers. Decreased DNA (5, 25, 180+ d) and increased protein/DNA ratio (180+ d) in lung homogenates. GSH enzyme activities: GSH-6-phosphate dehydrogenase decreased in 15 day olds and increased in weanlings and nursing mothers. GSH reductase increased at 25 d and older. GSH peroxidase decreased at 5 d, increased at 35 d and 180 + d.	Tyson et al. 1982

9.6 Controlled Exposure Studies

9.6.1 Characteristics of Controlled Exposure Studies

9.6.1.1 Human volunteers

Experimental exposures of human volunteers to air pollutants under controlled laboratory conditions can provide useful pathophysiological information directly relevant to standard-setting. Controlled human exposure studies all follow a similar general structure. Volunteers are usually exposed to one or more carefully measured pollutants through a mouthpiece (oral breathing only), a face-mask (oronasal breathing), or in a chamber (oronasal breathing). A study subject may also utilize oral breathing if he or she wears a nose-clip. Nasal breathing may also be effectuated by keeping one's mouth closed in a chamber or face-mask experiment. Water-soluble pollutants, such as sulfur dioxide, tend to be scrubbed from incoming air in the nasal passages, so the mode of breathing may make a marked difference in the health effects observed. However, for ozone, the mode of breathing, per se, does not appear to have a marked effect on lung function or symptoms reported by subjects (Hynes et al. 1988; Adams et al. 1989). Study subjects may breathe quietly at rest, or they may be required to hyperventilate or exercise in order to increase the rate of pollutant delivery, the total dose received, or both.

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

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

Exercise on a treadmill or stationary cycle is often incorporated in the exposure protocol in order to increase ventilation rates (and hence the dose of the pollutant delivered to the respiratory tract), to simulate the effect of active outdoor work or recreation. Indeed, multi-hour ozone exposure protocols utilized by the U.S. EPA were intended to simulate the exertion of outdoor manual (Folinsbee 1989). It should be noted, however, that exercise may itself modify the outcomes of

interest in studies of individuals with asthma. Many asthmatics experience coughing and chest tightness during or shortly after exercise, accompanied by measurable decreases in lung function. Such exercise-induced bronchospasm (EIB) is usually self-limited and reversible within an hour, but may require inhalation of bronchodilator medication to alleviate symptoms. In analyzing the results of chamber studies of asthmatics, one can control for the effects of EIB by comparing lung function after pollutant exposure to that found after filtered air exposure on another day. However, the magnitude of EIB in a given individual may vary from day to day, which may obscure somewhat any pollutant-associated effects, even with such statistical control.

9.6.1.2 Typical Outcomes Assessed in Human Controlled Exposure Studies

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

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

Although it has been recognized for decades that acute and chronic ozone exposure in animals injures the airway epithelium and causes inflammation, this effect was first demonstrated in a controlled exposure study of humans using a relatively high dose in 1986 (Seltzer et al. 1986). Inflammation represents an orchestrated sequence of biological responses to injury or infection, involving (among other things) the release of pro-inflammatory mediators (such as IL-8),

which attract white blood cells known as polymorphonuclear cells [PMNs] to the site of injury. Inflammatory changes also include increased local vascular permeability, and influx of inflammatory cells, including PMNs and other types of lymphocytes. A number of biochemical markers of injury or inflammation have been linked with ozone exposure (e.g., Devlin et al. 1991; Seltzer et al. 1986) and will be discussed in the context of specific studies. One function of inflammation is to kill foreign pathogens with highly reactive oxidants generated by various white blood cells. However, these chemicals can also attack and injure host tissue as well. Thus, the inflammatory process may amplify ozone-associated toxicity.

Investigators have also measured ozone-related effects on host defenses. Alveolar macrophages (AMs) are mobile phagocytic cells responsible for maintaining the sterility of the deep lung. Under normal circumstances, AMs represent about 95% of the immune cells retrieved in BAL fluid. A variety of tests of AM function (e.g., mobility, phagocytosis, and ability to generate reactive oxygen species) can be conducted on such cells obtained from human subjects' BAL to assess functional changes due to ozone exposure.

9.6.1.3 Typical Exposure Protocols

The protocols used in controlled human exposure studies have been largely standardized over the past 30 years. There are three basic protocols: one hour continuous exercise, two to four hour intermittent exercise, and six to eight hour quasi-continuous exercise. Each of these protocols was designed to simulate a common outdoor exposure pattern, and the ventilation rates used are representative of activities common to the exposure durations.

The one hour continuous exercise protocol examines responses to ozone in subjects performing moderate to heavy exercise continuously for one hour, with endpoint measurements before and after exposure. A variation on this protocol in studies primarily investigating the impact of ozone exposure on exercise performance adds a sprint to exhaustion at the end of the hour exposure. This protocol simulates short-term, moderate to heavy exercise exposures, and is representative of the activity patterns of people engaged in athletics, personal exercise programs, and after-school endurance sports. This protocol investigates responses to short, peak concentration exposures.

The two hour intermittent exercise protocol was designed to simulate somewhat longer exposures during which the activity level was of moderate, noncontinuous intensity. It was designed to simulate longer-term, less intense activity patterns, such as personal exercise programs and athletic training where activity occurs in alternating periods with rest, and outdoor home maintenance and yardwork. Endpoints are typically measured during the rest periods, as well as before and after exposure, except for those measured by bronchoscopy.

Recognition that some areas of the country had a wide, lower concentration pattern of ozone concentrations led to development of the 6.6 hour quasi-continuous exercise protocol. Several recent studies have extended this protocol to eight hours. This protocol simulates a day of outdor work, and the

exercise level, at 50 minutes per hour, is representative of that which can be maintained for a full day of work. As such, this protocol is a simulation of outdoor labor, such as construction, landscaping, or highway work. It is also representative of weekend and vacation exposure of children and adults. Typically, endpoints, except those measured through bronchoscopy, are measures before, and after exposure, as well as during the 10 minute rest periods each hour.

9.6.1.4 Strengths and Limitations of Controlled Exposure Studies

The principal advantage of controlled human exposure studies over epidemiological studies is that exposures to the pollutant(s) of interest can be precisely measured, and therefore exposure-response relationships can be determined with some degree of accuracy. Controlled exposure studies can, under some circumstances, identify a threshold exposure for certain outcomes on an individual subject basis, and thus they can provide useful information for the standard-setting process. However, due to inherent limitations, as described below, controlled studies can not identify a threshold concentration on a population level with certainty given the well know wide range of individual responses to ozone (see Section 9.6.2.1.5). Controlled studies in a clinical setting can also include invasive, labor-intensive procedures, such as bronchoalveolar lavage (described above), allowing for the examination of outcomes that would not be feasible in epidemiological investigations. While exposure conditions can also be controlled in animal experiments, the obvious advantage of human chamber studies in relation to the latter is that no cross-species extrapolation is required.

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

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

9.6.1.5 Ozone Analyzer Calibration Methods and Health Data Interpretation

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

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

9.6.2 Short-term Exposure to Ozone

9.6.2.1 Responses of Human Subjects

9.6.2.1.1 <u>Introduction</u>

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

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

The degree of response to ozone is related to three factors: 1) the ozone exposure concentration, 2) V_E, the ventilation rate, and 3) T, the duration of exposure (see Section 9.2.5). The consensus of available research is that ozone concentration is the most important of the three factors. Normal, healthy people exposed to ozone concentrations $\geq$ 0.12 ppm (the federal one-hour standard) develop significant, transient reversible decrements in pulmonary function if V_E or T are increased sufficiently. Host factors are also important in determining individual responses to ozone exposures: there is a large degree of variability (up to about ten-fold) among individuals in the magnitude of acute responses to a given inhaled dose of ozone. These responses appear to be characteristic of a given individual, as they are reproducible over periods of many months (see Section 9.6.2.1.5).

9.6.2.1.2 <u>Responses With One- to Three-Hour ozone Exposures</u>

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

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

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

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

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

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

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

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

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

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

Collectively, this literature indicates that one to three hour exposures to ozone concentrations as low as 0.12 ppm with moderate to heavy exercise can induce decrements in pulmonary function and increases in respiratory symptoms. Figure 9-1, derived from Seal et al. (1993), provides an illustration of the relationship between changes in FEV1, Sr_{aw} and cough and ozone concentration (two-hour exposures with intermittent exercise).

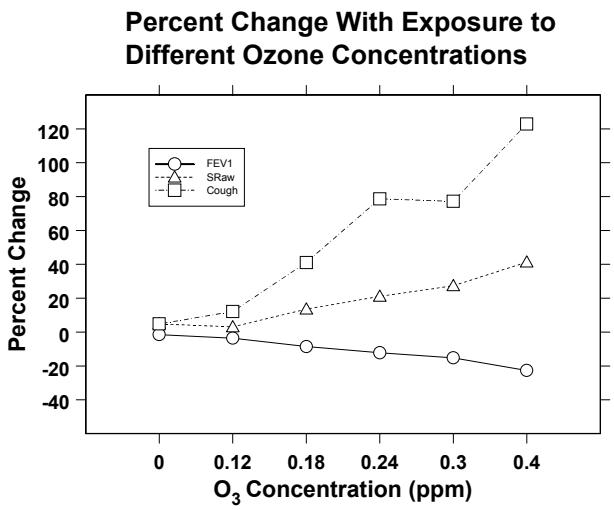

Figure 9-1 Percent change in FEV1, Sr_{aw} and cough with two-hour intermittent exercise exposures to FA, 0.12, 0.18, 0.24, 0.3, and 0.4 ppm ozone. Derived from Seal et al., 1993.

Investigators at the US EPA health effects research laboratory have published several papers that modeled the concentration-response function for the pulmonary function effects of ozone exposure. The first paper, McDonnell et al. (1983) developed an iterative least-squares method to fit a four parameter logistic function curve to the mean changes in FVC, FEV1 and sRaw of 138 young adult males exposed 2.5 hours to one of six ozone concentrations. The subjects performed heavy exercise in alternating 15 min periods. The four parameters represented the minimum and maximum responses, and the slope

and location of the rapidly changing segment. The dose-response curves for the group mean decrements in FVC and FEV1 were sigmoid in shape, with a large increase in mean response occurring between 0.18 and 0.24 ppm, with a plateau at higher ozone concentrations, while that for sRaw was exponential in shape. The small group mean decrements in FVC, FEV1 and FEF25-75% and the increase in sRaw were statistically significant at 0.12 ppm ozone, the lowest ozone concentration studied. There were no changes in these parameters with filtered air exposure. The authors concluded that the different shapes of the exposure-response function for the spirometric variables and for sRaw indicated that different biological mechanisms were responsible for the changes observed.

McDonnell et al. (1993) validated the sigmoid-shape of the ozone concentration-response function for FEV1. As these investigators noted, this shape is plausible from a biologic perspective as many biochemical and physiologic systems respond to stimuli is a similar manner. Biologic functions typically exhibit a stimulus threshold below which responses do not occur because the body has compensating functions that prevent systemic changes from occurring. There is also frequently a stimulus range over which response varies with the intensity of the stimulus, and a level of stimulus beyond which response is maximal and does not increase further. This general pattern of response is characteristic of many enzyme- and neurally-mediated responses. The sigmoid logistic function also predicts the curvilinear, concave upward shape to the concentration response function in the low ozone concentration range reported by Avol et al. !1984), Kulle et al. (1985), Hazucha (1987) and Linn et al. (1986). Evidence supporting the concave downward shape for the concentration response function at higher inhaled doses comes from Gliner et al. (1983) at 0.50 ppm ozone, which report that over 70% of the FEV1 decrement during two hour intermittent exercise exposures occurred during the first hour of exposure. It should be noted that this shape for the concentration response function is valid only for spirometric variables, at ozone concentrations up to about 0.40 ppm, since this is the data range used by McDonnell et al. (1993). It is well known that exposure to much higher concentrations of ozone induces pulmonary edema that will impact FEV1 and other spirometric variables through mechanisms other than those under consideration in the studies described here.

McDonnell et al. (1994) also performed a study that modeled acute changes in FEV1 as a function of exposure rate and total inhaled dose. Data on 374 young adult males who participated in 504 exposures to several different ozone concentrations for 2 or 6.6 hours formed the data set. A sigmoid function described the observed mean responses in terms of exposure rate and total inhaled dose over a wide range of concentrations and times. The function was based on a three-compartment biological model. Compartment 1 represented substances in the epithelial lining fluid of the airways. Compartment 2 represented the bronchial c-fiber neural receptors, vagal afferents, the central nervous system, and the phrenic and intercostal nerve efferents, which directly modulate reduction in FEV1 through inhibition of full inhalation. Compartment 3 included nonneural airway cellular constituents and their products. According to the model, inhaled ozone enters compartment 1 where it initiates a series of

reactions producing a set of mediators that directly stimulate C-fibers in compartment 2 and initiate release of a second set of mediators that result in epithelial damage and further mediator release in compartment 3. While some aspects of the proposed biological model remain unresolved, others are in agreement with known biological mechanisms (see 9.3). A subsequent analysis by McDonnell et al. (1997) reached similar conclusions as to the sigmoid shape of the exposure response relationship.

9.6.2.1.4 Small Airway Effects

The primary pulmonary function parameter reported in the controlled human exposure literature has been FEV1. This is largely due to its low coefficient of variation, the fact that it is largely effort-independent, being primarily influenced by vital capacity, and the relative ease in determining whether or not a subject has made a maximal effort in performing the test. However, some papers also report lung function test measurements that reflect the function of the small airways, primarily various measures of flowrate at low lung volumes. The primary variable in this category reported in the ozone literature is FEF25-75%, although a few investigators report other measures. FEF25-75% represents the average expiratory flow rate during the middle half of the forced vital capacity maneuver. FEF25-75% (as well as FEV1) can be reduced by several different mechanisms, which may interact with each other. The primary reasons for reduction in these pulmonary functions are an increase in airway resistance or a reduction in elastic recoil of the lung.

Several studies that utilized high exercise intensities and ozone concentrations less than 0.25 ppm, have reported larger percent changes in FEF25-75% than in FVC or FEV1, suggesting a degree of bronchoconstriction (Adams and Schelegle, 1983; Kulle et al., 1985; Folinsbee et al., 1984). Hazucha et al. (1973) reported evidence of effects in the small airways suggestive of bronchoconstriction in subjects who performed light exercise for 2 hours while breathing 0.37 ppm or 0.75 ppm (KI) ozone. Gliner et al. (1983) found no significant small airway effect in male and female subjects exposed for 125 min to 0.2 ppm ozone while performing moderate, intermittent exercise (exercise V_E of 30 l/min for males and 18 l/min for females). However, when the same subjects underwent the same protocol while breathing 0.42 or 0.5 ppm ozone, the decrements in FEF25-75% were disproportionately large compared to those in FVC or FEV1, suggesting a degree of bronchoconstriction.

Frank et al. (2001) reported on responses of healthy adults exposed to 0.25 ppm ozone for 2-hour on four consecutive days. FVC and FEV1 returned toward baseline across the four days of exposure, with the greatest decrement on day 2, consistent with the findings of others. The unique contributions of this study are that the investigators evaluated small airway function following each exposure, measured as SAWgp (a derived measure based on several measures of small airway function). The investigators interpreted the results as being likely due to reduction in small airway caliber. It is important to note that decrements in SAWgp did not resolve by the next morning after ozone exposure, suggesting that effects of ozone on small airways may be more long lasting than those in the

larger airways. Weinmann et al. (1995a,b) also reported decrements in small airway function, measured as isovolumetric FEF25-75% that persisted for at least 24 hours after two hour exposure to 0.35 ppm ozone with intermittent exercise. The observation that the effect persisted during the time frame of airway inflammation led the investigators to speculate that the persistent reduction in isovolumetric FEF25-75% be a marker for airway inflammation, although no studies have investigated this hypothesis.

Collectively, the results of these studies suggest that small airway effects are more likely to occur with inhalation of relatively high effective doses of ozone. The study by Frank et al. (2001) utilized a more moderate exercise load, although the ozone concentration was higher than current levels. However, the observation of a persistent effect on small airway function is of concern from the perspective of possible long-term effects related to repeated acute exposures, and suggests a need for further research.

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

Two related key issues in setting ambient air quality standards are the variability of responses between individuals, and the stability of individual responses. These issues are a consideration in selection of ozone concentration, since State law requires that ambient air quality standards protect sensitive people. In 1972 Bates et al. reported that there was a large degree of inter-subject variability in response to ozone. These observations were subsequently confirmed by numerous other investigators (e.g., Adams et al. 1981; Folinsbee et al. 1978; Folinsbee et al. 1978; McDonnell et al. 1985b, McDonnell et al. 1983; McDonnell et al. 1993; Kulle et al. 1985; Aris et al. 1995; Holz et al. 1999). It remains unclear what factors account for the variability in responsiveness among individuals.

Variability of individual responses at ozone concentrations near the proposed standards is most relevant to assessing the adequacy of a proposed standard. Several studies that investigated responses to 0.12 ppm ozone reported individual level data that can be used to give an indication of the range of individual responses likely to occur in FEV1 with one to three hour exposures (McDonnell et al. 1983; McDonnell et al. 1993; Schelegle and Adams, 1986; Gong et al., 1986). These data suggest that while some individuals do not develop decrements in lung function, some subjects studied with moderate to heavy exercise had decrements in FEV1 in excess of 20%.

Folinsbee et al. (1991) combined the data from four studies performed at the US EPA Health Effects Research Laboratory, which all used the same 6.6 hour. exposure protocol. He then reported on the distribution of change in FEV1 in response to exposure to FA and 0.08, 0.10, and 012 ppm ozone (Figure 9-2).

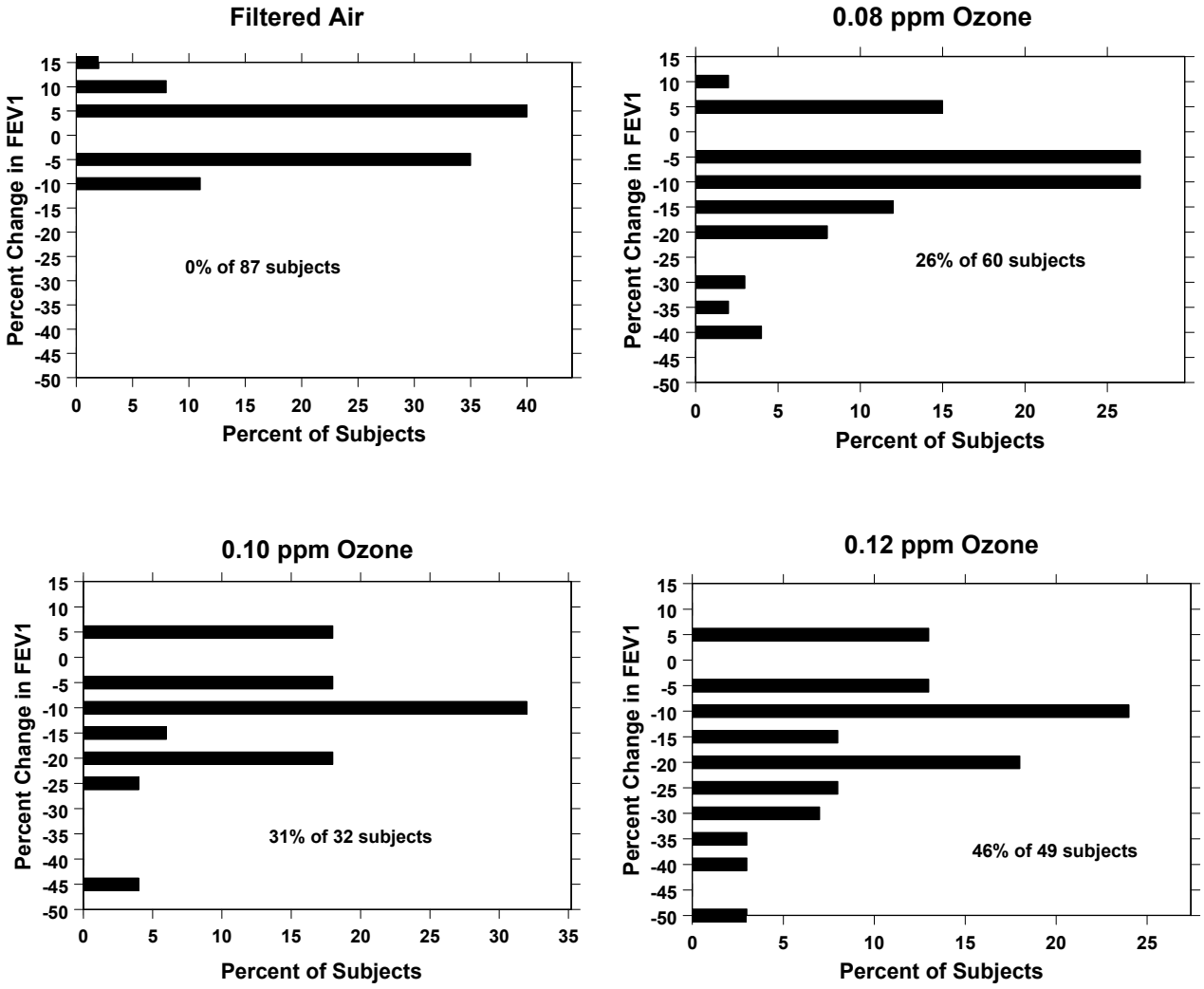

Figure 9-2 Distribution of response for 87 subjects exposed to FA and at least one of 0.08, 0.10, or 0.12 ppm ozone. All exposures were for 6.6-hour, with exercise for 50 min of each hour. Decrease in FEV1 is presented as the change from baseline. Each panel also includes the number of subjects, and the percentage of that number who had a decrease in FEV1 in excess of 10%. Derived from Folinsbee et al., 1991.

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

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

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

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

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

In summary, the data indicate that some individuals exposed to 0.12 ppm for one to three hours or to 0.08 ppm ozone for 6.6 hours while performing moderate to

heavy exercise will develop decrements in FEV1 of greater than 20%. Further, these studies show that individual responsiveness to ozone is relatively stable, at least for periods of at least a year or two. The data also indicate that each individual has a characteristic degree of responsiveness to ozone that is related to innate factors that remain to be elucidated. Relationship Between Endpoints: Pulmonary Function, Airway Reactivity, Inflammation and Symptom Responses

Several studies have investigated the relationships among the various endpoints that show changes with acute ozone exposure (pulmonary function responses, symptoms, airway inflammation, and airway reactivity). This subject is discussed in Section 9.2.1).

Table 9-5: Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone – Pulmonary Function

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.25, 0.50, 0.75 (KI)	2 hour at rest	Healthy adults, (8 M, 5 F), 21-22 yrs	Decreased FVC with 0.50 and 0.75 ppm exposure compared to FA. Small (NS) decrease of 4% in mean VO_{2max} following 0.75 ppm, compared to FA.	Horvath et al. 1979
0.37, 0.5, 0.75 (KI)	2 hour at rest	Healthy adults, (20 M, 8 F), 19-29 yrs	Decreased FEV1, V_{25} and V_{50} with 0.75 ppm exposure, compared to FA.	Silverman et al. 1976
0.50 (KI)	2 hour at rest	Healthy adults, (40 M), 18-28 yrs	Decrease in forced expiratory volume and flows.	Folinsbee et al. 1978
0.08, 0.10, 0.12, 0.14, 0.16	2 hour IE (4x15 min at V_E = 68 L/min)	Healthy adults, (24 M), 18-33 yrs	No significant changes in pulmonary function.	Linn et al. 1986
0.10, 0.15, 0.20, 0.25	2 hour IE (4x14min at V_E=70 L/min)	Healthy nonsmokers, (20 M), 25.3 ±4.1 (SD) yrs	FVC, FEV1, FEF25-75%, SG_{aw}, and TLC all decreased with increasing ozone concentration and increasing time of exposure. Threshold for response was above 0.10 ppm, but below 0.15 ppm ozone.	Kulle et al. 1985
0.12, 0.18, 0.24	1-hour competitive simulation at mean V_E=87L/min	Highly trained competitive cyclists, (10 M), 19-29 yrs	Decreased FVC and FEV1 with exposure to 0.18 and 0.24 ppm ozone, compared to FA exposure. Decrease in exercise time for subjects unable to complete the competitive simulation at 0.18 and 0.24 ppm.	Schelegle and Adams 1986
0.12, 0.18, 0.24, 0.30, 0.40	2.5 hour IE (4x15 min TM exercise at V_E=65 L/min); 1 exposure/subject	Healthy adults, (20 – 29 M/group), 18-30 yrs	Significant decreases in FVC, FEV1 and FEF25-75% at 0.12 ppm ozone; decrease in V_T and increase in f_R and SR_{aw} at 0.24 ppm ozone.	McDonnell et al. 1983

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-5 (cont.): Controlled Human Exposure Studies - One to Four Hour Exposures to Ozone-Pulmonary Function

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

Table 9-3: Airway Responsiveness Following Ozone Exposures

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

Table 9-6 (cont.): Airway Responsiveness Following Ozone Exposures

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12	45 min, IE (15 min ex at V_E=3 times resting)	Physician diagnosed asthma; SO_2-induced airway hyperreactivity. (12 F, 5 M), 19-38 yrs	Dietary supplementation for 4 weeks before, and during the experimental period (approximately an additional 2 weeks) with 400 IU vitamin E + 500 mg vitamin C reduced airway responses to a challenge with 0.10 or 0.25 ppm SO_2 post-ozone exposure. Supplementation with vitamins C and E had no effect on pulmonary function responses to ozone exposure.	Trenga et al. 2001
0.125 0.250 0.125 (on 4 consecutive days)	3 hour, IE (10 min rest/15 min ex), V_E=30 L/min	Mild bronchial asthma: (5 F, 6 M), 20-53 yrs Allergic rhinitis: (6 F, 16 M), 19-48 yrs	Mean early-phase FEV1 response and number of subjects with ≥20% reduction significantly greater after 0.25 ppm or 4 x 0.125 ppm ozone exposures. Most of the ≥15% late-phase responses occurred after exposure to 4 x 0.125 ppm ozone, along with significant inflammatory effects, as indicated by increased sputum eosinophils (allergic rhinitis and asthmatic groups) and in increased sputum lymphocytes, mast cell tryptase, histamine and LDH (asthmatics only).	Holz et al. 2002
0.20 0.40 0.40	2 hour, IE, (V_E=2 times resting) 2 hour/d for 3 days, IE (V_E=2 times resting)	Healthy nonsmokers, (12 M, 7 F), 21-32 yrs	Increased sensitivity to inhaled histamine aerosol challenge after exposure to ozone at 0.40 ppm, but not after exposure to 0.20 ppm. Increased sensitivity attenuated over the 3 day exposure period.	Dimeo et al. 1981

Table 9-6 (cont.): Airway Responsiveness Following Ozone Exposures

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

Table 9-6 (cont.): Airway Responsiveness Following Ozone Exposures

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.25	3 hour, IE, (15 min ex/10 min rest/5 min without ozone during each 30 min of exposure), (V_E=30 L/min)	Nonsmokers: mild atopic asthmatics, (11 F, 13 M); mean age = 26 yrs Allergic rhinitics (6 M, 6 F); mean age = 25 yrs Healthy controls (5 M, 5 F); mean age 23 yrs	Asthmatic and allergic subjects had increased bronchial responsiveness to allergen after ozone exposure, compared to FA exposure. .	Jorres et al. 1996

Table 9-6 (cont.): Airway Responsiveness Following Ozone Exposures

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

Table 9-7: Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
Ambient	variable, outdoor jogging	Healthy nonsmokers, recreational joggers, (18 M, 1 F), 23-38 yrs All completed initial BAL, 15 underwent BAL during the subsequent winter, and 6 completed BAL the following summer	BALF levels of LDH, IL-8, and PGE_2 increased during the summer smog season.	Kinney et al. 1996
0.20	2 hour face mask exposure, IE (15 min ex/15 min rest), (V_E=30 l/min)	Healthy nonatopic, nonsmokers (10 M, 2 F), mean age 28 yrs	Trend for increased PMN and ciliated epithelial cells in BALF 6 hour post-ozone exposure. Decreased substance P immunoreactivity in submucosa inversely related to changes in PMN and decrease in FEV1.	Krishna et al. 1997
0.30	1-hour (mouthpiece), CE (V_E=60 L/min)	Healthy nonsmokers (5 M)	Significantly increased PMN's in BALF at 1, 6 and 24 hour post ozone exposure, with the peak increase at 6 hour.	Schelegle et al. 1991
0.40	2 hour IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	BALF at 18-hour post-ozone exposure showed a significant increase in PMNs, protein, albumin, IgG, PGE_2, plasminogen activator, elastase, complement C3a, and fibronectin.	Koren et al. 1989a
0.40	2 hour IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	Macrophages removed 18-hour after ozone exposure showed changes in the rate of synthesis of 123 different proteins, as assayed by computerized densitometry of two-dimensional gel protein profiles.	Devlin and Koren 1990
0.40	2 hour IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy nonsmokers (11 M), 18-35 yrs	BALF obtained 18-hour after ozone exposure contained increased amounts of coagulation factors, tissue factor, and factor VII. Macrophages in the BALF had elevated tissue factor mRNA.	McGee et al. 1990

Table 9-7 (cont.): Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

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

Table 9-7 (cont.): Inflammatory Effects Measured in Bronchoalveolar Lavage Fluid After Controlled Human Exposures to Ozone

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

Table 9-8 : Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

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

Table 9-8 (cont.): Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.21	1-hour CE cycle ergometry (mean V_E=80 L/min)	Highly fit endurance cyclists, (14 M, 1F), 16-34 yrs	Pretreatment with albuterol had no significant effect on metabolic, ventilatory or pulmonary functions, airway reactivity, or exercise performance compared to pretreatment with placebo. ozone inhalation induced a decrease in V_{Emax}.	Gong et al. 1988
0.25	1-hour CE cycle ergometer (V_E=30 L/min/m^2 BSA)	Healthy nonsmokers, (5 M, 2 F), 22-30 yrs	12.4% decrease in FEV1, significant elevation of substance P and 8-epi-PGF2α in segmental airway washing, but not bronchoalveolar lavage fluid	Hazbun et al. 1993
0.30	1-hour CE cycle ergometer (mean V_E=60 L/min)	Healthy, (5 M), age not given	Decrease in FVC and FEV1, increase in SR_{aw} 1-hour post-ozone exposure. Increased percentage PMNs at 1, 6 and 24 hour post-ozone exposure in bronchial fraction of BALF, compared to FA exposure. PMNs peaked at 6 hour post-ozone in the bronchial fraction of BALF. Percent PMNs elevated at 6 and 24 hour post-ozone in pooled samples.	Schelegle et al. 1991
0.40	2 hour, IE (15 min ex/15 min rest), (V_E=70 L/min)	Healthy males (N=11), 18-35 yrs	NL performed immediately before, immediately after and 22 hour after exposures. Increased number of PMNs at both post-ozone exposure times. Increased tryptase immediately after ozone exposure and increased albumin at 22 hour after ozone exposure.	Graham and Koren 1990 Koren et al. 1990
0.40	2 hour at rest	Nonsmoking, mild asthmatics, (N=10), 18-35 yrs	Nonsignificant increase in response to allergen after ozone exposure. PMN and eosinophils increased after ozone+allergen challenge. ozone alone increased nasal inflammation. An eleventh subject dropped out before completing the protocols.	Peden et al. 1995
0.40	2 hour IE treadmill exercise (V_E=50-75 L/min)	Healthy, (8 M), 18-27 yrs	Decreases in FVC, FEV1, VI and TLC, and increases in SR_{aw} and f_R with ozone exposure compared to FA. Pretreatment with atropine abolished the ozone-induced increase in SR_{aw} and attenuated the FEV1 and FEF25-75% response.	Beckett et al. 1985

Table 9-8 (cont.): Other Controlled Human Exposure Studies of Inflammatory and Host Defense Effects

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.40	2 hour IE, alternating 15 min rest and treadmill exercise periods (V_E=35 L/min/m^2)	Healthy nonsmokers (11 M), 18-35 yrs	No correlation between pulmonary function and inflammatory endpoints measured in BAL fluid obtained 18-hour after exposure. At 18-hour post-ozone exposure there was an increase in the percentage of PMNs, total protein, albumin, IgG and neutrophil elastase, and a decrease in the percentage of macrophages, compared to following FA exposure.	Koren et al. 1989b
0.40	2 hour IE, alternating 15 min rest and treadmill exercise periods (V_E=35 L/min/m^2)	Healthy nonsmokers, (10 M), 18-35 yrs	PMN, PGE_2 and IL-6 higher in BAL fluid obtained 1-hour post-ozone exposure compared to in BAL obtained at 18-hour post-ozone exposure. Fibronectin and urokinase-type plasminogen activator higher at 18-hour post-ozone exposure than at 1-hour post-exposure.	Koren et al. 1991
0.40	2 hour IE, alternating 15 min rest and cycle ergometry (V_E=30 L/min/m^2 BSA)	Healthy nonsmokers, (13 M), 18-31 yrs	Indomethacin pretreatment attenuated ozone-induced decrements in FVC and FEV1, compared to ozone exposure alone. Indomethacin pretreatment did not significantly affect airway hyperresponsiveness.	Ying et al. 1990
0.40 0.60	2 hour IE, cycle ergometry (100 W for males; 83 W for females)	Healthy nonsmokers, (7 M, 3 F), 23-41 yrs	Increased airway responsiveness to methacholine, and in mean percentage of neutrophils, PGF2α, TXB_2 and PGE_2 concentrations in BALF 3 hour after both ozone exposures, compared to after FA exposure.	Seltzer et al. 1986
0.50	2 hour IE, alternating 15 min rest and treadmill exercise (V_E=40 L/min)	Healthy, (18 M), 20-30 yrs	Decrease in FVC, V_T, and maximal transpulmonary pressure, increase in SR_{aw} and f_R with ozone exposure, compared to FA exposure. Lidocaine inhalation partially reversed the decrease in FVC.	Hazucha et al. 1989

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

9.6.3.1 Introduction

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

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

9.6.3.2 Healthy Subjects

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

even at rest, can lead to adverse effects. Further, the effective dose of ozone inhaled by these subjects is in the range used in more recent studies that addressed the effects of exposure to a lower concentration of ozone over a simulated day of active work or play.

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

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

Horstman et al. (1990) expanded the database on multi-hour ozone exposures by exposing healthy young males to filtered air, 0.08, 0.10, and 0.12 ppm ozone using the 6.6-hour protocol. Each subject served as his own control. The three primary variables investigated were FEV1, pain on deep inspiration and the provocative dose of methacholine inducing a 100% increase in SR_{aw} (PD100). There were significant changes in all three primary variables following all three ozone exposures. The decrease in FEV1 became significant after the 5th hour of the exposure to 0.08 ppm ozone, the 4th hour of the exposure to 0.10 ppm, and the 3rd hour of the exposure to 0.12 ppm ozone. The range of subject responses and the mean decrements were somewhat smaller than in Folinsbee et al. (Folinsbee et al. 1988), but the overall findings were similar. Several subjects did not respond to any concentration of ozone, while others were responsive to all the ozone concentrations used. Subjects returned to the laboratory the next day for pulmonary function measurements. At 20 to 25 hours post-ozone exposure, FEV1 had returned to within 1% of baseline in these subjects. There was an inverse relationship between PD100 and ozone concentration, indicating that ozone exposure increased nonspecific airway reactivity in a dose-dependent manner. Larsen et al. (1991) used the data from Horstman et al. (1990) to develop a

“dose-response” relationship for percent change in FEV1 as a function of ozone concentration and exposure duration. The analysis suggested that FEV1 responses were approximately linear with exposure duration, and that ozone concentration played a slightly more significant role in predicting the effect size.

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

The fourth study in the US EPA series of multi-hour studies (Folinsbee et al. 1994) reported on the responses of 17 young males exposed to 0.12 ppm ozone for 6.6-hour on 5 consecutive days. The study was designed to evaluate whether the attenuation responses observed in studies performed at higher ozone concentrations for 1-2 hours (see Section 9.6.9) also occurred with multi-hour exposures to lower levels of ozone. The day-1 results were similar to those of the three studies described above, with the day-1 and day-2 changes in FEV1 averaging -11.9% and -6.23%, respectively. On day-3 through day-5 the changes in FEV1 were not different from the FA condition. Individual decrements in FEV1 ranged from +0.3% to –34.3%, and +2.9% to –33% on day-1 and day-2, respectively. Although most of the subjects had little or no change in FEV1 on day-3 through day-5, there were five subjects who had decrements on FEV1 between 5% and 10%. Reports of lower respiratory symptoms were increased only on day-1. Interestingly, responsiveness to methacholine was increased following all five ozone exposures compared to the FA exposure, but it was not correlated with either symptoms or with the change in FEV1. Fifteen of the subjects were also exposed to 0.16 ppm ozone for 4 hour while exercising for 50-min per hour at the same workload as used in the 6.6-hour exposures described above. The group mean decrements in FVC and FEV1 were -9.53% and -16.6%, respectively. Analysis of nasal lavage failed to show evidence of nasal inflammation. The larger decrements in measures of pulmonary function following the 4 hour exposure to 0.16 ppm ozone compared to the 6.6 hour exposure to 0.12 ppm ozone highlights the greater significance of the ozone concentration compared to exposure duration in inducing pulmonary function decrements. The results also confirm previous findings that pulmonary function responses and non-specific airway responsiveness to ozone exposure have different biological mechanisms (see Section 9.3).

Folinsbee et al. (1991) combined the FEV1 response data from the studies conducted at the EPA Health Effects Research Laboratory (Folinsbee et al. 1988; Horstman et al. 1990; McDonnell et al. 1991), and examined the distribution of responses among the

subjects at 0.0, 0.08, 0.10 and 0.12 ppm ozone. The results of the analysis indicated that 46% of the subjects studied had decrements in FEV1 greater than 10% after a 6.6-hour exposure to 0.12 ppm ozone. The percentages of subjects having greater than 10% reduction in FEV1 were 0%, 26% and 31% for FA, 0.08 and 0.10 ppm ozone exposures, respectively. Furthermore, the analysis showed that FEV1 decrements as large as 30% to 50% occurred in some individual subjects undergoing 6.6-hour exposure to ozone concentrations less than 0.12 ppm, confirming the previously observed wide range of individual response to ozone exposure, as well as adverse effects at levels typical of widespread ambient exposures (see Figure 9-2 above).

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

To explore the relative influences of ozone concentration, ventilation rate, and exposure duration on response magnitude, Hazucha et al. (1992) designed a protocol utilizing 8-hour exposures with two different ozone concentration profiles. The study compared responses to exposure to a constant ozone concentration of 0.12 ppm, and to a variable concentration profile (linear increase from 0 to 0.24 ppm over four hours, followed by linear decrease from 0.24 to 0 ppm over 4 hours). The results are illustrated in Figure 9-3, below. The total inhaled effective dose of ozone was equivalent for the two exposures (difference $<$ 1%). Exposure to the constant ozone concentration induced a group mean decrement in FEV1 of approximately 5% by the fifth hour of exposure, which did not change over the remainder of the exposure, indicating a response plateau, consistent with Horstman et al. (1990). In contrast, with the variable concentration protocol, the response over the first three hours was minimal, followed by a mean decrease in FEV1 over hours 4 through 6 that peaked at approximately 10%. There appears to be a lag in development of the maximal response, since the maximal ozone concentration occurred

at hour 4, yet the maximal FEV1 response occurred at hour 6. FEV1 improved during the last two hours of the exposure, and by the end of the exposure the FEV1 decrement was nearly identical to that following the constant concentration exposure. Adams (2003a) also compared responses of healthy young adults to two ozone concentration profiles: (1) a constant ozone concentration of 0.08 ppm, and (2) a triangular ozone profile where the ozone concentration increased from 0.03 ppm to 0.15 ppm over four hours, and then decreased to 0.03 over the next 2.6 hours (mean ozone concentration = 0.08 ppm). The total inhaled dose of ozone was equivalent for both protocols. The group mean decrement in FEV1 was smaller, at least partly due to the lower ozone concentration, compared to Hazucha et al. (1992), the maximal decrement occurred at the time of the peak ozone concentration with the triangular profile, but after six hours in the constant concentration exposure. The results of both of these studies illustrate that the FEV1 response is dependent on the dose rate as well as the cumulative dose of ozone inhaled, at least when the ozone concentration is variable. Furthermore, findings from both Hazucha et al. (1992) and Adams (2003) confirm that ozone concentration is the most important factor in determining responses to ozone exposure, compared to either exposure duration or ventilation.

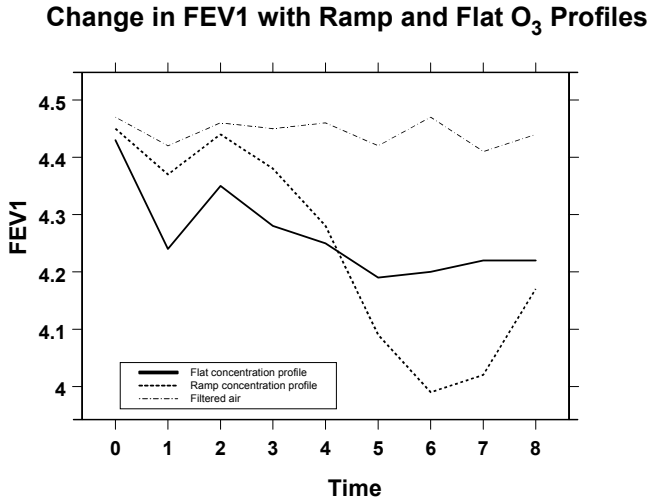

Figure 9-3 Comparison of the change in FEV1 with ramp and flat ozone concentration profiles. Derived from Hazucha, 1993.

Adams (2000b) investigated whether different equivalent ventilation rates (EVR) between subjects altered responses to 0.12 ppm ozone using the USEPA 6.6 hour protocol. EVR is a means of normalizing ventilation by scaling it to body surface area (V_E/BSA). Healthy young adult males and females completed nine ozone exposures involving varying ozone concentrations, exposure durations, and several EVRs, as well as one FA exposure. The results showed that because of the EVR methodology, the smallest subjects had lower exercise V_E rates (~26 L/min) than the largest subjects (~44 L/min), leading to smaller subjects inhaling lower doses of ozone than larger subjects.

Changes in FEV1, V_T, f_R and symptoms following ozone exposure were correlated with V_E, and not with lung or body size. These results lead to the conclusion that responses to ozone exposure are related to total inhaled ozone dose, and are not a function of lung or body size, in accord with reports by McDonnell et al. (1997), and Messineo and Adams (1990). Figure 9-4, below, illustrates the mean hourly change in FEV1 and total symptoms score at each of the inhaled ozone doses studied.

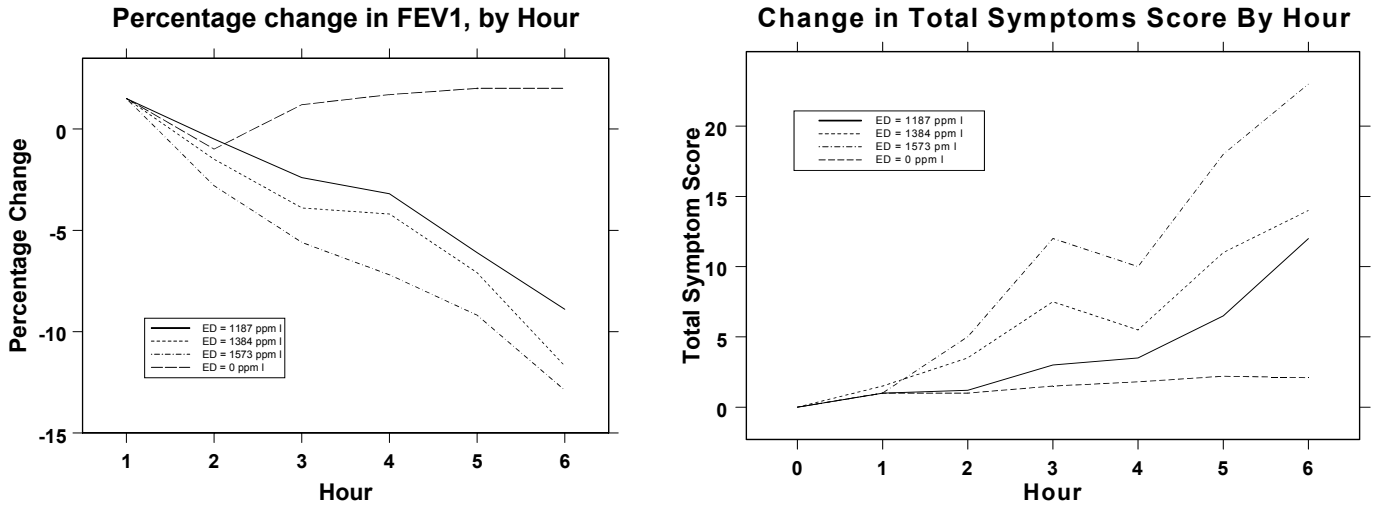

Figure 9-4 Percentage change in FEV1 and total symptoms score by hour with exposure to FA and three inhaled effective doses (ED) of ozone. The ozone concentration was 0.12 ppm, and effective dose was varied by changing V_E (17, 20, or 23 L/min/m^2 BSA). Derived from Adams, 2000b.

Adams (2002) compared the responses of healthy adults who were exposed to ozone in an environmental chamber and through a facemask system that allowed natural breathing while they completed the 6.6 hour protocol. The subjects inhaled FA and 0.12 ppm ozone in the chamber, and 0.04, 0.08 and 0.12 ppm ozone via the face mask system. Results with the facemask system were comparable to those obtained on the same subjects exposed in an environmental chamber. The changes in FEV1 were +2%, +1%, -4% and -13% for the FA, 0.04 ppm, 0.08 ppm and 0.12 ppm ozone conditions, respectively (Figure 9-5). The decrement at 0.08 ppm ozone was significantly larger than that following the FA exposure, and was significantly smaller that that at 0.12 ppm ozone. The change following the 0.04 ppm ozone exposure was not different from that with FA exposure. However, although the group mean decrement following the 0.04 ppm was not statistically significant, the range of changes in FEV1.0 for the group was +7.8% to –8.2%. Total symptoms score was statistically significant following the exposure to 0.08 ppm ozone, but symptoms following the 0.04 ppm ozone exposure were not different from following the FA exposure. Pain on deep breath was only significantly greater than following FA exposure for the 0.12 ppm ozone exposures. The subjects in this study demonstrated somewhat smaller responses to the exposure to

0.08 ppm, compared to similar studies conducted at the USEPA laboratory (Folinsbee et al., 1988; Horstman et al., 1990; McDonnell et al., 1991). This discrepancy may be due to differences in the individual sensitivity of the subjects who participated in the studies, or to the difference in exposure mode (face mask vs. chamber). The subjects in Adams (2002) completed the 0.08 ppm ozone exposure with the face mask system only, so the latter issue can not be resolved with the available data.

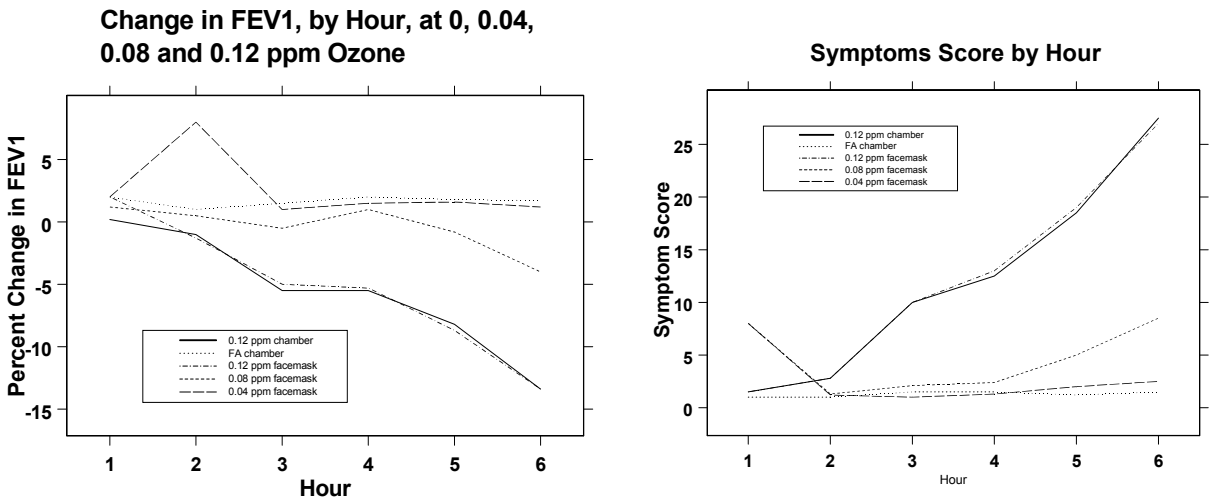

Figure 9-5 Change by hour in FEV1 and total symptoms score with four different inhaled ozone doses. Derived from Adams, 2002.

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

Table 9-9: Pulmonary Function Effects with Prolonged Exposures to Ozone

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

Table 9-9 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

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

Table 9-9 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

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

Table 9-9 (cont.): Pulmonary Function Effects With Prolonged Exposures to Ozone

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

9.6.3.3 Summary

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

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

9.6.3.4 Cellular and Biochemical Responses to Multi-Hour ozone Exposures

Several studies have investigated the cellular and biochemical changes induced in the lungs by multi-hour ozone exposure. In one study, subjects underwent fiberoptic bronchoscopy with bronchoalveolar lavage 16-18-hours following exposure to FA (N=18), 0.10 ppm (N=10) or 0.08 ppm (N=18) ozone using the 6.6 hour protocol described above (Devlin et al. 1991). Analysis of the BALF recovered from these subjects showed evidence for neutrophilic inflammation in the alveolar region, epithelial damage, and cell wall disruption. These conclusions are substantiated by the statistically significant increase in neutrophils, PGE_2, IL-6, and α_1-antitrypsin measured in the BAL fluid following exposure to 0.08 ppm ozone, compared to following FA exposure. Exposure to 0.08 ppm ozone also decreased the ability of alveolar macrophages to phagocytize microorganisms via the complement receptor. This has implications for defense against microbial pathogens in humans. These changes are indicative of pulmonary inflammation, and activation of inflammatory cells. A consideration in interpreting this study is that the data were analyzed with single-tailed paired T-tests (each subject served as his own control), without correction of the P-value for the multiple tests. The investigators addressed this issue by limiting the primary hypothesis tested formally to the change in neutrophils following both concentrations of ozone. The other analyses were performed as exploratory analyses, and the investigators acknowledged that some of these may appear significant at the P=0.05 level due to random chance. The data also suggest that ozone exposures at these concentrations could lead to fibrotic changes in the lung tissues, based on the increased fibronectin and protein recovered following the exposure to 0.10 ppm ozone. There was a considerable range of response magnitude between individual subjects in the changes in the cellular and biochemical markers measured, suggesting that there is

a sub-fraction of the population that is very sensitive to the inflammatory effects of ozone. Generally, changes after exposure to 0.10 ppm ozone were larger than those after 0.08 ppm ozone.

Table 9-10: Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

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

Table 9-10 (cont.): Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

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

Table 9-10 (cont.): Airway Hyperresponsiveness and Inflammatory Effects With Multi-Hour Ozone Exposures

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

9.6.3.5 Responses of Allergic Asthmatic Subjects to Multi-Hour ozone Exposures

Four studies have investigated the responses of allergic asthmatics to multi-hour ozone exposures. The first, Linn et al. (1994), utilized the 6.6-hour protocol in an evaluation of the responses of 15 healthy and 30 asthmatic subjects to FA, or 0.12 ppm ozone, and 100 μg/m^3 sulfuric acid, singly and combined, with each atmosphere inhaled on two consecutive days. When the pulmonary function changes were analyzed on a percentage change basis to remove the influence of differences in baseline lung function, the responses of the asthmatic and nonasthmatic groups were similar. However, the changes in the asthmatic group were superimposed onto generally reduced pulmonary function at baseline, the asthmatic subjects having reduced FEV1 even with FA exposure. The reduction in FEV1 and increase in SR_{aw} became significant after the third hour of exposure, and the subjects reported increased symptoms during the last two hours of the day-1 ozone exposure. Both the normal and asthmatic groups had small, statistically significant mean decrements in FEV1 following the day-1 ozone exposure, which were accompanied by increased nonspecific airway responsiveness. However, there were no statistically significant differences between the decrements in FEV1 of the normal (-1.7%) and asthmatic groups (-7.8%) due to the wide range of variability between the individual subjects in both groups. FEV1, SR_{aw} and symptom responses were smaller on day-2 of exposure, in agreement with the findings of Horvath et al. (1991) and Folinsbee et al. (1994). The smaller mean decrements observed on day-1 in this study compared to Folinsbee et al. (1988;1994), and Horstman et al. (1990) may be related to previous ambient ozone exposure in these Los Angeles residents, as discussed in Section 9.6.9, or to a larger number of ozone-insensitive subjects in the group.

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

of inhalation, and thus reduced FVC, which in turn reduces FEV1, although bronchoconstriction was evident to a greater degree in the asthmatic than nonasthmatic subjects.

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

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

challenge, followed the next two mornings (days 3 and 4) by challenge with aerosolized histamine and lung function testing, in order to assess the duration of enhanced airway hyperresponsiveness. Seven of the nine subjects responded to a lower PD20 of allergen after ozone exposure (0.58 mean dose shift in the doubling concentration of allergen; P=0.03). After the allergen challenge, subjects were monitored in the laboratory until their pulmonary function was stable, at which time they were admitted to the University General Clinical Research Center, where their lung function was monitored hourly. Three of the subjects had late-phase responses, defined as a greater than 20% decrement in FEV1 more than four hours after the allergen challenge, after both allergen challenges (i.e., allergen following FA or ozone), and one had a late-phase response only after the FA/allergen exposure. The investigators had hypothesized that airway inflammation consequent to ozone exposure and allergen challenge would increase nonspecific airway hyperreactivity, providing a possible mechanism for epidemiological findings suggesting a correlation between asthma exacerbation and ozone concentration on the previous day. However, contrary to expectations, airway reactivity, measured as bronchial reactivity to the nonspecific bronchoconstrictor histamine, was increased after the FA/allergen exposure, but not after the ozone/allergen exposure, although the subjects received less allergen after the ozone than the FA exposure. Histamine reactivity was not assessed immediately after the FA and ozone exposures (or before the allergen challenge), so the study does not speak to whether or not ozone exposure alone alters bronchial reactivity.

9.6.3.5.1 Summary

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

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

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

9.6.3.6 Responses of Animals to Controlled Concentrations of Ozone

9.6.3.6.1 Introduction

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

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

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

9.6.3.6.2 Ventilatory and Breathing Pattern Responses

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

In rats, repeated daily inhalation of ozone of concentrations up to 1.0 ppm have shown that the first and second day of exposure are characterized by a rapid shallow breathing pattern and that this breathing pattern response is reduced on each consecutive day of ozone inhalation (Tepper et al. 1989; Schelegle et al. 2003). If this cycle of five consecutive days of ozone inhalation is made part of a repeated episode regimen with 5d of exposure followed by 9d of recovery in filtered air, the same pattern of initial rapid shallow breathing, followed by response attenuation, was reproduced with up to 4 consecutive episodes (Schelegle, et al., 2003). Interestingly, this pattern of breathing pattern response consistently occurred in each cycle of ozone inhalation, despite the observation that the pattern of injury and inflammation was different with each consecutive episode (Schelegle, et al., 2003). When daily ozone exposure was continued for 1, 3, 13, 52 and 78 weeks, Tepper et al. (1991) observed a decreased ventilatory response to CO_2 challenge that was associated with an increase in expiratory resistance at all time points, but was greatest at 78 weeks. Wiester et al. (1995) used a similar daily exposure for 52 and 78 weeks and observed that the rapid shallow breathing response induced by a 2 hour inhalation of 1.0 ppm ozone was attenuated compared to FA controls. In addition, Wiester, et al. (1995) observed that the rapid shallow breathing response to a similar acute ozone challenge returned when the rats were allowed to breath filtered air for 4 months following the 52 or 78 week daily exposure protocol.

Table 9-11: Ventilatory and Breathing Pattern Responses

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

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

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

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

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

9.6.3.6.3 Lung Mechanics Responses

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

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

Table 9-42: Lung Mechanics

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

Table 9-12 (cont.): Lung Mechanics

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

Table 9-12 (cont.): Lung Mechanics

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

Table 9-12 (cont.): Lung Mechanics

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

9.6.3.6.4 Airway Responsiveness

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

Table 9-13: Airways Responsiveness

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

Table 9-13 (cont.): Airway Responsiveness

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

9.6.3.6.5 Host Defense – Animal Models

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

9.6.3.6.6 Inflammatory and Biochemical Responses in Animal Models

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

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

9.6.3.6.7 Effects of Ozone on Lung Development

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

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

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

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

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

airway. This phenotype was characterized by increased HDMA-induced histamine release as measured by serum histamine, elevated BAL eosinophils, and increased airway resistance and reactivity. The increased levels of serum HDMA-specific IgE is consistent with the concept that 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). These changes were measured by immunohistochemical staining for the presence of nerve cells. The changes noted included significant decreases in the density of epithelial nerves in the midlevel airways (between the sixth and seventh intrapulmonary airway generations) upon ozone exposure; these decrements increased in the ozone plus HDMA treated group. These changes were also accompanied by the appearance of abnormal streaks and clusters of nerve cells in the airways just proximal to the midlevel generations. The authors conclude that these effects represent either neural regression or stunted nerve development in the airway. Summary

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

9.6.4 Subjects with Pre-Existing Disease

9.6.4.1 Subjects with asthma

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

couple of decades. Asthma is the most common chronic disease of childhood, and has been estimated to be responsible for about 14,000,000 missed school days per year (Centers for Disease Control and Prevention National Center for Environmental Health 2004). Children are disproportionately impacted by asthma as they have higher prevalence rates and the highest asthma hospitalization rates are for 0-4 year olds. This is likely due at least partially to physics –airway resistance is inversely proportional to the 4th power of the radius. Thus in a small child a small degree of airway constriction can result in serious breathing difficulty.

Because of the underlying airway inflammation and hyperresponsiveness characteristic of this disease, asthmatics may have exacerbations of their disease upon exposure to respiratory irritants, such as ozone. Although asthmatic and ozone-induced airways inflammation are mediated through different biological mechanisms, it is likely that ozone-induced inflammation represents an additive effect. As a result, asthmatics have been considered a potentially susceptible subpopulation in setting air quality standards, and there have been a considerable number of studies intended to examine differences in response to ozone between asthmatics and nonasthmatics.

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

Many of the initial controlled ozone exposure studies focused on identifying differential symptom reporting and lung function changes between asthmatics and nonasthmatics. However, in the 1987 review of the ozone standard, the Staff

Report concluded that, based on controlled exposure studies published up to that time, one could not draw definitive inferences regarding whether individuals with asthma (or COPD, see below) were more sensitive to the acute effects of ozone than healthy individuals. Less demanding study protocols (resulting in lower breathing rates, and hence lower effective doses for asthmatics) and, in some studies, failure to control for medication use made it difficult to compare the results of these one- and two-hour exposure studies with those involving healthy subjects (California Department of Health Services 1987). Moreover, no studies involving such potentially susceptible populations had examined inflammatory responses. Studies conducted since 1987 have confirmed that low-level ozone exposures of short duration do not apparently result in lung function or symptomatic changes that differentially affect asthmatics participating in such comparative studies. In this regard it should be borne in mind not only that asthmatics have lower baseline lung function, which may be overlooked if one is only examining percentage changes in FEV1, for example. In addition, controlled exposure study protocols in general favor the selection of individuals who are not representative of the asthma spectrum. For instance, anyone who has had a recent respiratory infection will not be tested; on the other hand, in real life such infections are common causes of prolonged airway responsiveness in asthmatics. More importantly, there is likely to be a self-selection bias among asthmatics willing to be exposed to a known respiratory irritant.

Despite these limitations, several studies have demonstrated that asthmatics, whose airways already exhibit ongoing inflammation, experience a more vigorous ozone-induced inflammatory response, in both upper and lower airways, than nonasthmatics (Scannell et al. 1996; McBride et al. 1994). Higher effective doses, resulting from higher exposure concentrations and/or longer exposure durations, may cause greater airway obstruction in asthmatics relative to nonasthmatics. In addition, ozone exposure may potentiate the effects of exposure to other pollutants or to aeroallergens. Some of these results, particularly those indicating increased inflammation, may help explain the consistent epidemiological findings of an association between ambient ozone levels and increased asthma disease (see Sections 10.1.3, 10.2)

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

Investigations published since 1987 are consistent with the earlier reports in demonstrating that, for short exposures (30 min to 1-hour), asthmatics participating in controlled exposure studies with ozone concentrations modestly higher than the California standard do not appear to show lung function or symptomatic effects greater than nonasthmatics. Koenig et al. (1988a) exposed 24 adolescents, including 12 with mild asthma, to FA or 0.12 ppm ozone and 0.30 ppm NO_2, alone or in combination, for one hour of intermittent moderate exercise (mean ventilation rate = 32.5 L/min). No significant ozone-related changes were observed for FEV1, FVC, total respiratory resistance, peak flow, or maximal flow at 75% of FVC. However, a significant decrease was observed for maximal flow at 50% of FVC in asthmatic subjects only. In contrast, after the

combined ozone-NO_2 exposure no significant changes were observed for any of the lung function metrics in either group. The investigators suggested that the single statistically significant finding might have been due to chance (multiple comparisons). In another study, Koenig et al. (1987) studied responses of 10 healthy and 10 asthmatic adolescents exposed to 0.12 and 0.18 ppm ozone for 30 minutes at rest, followed by 10 minutes of moderate treadmill exercise. While there was an effect on total respiratory resistance in both groups at 0.18 ppm, there were no consistent responses in other lung function measures (FEV1, FVC, peak flow, or maximal flow at 50 % and at 75% of FVC) at either concentration.

Several other studies intended to examine joint effects of ozone with other exposures (aeroallergens, exercise, or sulfur dioxide,) have also demonstrated no effect of low ozone exposure concentrations on lung function or symptoms in asthmatics (0.12 ppm x 1-hour or less -- Ball et al. 1996; Molfino et al. 1991; Hanania et al. 1998; Fernandes et al. 1994; Koenig et al. 1990).

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

Since the publication of the Kreit study, most studies examining primarily the effects of ozone on asthmatics' lung function have involved larger effective doses than earlier investigations. However, the evidence regarding whether asthmatics show greater ozone-associated evidence of airway obstruction is mixed. For instance, Stenfors et al. (2002) found modestly greater effects on FVC and FEV1 in healthy relative to mildly asthmatic subjects exposed to 0.20 ppm ozone for 2 hour, with alternating 15-minute periods of rest and exercise, though there were

no significant between-group differences in the magnitude of the effects on lung function. Several others (reviewed in detail in Section 9.6.3.5) have used a 6.6-hour or 7.6-hour protocol. While not entirely consistent, these papers suggest differential susceptibility of asthmatics to an effective dose of ozone greater than in the earlier studies. For instance, Horstman et al. (1995) found greater spirometric changes in asthmatic than in nonasthmatic adults exposed to 0.16 ppm ozone for 7.6 hour, with intermittent exercise. Several of the asthmatics (6/17) required rescue medication to treat symptoms of bronchoconstriction. In contrast, Linn et al. (1994) did not find a difference between asthmatic and healthy subjects in a multi-hour exposure study in Los Angeles. In that study, the investigators exposed 15 healthy and 30 asthmatic subjects, aged 18-50, to FA or 0.12 ppm ozone and 100 $\mu g/m^3$ sulfuric acid, singly and in combination, for 6.5 hour on two successive days per exposure regimen. At least seven of the nonasthmatic subjects were allergic (by skin test) to common aeroallergens. Although asthmatic subjects withheld inhaled bronchodilators for at least 4 hour prior to the exposures, those taking oral bronchodilators or inhaled corticosteroids continued to do so. The well recognized anti-inflammatory effects of corticosteroids could well have blunted some ozone-associated effects. This investigation took place in the Los Angeles basin during the spring, and ambient ozone levels intermittently exceeded the experimental ozone exposure concentrations. Therefore, it is likely that, for at least some of the asthmatics and healthy subjects, the overall daily effective doses of ozone were greater than those designated by the study protocol (especially on the FA exposure days), which could have modified any effects of the experimental exposures. Moreover, the overall ozone-associated effects could well have been attenuated by repeated ambient exposures, even among asthmatics (Gong et al. 1997a). Overall, the percentage decreases in FEV1 and increases in SR_{aw} and symptom scores were similar in the asthmatic and the nonasthmatic subjects; however, the former group's lung function was lower and symptom scores higher at baseline. Graphically, the changes in lung function appeared to be progressive over the course of the exposure.

Basha et al. (1994) exposed five asthmatics and five nonasthmatics (all between the ages of 18 and 45) to 0.20 ppm ozone or FA for 6 hour, with alternating 30-minute periods of rest and exercise (target ventilation rate = 5 L/min/L vital capacity). All subjects were nonsmokers and had not had a recent respiratory infection. Information on regular medication use by the asthmatics was not provided, though they did not take inhaled medications for 12 hour or oral medications for 24 hour prior to exposures. The investigators measured lung function before, at hour intervals during, and shortly after the exposures; and measured SR_{aw} before and after the exposure. Symptom scores were measured before, once during, and immediately after the exposures. In contrast to other published studies, there were no significant changes in any measure of lung function (FEV1, FVC, FEV1/FVC, and FEF25-75%) before, during, or after the exposures. Results for SR_{aw} were not reported. The nonasthmatics recorded more ozone-related symptoms (itching or burning of the nose, dry mouth or throat, burning throat, urge to cough, and nausea) than the asthmatics, though

the mean symptom scores indicated that all of these were in the range of "perceptible" or "distinctly perceptible." The only symptom in asthmatics that was significantly increased was "urge to cough." Analysis of BAL fluid in this experiment is discussed below. The effective doses in this study were smaller than in the North Carolina and Los Angeles studies, which might explain the absence of any spirometric effects in either the healthy or asthmatic subjects. Moreover, the sample size was small, and the investigators did not provide sufficient baseline data on the subjects to allow for comparison with other studies.

Peden et al. (1997) and Kehrl et al. (1999) both studied small groups of allergic asthmatics without healthy subjects as controls, using 7.6-hour exposures to 0.16 ppm FA or ozone. These groups both experienced significant ozone-associated spirometric changes that were in the range of those experienced by healthy subjects doing moderate exercise using a 6.6-hour protocol (See Section 9.6.3). As their ventilation rates were lower than those of the healthy subjects (25 L/min during exercise versus about 40 L/min, respectively), the results of these studies are not directly comparable. However, these studies illustrate that asthmatics demonstrate spirometric responses at ozone concentrations observed in California, with an extended but plausible exposure duration for individuals doing outdoor labor. Again, ozone-associated effects on lung function are superimposed on pre-existing lung function deficits.

Some controlled exposure studies, as well as several field studies, suggest that there may be some interaction between ozone and other pollutants at low ambient concentrations; however, in general, most studies do not show effects greater than those attributable to ozone alone (Koenig et al. 1990; Koenig et al. 1994; Linn et al. 1995; Linn et al. 1997; see also Sections 9.6.12, 10.1, 10.2.5).

9.6.4.3 Ozone and inflammation in asthmatics

Several studies have examined the inflammatory impact of ozone exposure in asthmatics. Taking into account their underlying chronic airway inflammation, nonetheless, asthmatics still appear to demonstrate a greater inflammatory response to ozone exposure than nonasthmatics. Scannell et al. (1996) exposed 18 adult subjects (mean age = 26; range 18 – 36) with mild asthma to FA or 0.20 ppm ozone for four hours, with 50 minutes of moderate exercise alternating with 10-minute rest periods. The subjects had taken no asthma medications for at least 24 hours prior to the exposures, and no inhaled anti-inflammatory agents for at least 48-hours. The subjects completed symptom questionnaires and underwent lung function testing immediately before and after each exposure; lung function was also measured during the first few minutes of the rest periods. Fiberoptic bronchoscopy was conducted 18-hours after the exposures, with subsequent analysis of fluids obtained by proximal airway lavage (PAL) of the left mainstem bronchus and bronchoalveolar lavage (BAL, which was further subdivided into bronchial and bronchoalveolar fractions) of the right middle lobe. There were significant ozone-associated effects on lung function (FEV1, FVC, and SR_{aw}), lower respiratory symptoms, and inflammatory markers in the PAL (percent PMNs) and BAL (percent PMNs, total protein, IL-8, LDH, and several

others) among the study subjects. Compared with 81 healthy subjects who had participated in earlier phases of the same study, there were no significant differences in symptoms or lung function changes; however, the increases in percent PMNs and total protein in BAL were significantly greater among the asthmatics. In addition, the mean ozone-associated increase in SR_{aw} was more than twice as large in the asthmatics than in the nonasthmatic subjects (4.82 versus 2.24, respectively), but this difference was not statistically significant.

In a study of five atopic asthmatic and five nonasthmatic adults described above, Basha et al. (1994) reported significant differences between asthmatics and nonasthmatics with respect to inflammatory markers in BAL fluid. The subjects underwent bronchoscopy 18-hours after a six-hour exposure to either FA or 0.20 ppm ozone. Increases in PMNs/mL lavage fluid, percent PMNs, IL-6, and IL-8 were significantly greater in the asthmatics exposed to ozone compared with FA exposure and with the healthy subjects after either exposure. TNF and albumin were also increased among the asthmatics exposed to ozone, but these were not significantly different from the FA exposure or from the healthy subjects' results. Thus, despite this study's small size, the lack of significant changes in lung function and the relatively minor symptomatic responses, ozone exposure resulted in inflammatory changes in the asthmatics' lungs that were significantly greater than in the nonasthmatics.

Recently, Stenfors et al. (2002) examined early inflammatory responses to ozone in 15 mild asthmatics (mean age 24, range 19-31) and 15 healthy subjects (mean age 29, range 21-48), who were exposed to 0.20 ppm ozone or FA for two hour, with alternating 15-minute periods of rest and exercise (ventilation rate 20 $L/min/m^2$ body surface area). All subjects were never-smokers without a recent respiratory infection. All asthmatics were atopic, and used only bronchodilator medication, as needed. Though the manuscript indicates that the subjects did not take any anti-inflammatory or anti-oxidant drugs throughout the study, it is not clear whether bronchodilator medications were also withheld. The investigators measured lung function (FEV1 and FVC) pre- and post-exposure, and also had the subjects undergo bronchoscopy at six hours post-exposure (in contrast to the 18-hour interval in the other studies). During this procedure, the investigators performed bronchial biopsies, two bronchial washings (BW), as well as BAL. As noted above, there were no between-group differences in the magnitude of lung function changes. There were significant increases in PMNs in BW, but not BAL, in both groups. The PMNs of the asthmatics were also "activated", indicated by a significant ozone-related increase in the enzyme myeloperoxidase in their BW. The bronchial biopsies showed a significant increase in PMNs in the airways of the healthy but not the asthmatic subjects. At baseline, the asthmatics had higher levels of lymphocytes and mast cells than the healthy subjects; however, ozone exposure did not affect the numbers of these cell types in BW or BAL in either group. Eosinophils were present in BW, BAL, and the tissue samples of asthmatics, but were not affected by ozone exposure. Expression of vascular endothelial adhesion molecules (P-selectin and ICAM-1), which facilitate the recruitment of inflammatory cells to the airways, was upregulated in the healthy subjects, but not the asthmatics. Healthy subjects also experienced ozone-

associated increases of PMNs and mast cells in their airway walls, while the asthmatics did not. Under the conditions of this experiment, the asthmatic subjects did not experience greater inflammatory responses to ozone exposure than the nonasthmatics. However, the overall exposure was shorter and involved a lower dose of ozone than other studies examining inflammatory responses; moreover, the samples were taken much earlier after the exposures (at six hours rather than at 18-hours). Therefore, in the study by Stenfors et al. (2002), insufficient time might have elapsed prior to the BAL to be able to detect full inflammatory responses. In addition, they apparently did not measure levels of IL-8, a chemoattractant for PMNs, which might have given an indication of early inflammatory differences between the groups.

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

Peden et al. (1997) studied eight allergic asthmatics exposed to FA or 0.16 ppm ozone for 7.6 hour (see Section 9.6.3.5). All subjects had mild asthma and all were sensitized to dust mite allergen (*Dermatophagoides farinae*). Bronchoscopy and BAL were performed 18-hours after the FA and ozone exposures. Ozone exposure was associated with increased PMNs and eosinophils in BAL, but not lymphocytes, macrophages, or biochemical inflammatory mediators. In the BAL fluid, the mean PMN percentage nearly tripled between FA and ozone exposures (2.5 to 8.6), while the percentage of eosinophils increased by a factor slightly greater than two (1.8 to 3.6). These effects were more pronounced when the investigators examined the initial fraction of the BAL (mean percentage increases from 2.8 to 11.5 for PMNs, and from 2.9 to 17.8 for eosinophils). While other studies had previously demonstrated an influx of PMNs (or neutrophils) after ozone exposure, this study demonstrated such an effect with eosinophils, as well. Unlike the previous studies by Scannell et al. (1996) and Basha (1994), the subjects in this study also had a small percentage of eosinophils present in BAL fluid after air exposure. Eosinophilic infiltration into the airways and airway nerves is characteristic of allergic asthma, and plays an important role in asthma exacerbations (Costello et al. 2000; Green et al. 2002). Therefore, to the extent

that ozone exposure may exacerbate pre-existing eosinophilic airway inflammation among asthmatics, it may have important clinical implications. Eosinophilic inflammation in asthmatics has no equivalent in healthy, nonatopic individuals; therefore, when this phenomenon occurs it is by definition more severe in asthmatics than nonasthmatics.

Hiltermann et al. (1999) compared the values of inflammatory markers in induced sputum versus BAL fluid of 16 asthmatics exposed to 0.40 ppm ozone on two occasions for two hour with alternating 15 minute periods of exercise (20 l/min/m^2 body surface area). At this concentration, the investigators observed significant increases in several indicators of inflammation: total cells (by 1.6-fold), eosinophilic cationic protein (ECP – 1.8-fold), and neutrophil elastase (5-fold). Comparing BAL fluid obtained 18-hour after either FA or ozone exposure, they observed significant increases in albumin, ECP, elastase, percentage PMNs, and percentage lymphocytes. The ozone-associated changes in ECP and IL-8 (which increased in both induced sputum and BAL fluid, though these increases were not statistically significant) were highly correlated, as were the numbers and percentage changes in eosinophils in sputum and BAL fluid. The percentage change in eosinophils, but not PMNs, in induced sputum was also correlated with airway hyperresponsiveness (which was also measured in this study). This study demonstrated that induced sputum could be used in place of the more invasive bronchoalveolar lavage to measure ozone-associated inflammation in asthmatics.

Using induced sputum to measure inflammatory markers, Vagaggini et al. (2002) recently reported that ozone exposure can magnify eosinophilic responses caused by allergen challenge in allergic asthmatics. Previously, these investigators had found that exposure to ozone alone at 0.27 ppm for two hour resulted in enhanced neutrophilic, but not eosinophilic, inflammation in induced sputum of 11 subjects with mild asthma, which is consistent with the results of other investigations (Scannell et al. 1996; Basha et al. 1994 ; Vagaggini et al. 2001). However, in contrast to other studies examining whether ozone pre-exposure enhances the response to allergen in asthmatics (see below), these investigators reversed the order of exposure. In a randomized, single-blind study, 12 mild asthmatics (age range 18-31) were exposed to aeroallergen challenge, followed 24 hours later by either FA or ozone (0.27 ppm) for two hours with intermittent exercise (20 min/hour, ventilation rate 25 L/m^2 body surface area). The subjects had mild persistent asthma, but used only bronchodilators as needed (i.e., they did not take anti-inflammatory medications). Presumably medication was not withheld during this experiment, as the aeroallergen challenges alone caused substantial changes in lung function. Aeroallergens were dust mite (n=8), timothy grass (n=3) and a pollen (*parietaria*) (n=1). Six hours after the FA or ozone exposure, the investigators collected sputum induced with hypertonic saline. They reported that, while there were no differences in IL-8 concentrations or percentages of PMNs in induced sputum, there was a significant difference between the percentage of eosinophils after ozone exposure (27.5% [2.3-72.8%]) compared with FA exposure (9.9% [3.5-71.5%]) ($p = 0.04$). It is well established that allergen challenge elicits an eosinophilic influx

into the airways of asthmatics (Sulakvelidze et al. 1998; Gauvreau et al. 1999). Vagaggini et al. (2002) found that subsequent ozone exposure could in fact augment this eosinophilic response. These results may also help explain the enhanced eosinophilic response in the study by Peden et al. (1997), in that the subjects in that investigation were likely to have had ongoing regular household exposure to house dust mite allergen, which may in turn have been at least in part responsible for the underlying eosinophilic inflammation. Along with the study by Peden et al. (1997), this finding may have important clinical ramifications, as sputum eosinophil counts are a good predictor of asthma (Green et al. 2002). However, the ozone concentration used in this study is substantially greater than those typically found in the U.S. today. It would be useful to know whether such effects can be observed at lower ozone concentrations as well.

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

9.6.4.4 Interactions with other exposures

The effects of ozone plus other exposures, including exercise, respiratory irritants, and allergens have been conducted in controlled settings. Investigations of the effects of ozone plus other air pollutants are reviewed in Section 9.6.12. One potential concern among asthmatics sensitized to aeroallergens is that ozone could lower the “threshold” of response to a given allergen, potentially increasing individuals’ susceptibility to the effects of aeroallergens. There have been several studies examining this phenomenon, with somewhat inconsistent results. All such studies, however, used ozone doses that exceeded the current California ozone ambient air quality standard by at least 33%.

9.6.4.5 Ozone and allergen exposure

Allergy plays a large role in most cases of asthma. Among individuals sensitized to an aeroallergen, such as pollen, airway narrowing begins to develop within minutes of inhalation of allergen. This reaches a maximum within about a half-hour and often resolves within one to three hours. About half of adult asthmatics and 70-85% of children who experience this “early asthmatic response” to

allergen also develop a more, prolonged "late asthmatic response', in which airway constriction recurs after several hours, reaching a peak about 6 to 12 hours after the initial exposure (O'Byrne 1998). While both early and late asthmatic responses to allergen inhalation are mediated by IgE, the late response is associated with increased airway inflammation, notably with eosinophils, but PMNs and lymphocytes may also be increased, depending on the initial stimulus (O'Byrne 1998).

In an experiment conducted at the University of Toronto, Molfino et al. (1991) first reported that ozone could lower asthmatics' threshold of response to aeroallergens. Seven adults with mild asthma (mean age = 40, range 21-64) were exposed at rest to four exposure combinations: 0.12 ppm ozone or FA for an hour, followed by breathing either the allergen diluent (phosphate-buffered saline placebo) or the allergen. Allergens were selected according to the results of skin-prick tests for each individual. PFTs were measured for six hours (presumably once an hour) following each exposure. Airway responsiveness was measured the day before and the day after each exposure. Though the original study design called for a random allocation of exposures, the second subject experienced an increase in asthma symptoms and airway hyperresponsiveness that lasted three months after the ozone/allergen exposure. Therefore, the investigators administered this combination last to all the subsequent subjects, resulting in a nonrandomized, single-blind exposure protocol. For both allergen and the ozone/allergen combination, 3/7 subjects experienced a 15% decline in FEV1 three to six hours after the exposures. Overall, however, there did not appear to be any significant early- or late-phase change in FEV1 associated with exposure to the ozone-allergen combination in contrast to the allergen alone. However, the ozone pre-exposure resulted in a reduction of the dose of allergen required to elicit a given degree of airway responsiveness by more than half (mean (SD) PC_{15} = 0.013 (0.017) mg/mL after air-allergen and 0.0056 (0.0062) mg/mL after ozone/allergen ($p = 0.041$). However, as noted by Ball et al. (1996, discussed below), it is possible that the effects that Molfino and colleagues attributed to ozone might have been due to persistent effects of allergen on airway hyperresponsiveness, because the air/allergen exposure in this study preceded the ozone/allergen exposure for 6/7 subjects, and the time interval between the two exposures was four weeks or less for 6/7.

Investigators at the University of North Carolina (Chapel Hill) and the U.S. EPA attempted to repeat the work reported by Molfino (Ball et al. 1996), but could not reproduce the Toronto results. Following a double-blinded design, 15 adults (mean age 25.3, range 19-34) with mild asthma and allergy to grass or ragweed pollen were exposed at rest for one hour to either FA or 0.12 ppm ozone, followed by challenge with aerosolized allergen. These exposures took place outside of the pollen season. The subjects withheld bronchodilator use for 12 hour before and during testing. The subjects underwent lung function testing and answered symptom questionnaires before and after exposure to air or ozone, and were then administered an aeroallergen challenge. The next day their airway responsiveness was tested using progressively larger histamine doses until PD_{20} was attained. There was no ozone effect on FEV1 or SR_{aw}. Though the dose of

allergen required to elicit an early asthmatic response was lower in the ozone-exposed group, this difference was not statistically significant. There were no significant differences between the ozone and air exposures with respect to late asthmatic responses to allergen or airway hyperresponsiveness to post-exposure histamine challenge. Thus, Ball et al. (1996) were unable to confirm the results of Molfino et al. (1991) using a one-hour resting exposure to ozone. This may have been due, as noted above, to the nonrandom order of exposures plus the relatively short inter-exposure period in the Toronto experiment. In addition, though the subjects were generally similar in the two studies, those in the Molfino study had greater baseline airway hyperresponsiveness.

In light of the results reported by Ball et al. (1996), the University of Toronto research group repeated their experiment (0.12 ppm ozone or FA at rest for one hour, followed by aeroallergen challenge) with 15 mildly allergic asthmatic subjects rather than seven, as well as randomized and better controlled ozone exposures (0.11 to 0.13 ppm in the latter experiment versus 0.085 to 0.175 in the first) (Hanania et al. 1998). Under these conditions, they reported no effect of pre-exposure to 0.12 ppm ozone on airway responsiveness resulting from allergen challenge.

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

Kehrl et al. (1999) reported results similar to those of Jörres et al. (1996). In a double-blind, counterbalanced design, nine mildly asthmatic subjects (aged 20 – 35) were exposed to FA or to 0.16 ozone for 7.6 hour, with intermittent exercise (50 min/hour, ventilation 24 L/min), followed 18-hour later by challenge with dust mite allergen (to which all were sensitized). Seven of the nine subjects required

less allergen to attain a 20% fall in FEV1. As with the study by Jorres et al. (1996), the ozone exposure alone also evoked significant decrements in lung function. Therefore, they concluded that "ozone exposure conditions capable of inducing lung function decrements also increase airway responsiveness to specific allergen in subjects with mild atopic asthma." Moreover, in the Jörres study the increased airway responsiveness to allergen was observed shortly after (about 3 hour) the end of the ozone exposure; the report by Kehrl and colleagues indicates that this phenomenon can persist for at least 18-hours.

Finally, Jenkins et al. (1999) demonstrated that the ozone concentration is likely more important than exposure duration with respect to whether atopic asthmatics will experience enhanced airway responsiveness after a subsequent allergen challenge. Using a single-blind, randomized exposure protocol, these investigators exposed 11 mild asthmatic subjects (aged 18-45) to FA, 0.10 ppm ozone, 0.20 ppm nitrogen dioxide, or 0.10 ppm ozone plus 0.20 ppm NO_2, for six hours with intermittent exercise (for 10 out of each 40 min, ventilation 32 L/min), followed immediately by challenge with dust mite allergen. Ten of the 11 subjects were also exposed to the same gases, at twice the concentration, but for half the duration (3 hour), also followed immediately by allergen challenge. All exposures were separated by at least two weeks. Only the three-hour exposures to ozone (0.20 ppm), NO_2 (0.40 ppm), or their combination resulted in significant enhancement of airway responsiveness to allergen. Exposure for six hours at the lower pollutant concentrations did not have any significant effect, though the combination of ozone and NO_2 was of borderline significance ($p=0.067$). Thus, even though theoretically the total pollutant doses were equivalent, only the exposures involving the higher concentrations seemed to have had an impact on airway response to allergen. On the other hand, this study only examined immediate response to allergen administered after the gas exposures. In healthy subjects, an inflammatory response is clearly observable 18-hour after a 6.6-hour exposure to concentrations as low as 0.08 ppm ozone (Devlin et al. 1991). Thus, it is possible that had the allergen challenge been delayed until the day following the lower-concentration ozone exposure, increased airway hyperresponsiveness to allergen might have been observable. In addition, like many controlled exposure studies, this investigation had limited statistical power. At face value, however, this study suggests that ozone concentration may be a more important factor than duration of exposure in determining whether allergic asthmatics might experience increased responsiveness to allergen.

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

To the extent that ozone does potentiate the effects of allergen exposure in allergic asthmatics, there may be several mechanisms. In subjects with allergic rhinitis, high-dose ozone exposure by itself has been reported to cause an influx

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

9.6.5 Subjects with Allergic Rhinitis

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

McDonnell et al. (1987) investigated whether individuals (n=26, age range 18-30) with allergic rhinitis were more susceptible than those without this condition who had been tested earlier using an identical study protocol. The subjects' airway responsiveness was tested with histamine; they were then exposed for two hour to either FA or 0.18 ppm ozone, with alternating 15 minute periods of exercise (ventilation rate = 64 L/min) and rest. Exposures were counter-balanced and double-blinded. None had had symptoms of either allergic rhinitis or respiratory infection in the preceding four weeks. This population of allergic rhinitics had no history of wheeze, nocturnal cough or exertional dyspnea. Their baseline airway responsiveness was similar to that of subjects without allergic rhinitis. The

investigators took standard spirometric measurements (FVC, FEV1, FEF25-75%), airway resistance (SR_{aw}), lung volume metrics, and respiratory symptom scores. To validate the diagnosis of allergic rhinitis, the subjects were skin-tested for sensitization to common aeroallergens. FA exposure resulted in no significant changes in spirometric measures or symptoms, whereas ozone exposure resulted in significant decrements in FVC, FEV1, and FEF25-75%, and significant increases in lower respiratory symptoms (cough, pain on deep inspiration, and shortness of breath), SR_{aw}, and airway hyperresponsiveness. The degree of airway hyperresponsiveness was not associated with the magnitude of ozone-associated effects. Comparing the results of this experiment with a prior set of exposures of healthy subjects, there appeared to be little difference in response to ozone, with the possible exception of modest increases in SR_{aw} (0.46 cm H_2O*sec in the subjects with allergic rhinitis compared with 0.23 in the nonallergic subjects).

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

similar effective doses in other studies (see Section 9.6.2). Thus, the findings reported by Jorres et al. (1996) suggest that subjects with allergic rhinitis may represent a subpopulation indirectly susceptible to ozone, i.e., via the effect of ozone on their lower airway responses to allergen exposure.

Holz et al. (2002) examined the effect of single versus repeated ozone exposures on airway responses to allergen among 11 adults with allergic asthma (mean age 30+10 yr) and 22 with allergic rhinitis (mean age 29+7 yr). The subjects underwent four different exposures, which were administered in random order and were separated by at least four weeks. Each exposure lasted three hour, with alternating 15-min periods of rest and exercise. The four exposure protocols involved single exposures to FA, 0.125 or 0.25 ppm ozone, and one set of exposures to 0.125 ppm ozone on four consecutive days. Immediately post-exposure, exhaled NO was measured; one hour later, airway response to methacholine was measured (to PD_{15FEV1}). Twenty hours post-exposure, an aeroallergen challenge was administered with a single allergen dose (previously determined to result in PD_{15FEV1}). Exhaled NO was measured again one hour after the allergen challenge, and sputum induction took place six to seven hours post-allergen. PFTs were then measured hourly in the laboratory, followed by peak expiratory flow at home (for an unspecified duration). Early-phase response to allergen (measured by fall in FEV1) was significantly greater among subjects with allergic rhinitis after pre-exposure to 0.25 ppm ozone or after the repeated exposures to 0.125 ppm ozone, compared with FA pre-exposure. Ten of the 22 subjects with allergic rhinitis demonstrated at least a 20% fall in FEV1 in response to allergen after both of these ozone pre-exposures; one who showed a 29% FEV1 decrease reported “severe” symptoms after 4 x 0.125 ppm ozone. No significant effects were observed with respect to late-phase response to allergen. When compared with FA plus allergen exposure, there was a significant increase in eosinophils in induced sputum after allergen following four days of 0.125 ppm ozone exposure, but this was the only such finding among the subjects with allergic rhinitis. In contrast, asthmatics showed significant increases in a variety of indicators of inflammation in sputum after this exposure regimen. The investigators pooled the exhaled NO results for both sets of subjects, so one cannot ascertain whether there were ozone-related effects among the subjects with allergic rhinitis without asthma. These results support this group’s earlier finding that ozone may increase susceptibility to inhaled allergen (Jorres et al. 1996) and, in addition, suggest that repeated exposures to a low-level ozone concentration (0.125 ppm) among individuals with allergic rhinitis without asthma may be sufficient to enhance airway responses to allergen.

9.6.6 Subjects with Chronic Obstructive Pulmonary Disease

There have been few studies of ozone’s effects on individuals with chronic obstructive pulmonary disease (COPD), which refers to several diseases that all share the feature of airflow obstruction: chronic bronchitis, emphysema, and sometimes asthma. Recently, the U.S. Centers for Disease Control and Prevention estimated that about 10,000,000 adults in the U.S. had COPD, which was the principal cause of eight million cases of outpatient or physician visits,

119,000 deaths, 726,000 hospitalizations, and 1.5 million hospital emergency departments visits annually (Mannino et al. 2002). In the U.S., COPD is usually caused by cigarette smoking, though genetic predisposition and certain occupational exposures are also important factors in the etiology of this condition. Subjects with COPD have permanent loss of lung function and face varying degrees of disability related to respiratory impairment. Their pulmonary anatomy and physiology are distorted, leading to a nonuniform distribution of inhaled air in the lungs. Consequently, the more disease-affected regions of their lungs are poorly ventilated, and will receive a smaller local dose of ozone than better ventilated regions, which will receive a greater delivered dose.

Of the few studies examining ozone's impact on individuals with COPD, all have used exposure concentrations higher than the current standard. (Linn et al. 1982) exposed 25 older adults (mean age = 62, range 46-70), all but one of whom chronic COPD symptoms and substantial airway obstruction (FEV1/FVC mean = 50%, range 32 – 66%) to 0.12 ppm ozone or FA for one hour with alternating 15-minute periods of rest and mild exercise (mean V_E = 20 L/min during exercise). There were no ozone-associated changes in respiratory symptoms or pulmonary function. However, there was a slight but statistically significant difference in arterial oxygen saturation (SaO_2) measured by ear oximetry, comparing the mean change in SaO_2 on the ozone versus the control days. The biological significance of this change is uncertain; on average the subjects showed mild oxygen desaturation in both sets of exposures, suggesting that interference with blood oxygenation could carry clinically meaningful implications. However, as noted, there were no significant changes in symptoms in this experiment. Moreover, in a subsequent study of 28 subjects (mean age 60, range 45 –68) with COPD (FEV1/FVC mean = 59%, range 36 – 75 (Linn et al. 1983) were unable to replicate the finding of decreased SaO_2 using a similar study protocol with higher concentrations of ozone (0.18 and 0.25 ppm). Though this latter study group had somewhat less severe disease than in the earlier study by Linn et al. (1982), and the authors speculated that the subjects may have used a greater quantity of medication, nonetheless these subjects did receive markedly greater ozone doses, weakening the evidence of an ozone-associated effect on SaO_2.

In a study involving 13 subjects (mean age 56, range 43-69) with previously diagnosed COPD (FEV1/FVC mean = 57.5%, range = 46-70%), Solic et al. (1982) reported no significant ozone-associated effects on lung function or symptoms after 2-hour exposures involving 7.5 minutes of exercise (V_E = 20-30 L/min) at the end of each half hour. Like Linn et al. (1982), these investigators also observed a statistically significant difference in SaO_2 between the ozone and FA exposures. However, unlike the study by Linn et al., in which SaO_2 was measured before and at the end of each exposure, in this study, SaO_2 was measured only at the end of the exposure. Therefore, even though Solic et al. reported a small, but statistically significant exposure-related effect on SaO_2 (94.8 versus 95.3%, p=0.008), one cannot be sure that this difference can be entirely attributed to ozone because the investigators did not obtain pre-exposure oxygen saturation data. Subsequently, the same investigators conducted a similar experiment involving 13 subjects (mean age 58, range 44 –67) with mild-to-

moderate COPD (mean FEV1/FVC = 55.5%, range 37 –65) using an exposure concentration of 0.30 ppm ozone. As before, there were no significant changes in symptoms or lung function, but they did observe a similar exposure-related difference in SaO_2 (95.1% versus 94.2% at the end of the air and ozone exposures, respectively; p = 0.07 [because of an equipment malfunction only 8 of 13 had acceptable SaO_2 data]) (Kehrl et al. 1985). These investigators believed that the observed differences in end-exposure SaO_2 was due to ozone toxicity, and speculated that the difference in oxygen saturation was likely due to exaggeration of the pre-existing ventilation-perfusion mismatch characteristic of COPD.

Gong et al. (1997b) exposed nine men with severe COPD (FEV1/FVC mean = 36%, range = 20-73%) and 10 healthy age-matched controls to 0.24 ppm ozone or FA for four hours, with light exercise (V_E = 20/L/min) alternating with rest every 15 minutes. The subjects were selected to have COPD that was predominately emphysemic rather than predominately chronic bronchitic, because the latter disease characteristic would be more likely to have result in mucus secretion in the airways that could absorb inhaled ozone before it reaches the target tissue. Subjects were requested to withhold medications prior to the exposures, but some could not do so. Slightly more than half the subjects were exposed during conditions of high ambient ozone levels. Under these experimental conditions, ozone exposure resulted in a 19% FEV1 decline among COPD subjects, compared with 2% in healthy subjects (p=0.0002); however, because the COPD subjects also showed a decline of 11% during FA exposure, the overall between-group effect of ozone was not statistically significant. There were no significant ozone-associated effects on FVC, SR_{aw}, airway reactivity, SaO_2, or respiratory symptoms, though the COPD subjects' total symptom scores were modestly greater than those of the healthy subjects. The COPD subjects had lower overall mean SaO_2 (93.4% vs. 96.4%), and ozone did appear to reduce SaO_2 in both groups during the exposure period by about 1%; however, this difference had been reversed by the end of exposure. This experiment involved effective doses of ozone that were substantially greater than in the previous studies of individuals with COPD, and demonstrated a clear relationship with decreased FEV1 and an equivocal one with SaO_2. However, given the small number of individuals, the lack of detail about the extent to which medications were withheld, and the observation that more than half of the exposures occurred during a high ozone season, this study cannot be considered definitive.

In toto, these experiments involving individuals with COPD indicate that, even at concentrations substantially higher than the current standard, individuals with COPD are unlikely to experience marked respiratory effects. However, this generalization is subject to the caveats expressed in Section 9.6.1 about controlled exposure studies generally.

9.6.7 Subjects with Hypertension

In a small study of subjects with stable hypertension, (Gong et al. 1998) reported that 3-hour exposure to 0.30 ppm ozone, with intermittent 15-minute periods of rest and exercise, resulted in several ozone-associated changes in lung function

and hemodynamics. In this study (ten subjects with hypertension and six healthy subjects), these investigators measured hemodynamic variables after catheterization of the right heart and a radial artery. Though other studies have examined some cardiovascular variables in healthy subjects and some with cardiovascular disease, this was the first study to involve direct measurements using such invasive procedures. The subjects were catheterized in a hospital setting and exposed to FA on day one of the experiment, and to ozone on day two. There were no significant ozone-associated effects on numerous indicators of cardiovascular function, including blood pressure, ECG, serum enzymes (creatine phosphokinase, lactate dehydrogenase [LDH-1], and troponin T), blood hormones (norepinephrine and atrial natriuretic factor), vascular resistance (pulmonary and systemic), pulmonary artery pressure, ventricular function, arterial oxygen saturation (SaO_2), or cardiac index. However, there were statistically significant ozone-associated increases in heart rate and the rate-pressure product (product of heart rate x systolic blood pressure), and decreases in blood oxygenation, as measured by the alveolar-arterial oxygen difference (AaP_{O2}) and plasma epinephrine. In addition, the subjects experienced approximately 6% ozone-related decrements in both FEV1 and FVC. All ozone-associated changes did not differ between the hypertensive and healthy patients. The increased heart rate and AaP_{O2} suggest that, at least at this relatively high effective dose, ozone exposure may adversely affect pulmonary gas exchange, which theoretically could result in acute arterial hypoxemia for individuals with compromised oxygenating capacity. In these subjects, who had normal baseline oxygenation, no clinically meaningful untoward effects occurred. Gong et al. (1998) cite several earlier studies involving healthy subjects with inconsistent results regarding changes in AaP_{O2} (Islam et al. 1979; Linn et al. 1979); this study cannot resolve whether such changes are likely to occur in patients with cardiovascular disease at lower ozone exposure levels.

Table 9-14: Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

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

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

Ozone Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.12	One hour resting exposure to FA or 0.12 ppm ozone followed by challenge w/ allergen diluent or by allergen	7 adults w/ mild asthma (4 M, 3 F, ages 21- 64)	ozone+allergen exposure significantly reduced the amount of allergen required to elicit a given PC15. Otherwise no difference in lung function or symptoms attributable to ozone exposure.	Results may be due in part to consistent air +allergen exposure preceding ozone + allergen exposure in 6/7 subjects. Exposures single-blinded only.	Molfino et al. 1991
0.12	Separate days (3 weeks apart) exposed to FA or ozone, 1-hour at rest followed by allergen challenge to determine concentration leading to 15% fall in FEV1	15 adults with mild asthma (9 M, 6 F) 18 to 49 yrs. (mean 32.5)	No significant effect of pre-exposure to 0.12 ppm ozone on airway responsiveness to allergen challenge. No significant changes in spirometry after ozone exposure at rest.	No patients on anti-inflammatory medications. Bronchodilators withheld at least 6 hrs. Single blinded randomized. Tighter control of exposure than Molfino 1991.	Hanania et al. 1998
0.12 and 0.24	90 minutes alternating 15 min. exercise (23 L/min) and rest. Nasal lavage 10 min., 6 hour, and 24 hour post exposure.	10 atopic asthmatic (18-41 yr.) 8 non-asthmatic adults (18-35 yr.)	At 0.24 ppm asthmatics demonstrated significant increases in epithelial cells and PMNs at 10 min. and PMNs at 24 hour. At 0.12 ppm no significant changes were observed among asthmatics.	No significant changes were noted at either dosage among non-asthmatics.	McBride et al. 1994
0.12 and 0.18	30 minutes at rest	10 healthy and 10 asthmatic adolescents	Significant increase in total respiratory resistance in both groups at 0.18 ppm. No significant effect on FEV1, FVC, peak flow, or maximal flow at 50 or 75% FVC.		Koenig et al. 1987

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

Ozone Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.125 and 0.25	Single exposures to FA, 0.125 ozone, and 0.25 ozone, and 4 consecutive days to 0.125. 3 hour, w/ alternating 15-min intervals of exercise and rest. V_E=8.4 L/min (rest); 28.6 L/min (exercise)	11 asthmatics, 22 w/ allergic rhinitis	Significant decrease in FEV1, but not FVC after 0.25 ppm ozone only. No changes in methacholine airway responsiveness for any ozone dose. Increased numbers of cellular inflammatory indicators in induced sputum for 4x0.125 ppm ozone. No significant changes in early- or late-phase lung function responses to post- ozone allergen challenge.		Holz et al. 2002
mean 0.156	Ambient air (mean ozone = 0.156 ppm) , 1-hour, V_E= 38 L/min (mean)	Asthma (n=50) Healthy (n=48)	PFT changes not associated with ozone levels. Asthmatics had greater symptom scores. Variation in FEV1 increased with increasing ozone.	No discussion of asthmatics' medication status.	Avol et al. 1998
0.16	7.6 hour, IE (50 min light exercise/hour) (13 L/min/m^2) Allergen exposure 18-hour post ozone	Mild atopic asthmatics, house dust mite sensitive, (5 F, 4 M), 20-35 yrs	Provocative dose of dust mite allergen to elicit 20% decrement in FEV1 significantly decreased after ozone exposure.	7 of 9 patients. required less allergen after ozone. Mean dose shift 0.58 attributable to ozone in doubling conc.	Kehrl et al. 1999

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

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

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

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

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

Ozone Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.20	6 hour, alternating periods of 30 minutes of rest and exercise (5 L/min/L vital capacity)	5 adults w/ mild asthma; 5 healthy subjects (age range for all 18 – 45)	No significant changes in FEV1, FVC, FEV1/FVC, or FEF25-75 % in either group. Minor changes in ozone-associated symptoms, slightly greater in healthy than asthmatic subjects. Significant increase in "urge to cough" among asthmatics. Significant ozone–associated effects on inflammatory markers in BALF (% PMNs, PMNs/mL, IL-6 and IL-8).	Small study. Though pre- and post-exposure SR_{aw} was measured, results were not reported. Inflammatory changes occurred even in the absence of significant effects on lung function.	Basha et al. 1994
0.20	2 hour, IE V_E=20 L/min/m^2 BSA 50 min. exercise alternating with 10 minutes rest	15 healthy subjects (mean age 24, range 19-31); 15 mild, atopic asthmatics (mean age 29, range 21-48)	Significant decrements in FEV1 and FVC in healthy subjects, and in FVC in asthmatics. No between-group differences in magnitude of lung function changes.		Stenfors et al. 2002
0.22	2 hour; alternating 15 minute periods of rest and light exercise (twice resting level during exercise). Ambient air (mean ozone = 0.22 ppm). Purified air >3 wks post-ozone exposure.	Asthmatics (n=30); Healthy (n=34)	Significant increase in symptoms for normals, but not asthmatics. No significant difference in PFT changes between normal and asthmatics.	No discussion of asthmatics' medication status. Order of exposure not randomized. Many "healthy" subjects had history of allergy, which may have affected their responses to ozone..	Linn et al. 1980

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

Ozone Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.22	2 hour resting	17 asthmatics	Slightly lower FEV1 (post- versus pre-exposure) with ozone compared with filtered air exposure.	Most subjects on medication. No nonasthmatic control group.	Silverman 1979
0.24	4 hrs light exercise (V_E=20L/min) alternating 15 min with rest	9 men with severe COPD (59-71 yr.) 10 age matched control men	FEV1 declined 19 % in COPD patients. vs. 2% in healthy subjects. 11% decline with FA. SaO_2 reduced by 1% during exposure but resolved by end of exposure.	Half of individuals exposed during high ambient conditions. Some unable to withhold medication.	Gong et al. 1997a
0.25	3 hrs, 6 cycles of 10 minutes rest/ 15 min. exercise/ 5 min. PFTs (minute ventilation 29.7L/min ± 5.5L)	24 adults with mild atopic asthma, 12 with allergic rhinitis (no asthma), and 10 healthy subjects mean age 26 (21-35yrs)	Marked, statistically significant increase in airway responsiveness to subsequent allergen challenge. Compared to FA, ozone exposure required 3.3 times less allergen to attain a 20% fall in FEV1.	FA and ozone exposure separated > 4 weeks. No anti-inflammatory medications for at least 3 mo prior.	Jorres et al. 1996
0.27	Allergen challenge 24 hrs. before 2 hour exposure. Six hrs later induced sputum collected.	12 adults with mild atopic asthma.	Percentage of eosinophils but not neutrophils in sputum was significantly higher after exposure to ozone compared to air.	ozone exposure after allergen augments allergen induced influx of eosinophils into airways	Vagaggini et al. 2002

Table 9-14 (cont.): Controlled Studies of Subjects with Pulmonary or Cardiovascular Disease

Ozone Conc. (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Comments	Reference
0.30	Rt. heart and radial artery catheterization FA exp. one day, ozone next x 3 hrs. 15 min. ex. (V_E=30-40 L/min) alternating with 15 min. rest	10 non-medicated hypertensive males, 42-78 yrs. (mean 53, SD 10) 6 control males, 40 – 49 yrs. (mean 44, SD 3)	Mean pre-existing significant changes in rate-pressure product (1,353 beats/min/mm Hg), and heart rate (8 beats/min), FVC and FEV1 (-6%) and increased P_aO_2 with ozone. No significant differences between hypertensive and normotensive groups.		Gong et al. 1998
0.40	2 hour alternating 15 min. periods rest and activity (V_E 30L/min/m^2)	9 asthmatics, 9 normal (5 F, 4 M each group) 18-35 yr	Statistically significantly greater decrease in FEV1, FEV1%, and FEV_{25-75} in asthmatic subjects. No difference noted between groups for FVC (both decreased). Decrements increased with increasing length of exposure. Asthmatics SR_{aw} increased more than controls	Asthmatics discontinued medication 12 hrs prior to exposure	Kreit et al. 1989
0.40	2 separate 2 hour exposures FA and ozone followed by unilateral allergen challenge D. farinae increasing dosage 10 to 10,000 AU.	11 asthmatics (m/f) sensitive to D. farinae, age 18-35	ozone had intrinsic effect of influx of PMNs and Eosinophils independent of allergy. Less allergen required to produce nasal symptoms after ozone exposure.	Randomized, double blinded. Saline applied to contralateral side.	Peden et al. 1995

9.6.8 Possible Effect Modifiers: Influence of Anti-oxidant Vitamins, Gender, Age, Racial and Environmental Factors

9.6.8.1 Antioxidant Vitamins

Since ozone is an oxidant gas, and is known to activate antioxidant pathways, several researchers have investigated whether supplementation with antioxidant vitamins might alter responses to ozone. Animal studies investigating this topic are discussed in Sections 9.4.2. Several human studies have investigated the impact of antioxidant vitamin supplementation in healthy (Romieu et al., 1998; Grievink et al., 1998; Hackney et al., 1981) and asthmatic (Sienra-Monge et al., 2004; Romieu et al., 2002, 2004; Trenga et al., 2001) subjects.

Studies by Romieu et al. (1998, 2002; 2004) and Sienra-Monge et al. (2004) were field studies performed in Mexico City. These studies investigated the impact of supplementation with vitamins C and E on nasal inflammatory responses (Sienra-Monge et al., 2004), lung function responses (Romieu et al., 2002), and the influence of a genetic polymorphism in the antioxidant coding gene GSTM1 (Romieu et al., 2004) in asthmatic children exposed to polluted ambient air containing an elevated concentration of ozone. Romieu et al. (1998) investigated the influence of vitamin C and E supplementation on lung function of street workers in Mexico City. The results of this group of studies suggest a beneficial effect of the vitamin supplementation treatment on the endpoints examined in people exposed to high concentrations of ambient air pollutants, including ozone, although the ozone effect was not statistically significant for lung function in the study by Romieu et al. (2002).

Grievink et al. (1998) studied the influence of carotene and vitamin C and E supplementation on acute effects of ozone on pulmonary function in amateur cyclists. Pulmonary functions were measured after training sessions or competitive races on four to 14 occasions in supplemented and control groups in the summer of 1994 in The Netherlands. The results suggest that antioxidant vitamin supplementation may protect against acute effects of ambient concentrations of ozone on lung function in heavily exercising adults.

Trenga et al. (2001) investigated the effect of supplementation with vitamins C and E on ozone-induced bronchial hyperresponsiveness of adult asthmatics. The subjects underwent controlled exposures to air or 0.12 ppm ozone for 45 min with moderate intermittent exercise. Bronchial hyperresponsiveness was evaluated with 10 min exposure to 0.10 and 0.25 ppm sulfur dioxide inhalation challenges. The study used a double-blind crossover design. Subjects supplemented with vitamins C and E responded less severely to the sulfur dioxide challenge than subjects given placebo.

Hackney et al. (1981) studied healthy young adults supplemented with vitamin E daily for at least nine weeks prior to two hour exposure to 0.5 ppm ozone, with intermittent light exercise, and concomitant heat stress. The results showed no difference between the responses of the vitamin E and placebo groups in symptoms, lung function. This study was conducted at an ozone concentration level considerably higher than both the ambient levels in the field studies from

Mexico City discussed above and Trenga et al. (2001). It is possible that the ozone concentration in this study was sufficiently high as to overwhelm any protective effect of the antioxidant treatment.

Overall, the evidence suggests that antioxidant vitamin supplementation, especially in people who are deficient, may modulate responses to inhaled ozone.

9.6.8.2 Gender Differences

The question as to whether there is a difference in the responsiveness of males and females to ozone has been debated for some time. Although data on lung function responses of young adult females have been reported in a number of studies, there are considerably fewer data on compared to young adult males. A few papers have examined the lung function responses to ozone of older adult females. Several early papers suggested that males and females might differ in responsiveness to ozone (e.g., Horvath et al. 1979; Lategola et al. 1980; Gliner et al. 1983; Gibbons and Adams 1984; Lauritzen and Adams 1985), based on the observation that even though the male and female subjects had similar spirometric responses to ozone exposure, the females inhaled a lower total dose of ozone because of a lower exercise V_E. The first study to formally examine the comparative responsiveness of men and women to ozone was published by Horvath et al. (1986). The study compared the responses of healthy nonsmoking young adult female subjects exposed to 0.48 ppm ozone with a group of young adult male subjects who had previously completed the same two-hour intermittent exercise protocol in the same laboratory (Drechsler-Parks et al. 1984). Pulmonary function and symptom responses were similar for the two groups, although Horvath et al. (1986) noted that the female subjects had inhaled about 22% less ozone than the male subjects. This led to the suggestion that females might be more sensitive than males. However, when the ozone dose was normalized to body surface area or to FVC, the female subjects inhaled slightly higher relative doses of ozone than the males, pointing to the opposite conclusion.

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

responses of women relative to men depending on whether the comparison was based on the absolute or relative dose of ozone inhaled. Reisenauer et al. (1988) and Drechsler-Parks et al. (1989) reported similar findings for older adult women.

Messineo and Adams (1990) hypothesized that the possibly greater responsiveness reported for female subjects was related to the generally smaller lung size of women compared to men. They tested this hypothesis by exposing two groups of young adult women to FA, 0.18 and 0.3 ppm ozone for one hour while they performed continuous exercise at a mean V_E of about 47 L/min. One group consisted of women with a group mean FVC of 3.74 L, while the other group had a group mean FVC of 5.11 L. The mean percentage changes in pulmonary function were similar for the two groups, although the changes observed were approximately twice as large as those of a group of young adult males who had earlier completed a similar protocol in the same laboratory. The results suggest it is unlikely that differential responsiveness to ozone between males and females is related to differences in lung size.

Weinmann et al. (1995a) exposed young adult male and female subjects to FA and 0.35 ppm ozone for 2 hours while they exercised intermittently. There were no significant differences between the responses of the genders in any lung function at post-exposure when V_E was equivalent to ten times FVC. The average V_E was 29.6% lower for the female than the male subjects. This led the investigators to conclude when V_E was proportional to body size/lung size there was no significant gender difference in spirometric responsiveness to ozone, in contrast to Messineo and Adams' (1990) findings comparing females with large and small FVC.

Two studies have investigated possible explanations for the apparent differential responsiveness to ozone of males and females. Bush et al. (1996) investigated the longitudinal distribution of ozone absorption in the lung. On average women absorbed ozone higher up in the lung tree than men. However, since women have a smaller dead space volume than men, if absorption is expressed in terms of the ratio of the distribution of ozone absorption in terms of penetration volume (V_P) and dead space volume, the absorption distribution in men and women was not different. The study evaluated ozone dosimetry in terms of an intrinsic mass transfer parameter, and found that differences between ozone dosimetry could be explained by a correlation with anatomic dead space. Application of this result to measurements of ozone exposure response indicated that previously reported gender differences may be due to improper accounting for the tissue surface area of the conducting airways, and implies that gender differences are not due to differences in (normal) lung anatomy. Housley et al. (1996) reported that the nasal lavage fluid of females contains a smaller amount of the antioxidant uric acid than that of males. Since the primary source of uric acid is plasma, lower nasal concentrations would reflect lower plasma concentrations. The authors speculated that the lower plasma and nasal lavage fluid levels in females might make them more susceptible to oxidant injury because their lower uric acid levels might allow more ozone to penetrate deeper into the lung.

Overall, the currently available literature suggests that young adult females might be somewhat more sensitive to ozone if the comparison is made with the same absolute inhaled dose as young adult males. However, this is not likely of practical consequence since in most cases females exercise at a similar percentage of maximal as males, which would be the case with many body-weight bearing activities performed at the same speed. If males and females are required to perform at the same absolute workload and V_E, females are likely to experience somewhat greater effects than males for reasons that are not understood at this time.

9.6.8.3 Hormonal Influences

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

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

Table 9-15: Gender and Hormonal Differences

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

Table 9-15 (cont.): Gender and Hormonal Differences

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30	1-hour, CE, (V_E=50 L/min), mouthpiece	Healthy nonsmokers with regular menstrual cycles, (9 F), 20-34 yrs	FEV1 decreased 13.1% during the mid-luteal phase and 18.1%during the follicular phase. Decrement in FEF25-75% was significantly larger during the follicular phase than the mid-luteal phase. Changes in FVC were similar in both phases.	Fox et al. 1993
0.35	1.25 hour, IE, (2 30 min exercise periods), (V_E= 40 L/min)	Healthy, screened to all be ozone responders, (19 F), mean age = 22 yrs	FVC and FIVC both decreased 13%, FEV1 decreased 20%. ozone exposure increased airway responsiveness to methacholine. Small effects in both inspired and expired spirometry persisted past 18-hour post-ozone exposure. Chemoreceptors were not activated, but ventilatory drive was accelerated.	Folinsbee and Hazucha 2000
0.35	130 min	Healthy nonsmokers with regular menstrual cycles, (9 F) 18-35 yrs	Changes in FVC, FEV1 , FEF25-75%, $V_{max50\%}$ and $V_{max25\%}$ were similar during both the follicular and luteal phases.	Weinmann et al. 1995b
0.45	2 hour, IE, (mean V_E=28.5 L/min for men, and 26.1 L/min for women)	Healthy nonsmokers, (10 M) 60-89 yrs; (6 F) 64-71 yrs	Mean decrement in FEV1 = 5.7%. Decrements in FVC and FEV1 were the only pulmonary functions significantly altered by ozone exposure. No significant differences between responses of men and women.	Bedi et al. 1989
0.45	2 hour, IE, (V_E=27.9 L/min for men and 25.4 L/min for women)	Healthy nonsmokers, (8 M), 51-69 yrs; (8 F), 56-76 yrs	Range of responses in FEV1, 0 to –12% (mean decrement was 5.6%). No significant difference in responses of men and women, although women tended to have somewhat larger effects.	Drechsler-Parks 1987 Drechsler-Parks et al. 1987a
0.48	2 hour, IE, (V_E=25 L/min)	Healthy nonsmokers, (10 F), 19-36 yrs	Mean decrement in FEV1 = 22.4%. Significant decrements in all spirometry measurements. Results not significantly different from a similar study on males (Drechsler-Parks et al., 1984)	Drechsler-Parks et al. 1984 Horvath et al. 1986

9.6.8.4 Age Differences on Pulmonary Function Changes with Ozone

9.6.8.4.1 Children

There is evidence that ozone responsiveness, measured by pulmonary function and symptoms, varies with age. There are few controlled, clinical studies on subjects under age 18, although field and epidemiological studies (see Sections 10.1; 10.2) have suggested that children may be more responsive to ambient air pollution than young adults.

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

Avol et al. (1987) studied a group of 33 boys and 33 girls with mean age of 9.4 years who were exposed in an environmental chamber to purified air or outdoor ambient air that was drawn into the chamber. It should be noted that the exposure included the full range of air pollutants present in the outdoor air mix on the exposure days. The mean ozone concentration averaged 0.113 ppm on ambient air exposure days and 0.003 ppm on purified air days. These children had similar declines in FVC and FEV1 following both exposures. The results were similar to other studies using the same protocol that were performed by the same investigators, but with adolescent and adult subjects (Avol et al. 1985; Avol et al. 1984). It should also be noted that these studies were performed during the high ozone season with subjects who lived in a high ozone region (Los Angeles basin). Given the well-known attenuation of pulmonary function and symptoms responses to ozone with repeated exposures (see Section 9.6.9), it is not surprising that these subjects demonstrated little response consequent to these exposures. In addition, the authors noted that the children had difficulty performing consistent, reproducible pulmonary function tests, a factor that could also have impacted the results.

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

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

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

The effects of ozone on children have also been the subject of field studies (see Section 10.1), and long-term epidemiologic studies (see Section 10.3). These sections discuss acute and long-term effects attributed to ozone exposure in pediatric populations.

9.6.8.4.2 Middle-Aged and Older Subjects

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

Horvath et al. (1991) examined middle-aged subjects (30-43 yrs) exposed to FA and 0.08 ppm ozone on two consecutive days for 6.5 hours, extending the data base on multi-hour exposures to an age group likely to be well represented among outdoor workers, and among people who engage in prolonged active recreation outdoors. The subjects also underwent a one-hour continuous exercise challenge to 0.35 ppm ozone to characterize responsiveness. The mean reduction in FEV1 with the challenge test was –9.6% (range: 1-30%). The mean change in FEV1 following the first 6.5 hour ozone exposure was –2.2%, only 2 of 11 subjects having a decrement greater than 2%. The changes in FEV1 following the second consecutive ozone exposure were within the variability of the data. Seven subjects reported at least one symptom of respiratory irritation (cough, chest tightness, or pain on deep inhalation) by the end of the first ozone exposure, but only 2 subjects reported symptoms after the second 6.5 hour ozone exposure. None of the subjects reported symptoms after the FA exposure. These results are in contrast to those from studies on young adults who completed the same protocol. (see Section 9.6.2). The greater decrement in FEV1 after the 0.35 ppm ozone challenge test highlights the importance of concentration compared to ventilation rate and exposure duration.

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

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

9.6.8.4.3 Summary

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

Table 9-16: Influence of Age on Pulmonary Function Responses to Ozone Exposure

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

Table 9-16 (cont.): Influence of Age on Pulmonary Function Changes With Ozone Exposure

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

Table 9-16 (cont.): Influence of Age on Pulmonary Function Changes With Ozone Exposure

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

9.6.8.5 Ethnic and Racial Factors

Young white adults, particularly males, are the most frequently studied subjects in controlled studies. Lung size has been hypothesized as a factor possibly leading to gender differences in responsiveness to ozone (see Section 9.6.8.2), and also as a possible basis for racial differences in responsiveness. African-Americans tend to have smaller lungs than Caucasians for a given standing or sitting height (Rossiter and Weill 1974), a factor related to differences in typical body proportions. Consequently, an equivalent inhaled volume of ozone could result in a larger ozone dose per unit of lung tissue in African-Americans compared to Caucasians, potentially inducing greater effects in African Americans. Only one study has compared the responses of Caucasian and African-American subjects (Seal et al. 1993). The primary statistical analysis found no basis for concluding that there were any differences in the responses of African-American and white men and women. Several exploratory analyses using alternate statistical methodologies were also conducted. However the results of these analyses are not wholly consistent by either race or gender, and there was not a clear dose-response relationship when the subjects were compared by race-gender-concentration group. A significant limitation to this study is that although the study included a large number of subjects (n=372), each subject completed only one of 24 possible experimental combinations (ethnicity {n=2} x gender {n=2} x ozone concentration {n=6}). Consequently, it is possible that differences in the innate ozone responsiveness of the 24 experimental groups (n=15-17 subjects per group) could at least partially account for the inconsistent dose-response relationships when the 24 experimental groups were compared. In contrast, the primary analysis considered four race-gender groups (n=92-94 per group). The considerably larger group sizes used in the primary analysis would tend to balance out effects related to individual differences in responsiveness.

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

9.6.8.6 Environmental Factors

It has been hypothesized that concurrent heat exposure might increase responses to ozone due to a heat-stimulated increase in V_E. While heat exposure leads to increased V_E through panting in animals that do not regulate temperature through sweating, this is not a significant mechanism of temperature regulation in humans. Most controlled air pollution exposure studies have been conducted at typical indoor temperatures and humidities (20º - 22º C, and 50% relative humidity). However, ozone concentrations are typically highest on hot, sunny, dry days. Several investigators (Folinsbee et al. 1980; Folinsbee et al. 1977; Gibbons and Adams 1984; Gong et al. 1986; Gong et al. 1997a; Linn et al. 1982) have included heat stress in their exposure protocols to more closely simulate ambient conditions. These studies have used both two-hour intermittent exercise protocols (Folinsbee et al., 1977, 1980; Linn et al., 1982, 1988; Gong et al., 1997), and continuous heavy exercise protocols (Gibbons & Adams, 1984;

Gong et al., 1986). Generally, these studies reported pulmonary function and ventilatory symptoms after ozone exposures under hot conditions similar to those recorded after similar ozone exposure protocols completed at room temperature. Although, Folinsbee et al. (1977) found a non-significant trend toward larger decrements in pulmonary function following their most severe ambient condition (40º C, 50% relative humidity), there is no convincing evidence that ambient temperature or humidity alters pulmonary function or symptoms responses to ozone exposure. Gibbons & Adams (1984) also reported that ability to complete a given ozone exposure was shortened when subjects exercised under higher temperature conditions than when studies were performed under room temperature conditions. This may have implications for summer ozone exposures in California, depending on whether or not exposed people have developed heat adaptation responses.

9.6.8.7 Smoking

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

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

Torres et al. (1997) divided smokers and nonsmokers into three groups based on smoking status and response to an ozone challenge test. The subjects completed four-hour intermittent exercise exposures, once to FA and twice to 0.22 ppm ozone. Bronchoalveolar and nasal lavages were performed immediately after one ozone exposure, and 18-hour after the other. Half of the subjects underwent bronchoalveolar and nasal lavage immediately after the FA exposure, and the other half at 18-hour after the exposure. Analysis of cells obtained at nasal lavage showed a high degree of variability among subjects, and no statistical difference between subject groups. There was no correlation

between the cellular measurements from nasal lavage and bronchoalveolar lavage. Analysis of the bronchoalveolar lavage data indicated that smokers had considerably more inflammatory cells in BALF, even with FA exposure, than did nonsmokers, demonstrating ongoing lung inflammation even without pollutant exposure. All subject groups showed a decrease in cells when BAL was performed immediately after exposure, and an increase when BAL was performed at 18-hour after ozone exposure. IL-6 and IL-8 were highest immediately after ozone exposure, and the concentrations of these two cytokines were correlated with the late increase in PMN. The inflammation, as measured in fluid recovered from both the bronchial and alveolar regions of the lungs, was primarily neutrophilic in nature, although there were small increases in other immune cell types. The investigators also performed logistic regression analysis to attempt to isolate predictors of ozone responsiveness. The model included age, gender, provocative dose inducing a 50% increase in SR_{aw} (PD50), allergies, PMN's after FA exposure, and subject group. Age was inversely related to responsiveness. None of the other characteristics considered were associated with ozone responsiveness. Further, the results indicate that the inflammatory time course in smokers is similar to that of nonsmokers, and that ozone-associated inflammation can be present in smokers and nonsmokers in the absence of symptoms or changes in pulmonary function.

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

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

Table 9-17: Influence of Ethnic, Environmental and Other Factors

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

Table 9-17 (cont.): Influence of Ethnic, Environmental and Other Factors

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

Table 9-17 (cont.) : Influence of Ethnic, Environmental and Other Factors

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

Table 9-17 (cont.): Influence of Ethnic, Environmental and Other Factors

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

Table 9-17 (cont.): Influence of Ethnic, Environmental and Other Factors

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

Table 9-17 (cont.): Influence of Ethnic, Environmental and Other Factors

Ozone Concentration (ppm)	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.50 (KI)	2 hour, including 30 min ex during either the second or third half hour (40% V_{O2max}); 4 ambient conditions: (1) 25° C, 45% RH; (2) 31° C, 85% RH; (3) 35° C, 40% RH; (4) 40° C, 50% RH	Healthy nonsmokers (14 M), 20-25 yrs	ozone exposure induced similar decrements in pulmonary function regardless of which half hour subjects exercised. The largest reductions in lung function occurred immediately after the exercise period (826 mL in FVC and 937 mL in FEV1), with some recovery during the balance of the exposure (decrements of 388 mL in FVC and 426 mL at end of exposure). There was a trend toward greater reduction in pulmonary function under the most severe heat/RH conditions, but this was significant only for FVC. Concluded that heat stress may modify the overall effect of ozone on pulmonary function.	Folinsbee et al. 1977

9.6.8.7.1 Socioeconomic Status

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

9.6.8.8 Summary: Gender, Age, Racial and Environmental Factors and Responsiveness to ozone

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

9.6.9 Responses to Repeated Ozone Exposures

9.6.9.1 Responses to ozone exposures on consecutive days

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

The first study to systematically examine responses of human subjects exposed to 0.5 ppm ozone on consecutive days (KI) was performed by Hackney et al. (1977). One subject had little response on any exposure day, but the other five showed decrements

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

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

Frank et al. (2001) reported on responses of healthy adults exposed to 0.25 ppm ozone for 2-hour on four consecutive days. FVC and FEV1 returned toward baseline across the four days of exposure, with the greatest decrement on day 2, consistent with the findings of others. The unique contributions of this study are that the investigators evaluated small airway function following each exposure, and also presented details about pre-exposure lung functions on each day. The results, illustrated in Figure 9-6 below, indicate that small airway function, measured as SAWgp (a derived measure based on several measures of small airway function), followed a similar timecourse as FVC and FEV1 with ozone exposures on four consecutive days. The figure also illustrates that pre-exposure lung function on days two through five remained depressed, indicating incomplete recovery within a 24 hour period.

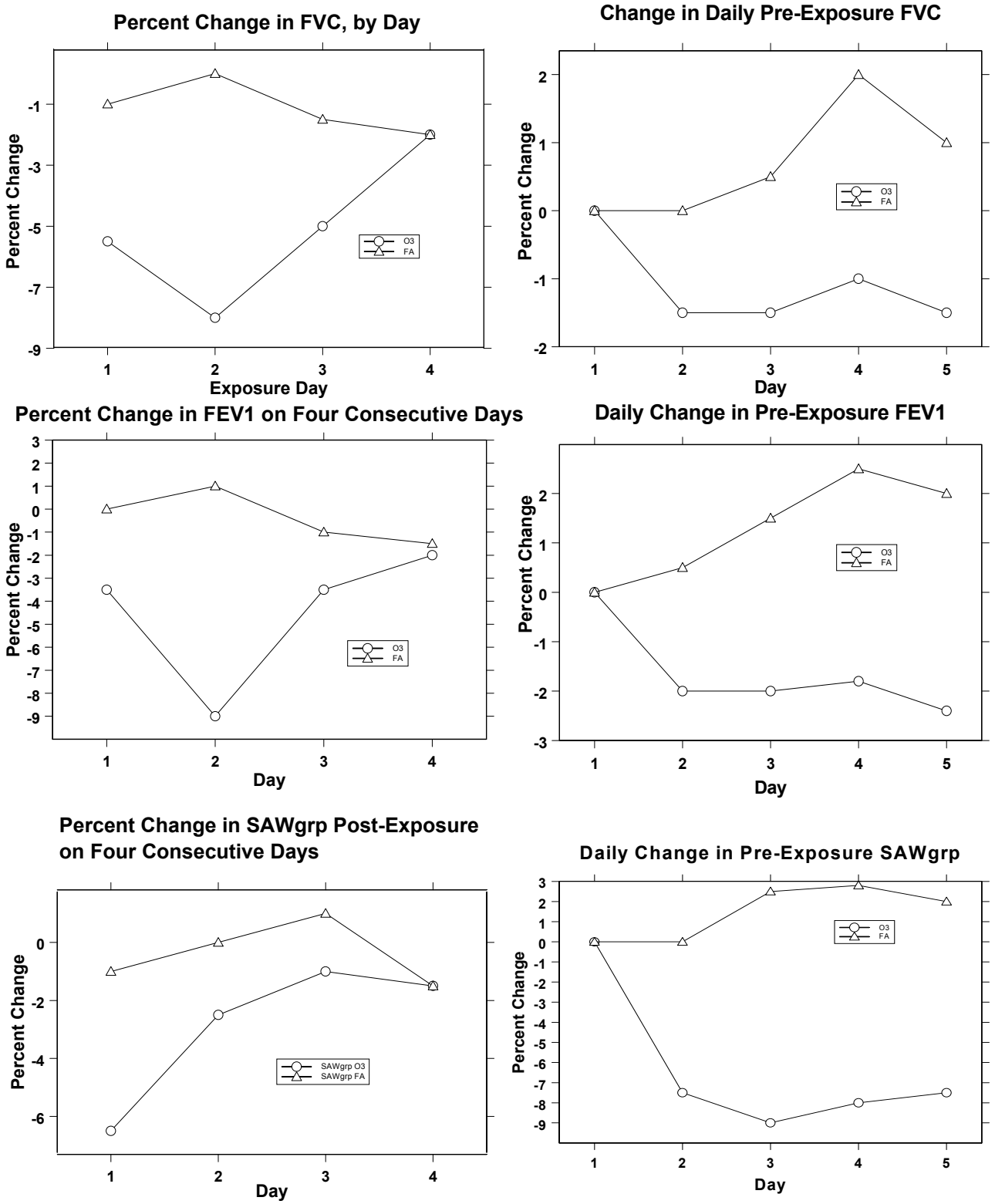

Figure 9-6 Percent change in FVC, FEV1 and SAWgp pre- and post-exposure. Derived from Frank et al., 2001.

Dimeo et al. (1981) sought to determine the lowest ozone concentration that increased airway reactivity, as measured by increases in SR_{aw} in response to histamine inhalation

challenge with repeated ozone exposures. When subjects were exposed to 0.4 ppm ozone (KI) on three consecutive days, the response to histamine was enhanced after the first ozone exposure, but was similar to the FA response following the second and third consecutive ozone exposures. This was in contrast to the time course of pulmonary function changes reported by others (i.e., larger response on the second day, followed by progressively smaller responses, e.g., Farrell et al. 1979). Although Dimeo et al. did not report pulmonary function data in the paper, the authors did state that the largest decrements in pulmonary function occurred after the second of the three consecutive ozone exposures. These results underscore that several mechanisms, with different time courses, appear to be involved in the various responses observed following ozone exposure (see Section 9.3). Further, the authors suggested that the threshold for increased bronchomotor reactivity with ozone exposure is somewhere between 0.2 and 0.4 ppm (KI). However, the notion of a “threshold” ozone concentration has been called into question by more recent studies involving lower ozone concentrations, but greater delivered doses due to longer exposure duration and increased exercise-associated V_E (e.g., Folinsbee et al., 1994). The more recent view is that thresholds likely exist on an individual level, but that the variability among individuals is great enough that on a population-wide basis, the threshold is impossible to determine.

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

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

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

9.6.9.2 Repeated Prolonged Ozone Exposures

Folinsbee et al. (1994) studied the responses of 17 healthy, young adults who completed 6.6 hour exposures to 0.12 ppm ozone on five consecutive days. There was a group mean decrement in FEV1 following the first ozone exposure of 11.9%, which was reduced to 6.2% following the second ozone exposure. Changes in FEV1 following the final three ozone exposures were not different from baseline measurements.

Methacholine responsiveness, on a group basis, was increased after all five ozone exposures, although it was greatest on day two. Cough and pain on deep inspiration were statistically increased only after the first ozone exposure. There was no correlation between symptoms or FEV1 and methacholine responsiveness, indicating that different mechanisms are responsible for each category of responses.

Table 9-18: Changes in Forced Expiratory Lung Volume After Repeated Daily Exposure to Ozone[a]

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

a. See Glossary for abbreviations and acronyms.
b. Listed from lowest to highest ozone concentration.
c. Exposure duration and intensity of intermittent exercise (IE) or continuous exercise (CE) were variable; V_E (in parentheses) in L/min or as a multiple of resting ventilation.
d. Subjects were especially sensitive on prior exposure to 0.43 ppm ozone, as evidenced by a decrease in FEV1 of more than 20%. These nine subjects are a subset of the total group of 21 subjects used in this study.
e. Bronchial reactivity to methacholine challenge was also studied.
f. Subjects had mild asthma.
g. Seven subjects completed the entire experiment.

Table 9-19: Pulmonary Function Effects With Repeated Exposures to Ozone

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

Table 9-19 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

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

Table 9-19 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

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

Table 9-19 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

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

Table 9-19 (cont.): Pulmonary Function Effects With Repeated Exposures to Ozone

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

9.6.9.3 Exercise Performance

It is well known (see Section 9.6.10) that single ozone exposures can limit maximal exercise performance. Foxcroft and Adams (1986) investigated whether the attenuation of pulmonary function decrements and symptoms responses with repeated ozone exposures would also extend to ozone-induced reduction in maximal exercise performance. Although pulmonary function changes were largest following the second of the consecutive exposures, these subjects had similar decrements in pulmonary function after the first and fourth days of exposure. In both cases the decrements were statistically significant when compared to the FA day. Thus, in terms of pulmonary function, these subjects did not develop the usual pattern of responses to repeated ozone exposure. However, symptomatology did decrease across the four exposures. Although there was some improvement in exercise performance time between the maximal exercise test following the first ozone exposure compared to that on the fourth day, exercise time remained significantly less on both days than following FA exposure.

9.6.9.4 Repeated ozone exposures in subjects with lung disease

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

Asthmatics also appear to manifest attenuated functional responses with repeated ozone exposure. Gong et al. (1997a) exposed adult asthmatics twice to FA, and then on five consecutive days to 0.4 ppm ozone (UV). The subjects showed a nonsignificant decline in preexposure pulmonary function across the

five consecutive days of ozone exposure, in agreement with other investigators examining subjects without respiratory disease (Gliner et al. 1983; Frank et al. 2001). Pulmonary function decrements were similar and statistically significant after the first two ozone exposures, and returned toward baseline over the following three exposures, a pattern similar to that reported for normal subjects (see Section 9.6.9). Responses after re-exposure to 0.4 ppm ozone four and seven days after the last consecutive ozone exposure indicated that response attenuation had begun to reverse after four days without experimental ozone exposure. Examination of individuals' responses confirms previous findings that those subjects who were initially most responsive showed slower development of response attenuation, and faster loss of attenuation (Horvath et al. 1981). Airway responsiveness to methacholine was at least partly independent of effects on lung function, with excess bronchial reactivity persisting after FEV1 responses had attenuated, in accord with the findings of Folinsbee et al. (1994). Methacholine reactivity was increased after all the ozone exposures. Although there was a trend toward reduction in airway reactivity to methacholine across the ozone exposure days, attenuation in this endpoint was not complete with five consecutive day exposures. Since this is the only study of this type with asthmatics, and since it is unknown whether the group studied is representative of the asthmatic population, it is unclear whether the findings can be generalized to the broader population.

9.6.9.5 Persistence of attenuation

As discussed above (Section 9.6.9.4), Gong et al. (1997a) reported that reduced pulmonary function and symptom responses with repeated ozone exposures only persist for a few days of nonexposure to ozone in asthmatics. Several studies with normal subjects that used similar exposure protocols have reported similar results (Horvath et al. 1981; Kulle et al. 1982; Linn et al. 1982).

9.6.9.6 Duration of hyperresponsiveness to ozone

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

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

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

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

9.6.9.7 Repeated natural ozone exposures and responses to controlled ozone exposures – seasonal variation

As noted above, it has been proposed that the reason long-term Los Angeles residents might be less responsive to ozone in controlled exposure studies than Canadians and new arrivals to Los Angeles might be attenuation due to regular exposure to ozone concentrations typical of Southern California. Linn et al. (1988) addressed one aspect of this hypothesis by evaluating whether there was a seasonal influence on ozone responsiveness in 38 Los Angeles residents. The subjects participated in exposures to FA and 0.18 ppm ozone in late spring, autumn, winter, and again the following spring. Subjects who were responsive to ozone showed seasonal variation in their degree of responsiveness. These subjects had the largest decrements in pulmonary function in the spring when ambient ozone concentrations had been low for several months, considerably smaller changes in autumn, representing the end of the high ambient ozone season, and intermediate changes in winter. Subjects who had minimal responses to the initial spring ozone exposure had similar responses at all follow-up ozone exposures. The results suggest that, among individuals who are responsive to ozone, long-term natural exposure to comparatively low ambient ozone concentrations alters responsiveness across seasons. This is in contrast to repeated controlled exposure studies at low concentrations (see Section 9.6.9), which did not induce changes in ozone responsiveness across the consecutive exposure days. These findings suggest that due to regular, repetitive ambient ozone exposures, responses of subjects native to the Los Angeles Basin may not be representative of the population whose ozone exposures are more infrequent and episodic. This must be considered in the interpretation of all ozone exposure studies conducted in Los Angeles relative to studies conducted elsewhere.

9.6.9.8 Repeated ozone exposures and airway inflammation

Frank et al. (2001) performed bronchoscopy with BAL 24 hours after subjects had undergone four consecutive daily exposures to 0.25 ppm ozone. Ozone exposure induced changes in FVC, FEV1 and symptomatology that followed the typical pattern of increased effects on day-two compared to day-one, followed by response attenuation. Frank et al. were the first to specifically examine the effect of repeated ozone exposure on small airway function (SAWgp -small airway group: a composite index of several measures of small airway function). The results showed the greatest decrements in SAWgp on the first ozone exposure day. The subjects also demonstrated increased f_R and decreased V_T. Further, daily pre-exposure FVC, FEV1 and SAWgp progressively decreased across the four ozone exposure days, although only the decrease in SAWgp was statistically significant. The authors concluded that the BALF results after the fourth ozone exposure indicated pulmonary inflammation. However, since the only statistically significant endpoint in the BALF analysis was an increase in neutrophils, there being no significant changes in albumin, fibrinogen, kinins or other types of cells, this is a tenuous conclusion. Given that there were only 8 subjects, the group may have been too small to determine statistical significance, and thus these results can only be considered qualitatively.

Christian et al. (1998) performed a more extensive analysis of BALF obtained at bronchoscopy about 20 hours after the subjects completed a single four-hour exposure to 0.2 ppm ozone with intermittent exercise (V_E = 25 l/min/m^2), and after they completed the same protocol on four consecutive days. The pattern of responses in FVC, FEV1 and SR_{aw} was similar to that reported by others who have used similar single- and multi-day. Changes in V_T, f_R and symptoms were also similar to previous reports (see Sections 9.6.2 and 9.6.3). The significance of this paper is the extensive BALF analysis. Contrary to the hypothesis of the study, the BALF results suggested that inflammation did not progress with consecutive days of ozone exposure, as evidenced by decreased PMNs and reduced fibronectin concentration in the bronchial fraction of the BAL after the 4 consecutive days of ozone exposure compared to after the single-day ozone exposure. In the bronchoalveolar fraction, there were significant decreases in the number of PMNs, fibronectin and IL-6 after the four-day exposure, compared to the single-day exposure. These results suggest attenuation of the ozone-induced inflammatory response in both proximal airways and in the distal lung with repeated ozone exposures. However, even though inflammation appeared attenuated after the four-day exposure, the BALF analysis indicated that inflammation was still present. How long such inflammation persists is unknown. Moreover, chronic inflammation may lead to airway remodeling and structural damage to the respiratory tract. No information addressing these issues is currently available on human subjects, although animal studies (see Section 9.4.3) suggest that chronic exposure to ozone can lead to morphological changes in the lung tissues, and to reduction in inflammation after a few days of exposure.

Jorres et al. (2000) investigated the effect of repeated ozone exposures on inflammatory markers in BALF and mucosal biopsies. The study involved 23 healthy adults who underwent single exposures to FA and 0.2 ppm ozone, as well as four-hour exposures to 0.2 ppm ozone on four consecutive days. BAL was performed and mucosal biopsies were obtained 20 hour after the single and the fourth ozone exposures. Changes in FEV1 followed typical patterns, that is, similar responses following both the single ozone exposure and the first of the consecutive ozone exposures, and somewhat larger decrements after the second consecutive exposure. This was followed by smaller changes in FEV1 following the third and fourth ozone exposures. The pro-inflammatory markers IL-6 and IL-8, along with total protein, were increased after the single ozone exposure, but only total protein remained elevated after the four-day protocol. IL-10, an anti-inflammatory cytokine, was only increased after the four-day protocol, suggesting that it may have a role in the attenuation process. Macrophages in BALF decreased with the single ozone exposure, compared to FA, but had returned nearly to the FA level after the four-day protocol. Neutrophils increased three-fold after the single ozone exposure, and decreased toward the FA level with four days of ozone exposure, although they were still about twice the baseline levels after four days of exposure. The results of the bronchial biopsies showed mucosal inflammation that was greater after the four-day protocol than after the single ozone exposure, in contrast to the BALF analysis, which suggested that airway inflammation was waning. These results suggest that BALF analysis may not accurately reflect airway tissue effects. It is important to note that the biopsy analysis indicated the presence of increasing airway inflammation, even though pulmonary function changes had attenuated, and some markers of inflammation, as measured in BALF, were returning to baseline levels.

9.6.9.9 Summary

Although the term “adaptation” has been used to describe the reductions in pulmonary function and symptom responses that develop with repeated exposures to relatively high ozone concentrations (i.e., over 0.2 ppm), the database as a whole suggests that this term is a misnomer. There is evidence that some aspects of airway inflammation continue, or even increase, with repeated ozone exposures. Baseline pulmonary function decreases with repeated ozone exposure. Attenuation of pulmonary function and symptom responses to a fixed ozone concentration does not necessarily alter responses to a subsequent exposure to a higher or a lower ozone concentration. And finally, pulmonary function and symptom response attenuation begins to reverse after only a few days of nonexposure, at least under controlled laboratory conditions. In contrast, Linn et al. (1988) paper suggests that under ambient exposure conditions the attenuation effect may be seasonal in nature. When all the evidence is considered, one must conclude that this pattern represents attenuation of some, but not all, of the responses elicited by ozone exposure, since some adverse responses persist, even with attenuation of symptom and pulmonary function responses. This indicates that “adaptation” is an inappropriate characterization of these phenomena. In particular, the persistence

of airway hyperreactivity and inflammation suggests that long-term exposure to ozone may result in chronic morphological effects, such as tissue remodeling, and chronic inflammation similar to those changes observed in animals (see Section 9.4.3). In addition, evaluation of the individual data presented in the papers reviewed above indicates that not all subjects demonstrated attenuation of pulmonary function and symptom responses across consecutive days of ozone exposure, suggesting that this response is not universal. Furthermore, it remains unclear whether or not attenuation represents a beneficial response.

9.6.10 Effects of Ozone Exposure on Exercise Performance

9.6.10.1 Human Studies

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

Subsequently, Folinsbee et al. (1977) reported reduced work capacity, as indicated by reduction in maximum oxygen uptake ($V_{O_{2max}}$), maximum attained workload, maximum ventilation (V_E), and maximum heart rate, in subjects who performed a maximal exercise test after they had completed a two-hour exposure to 0.75 ppm ozone (KI) with light intermittent exercise. These findings were coupled with a significant reduction in FEV1 and a significant increase in symptoms of cough and chest discomfort. In contrast, Horvath et al. (1979) and Savin and Adams (1979) did not observe changes in exercise performance, however, the exposure protocols in these studies both resulted in substantially lower effective doses of inhaled ozone than those inhaled by the subjects in the study by Folinsbee et al. (1977). The participants in the study by Horvath et al. (1979) performed the maximal exercise test after a two-hour resting exposure to 0.75 ppm ozone (KI), while those in the study by Savin et al. (1979) were exposed to 0.3 ppm ozone only during the maximal exercise test. The differences among these findings can therefore be attributed, at least in part, to the significantly larger dose of ozone inhaled by the subjects in the study by Folinsbee et al. compared to those of Horvath et al. (1979) and Savin and Adams (1979).

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

Linder et al. (1988) reported small, statistically significant reductions in exercise performance during a progressive maximal exercise test at ozone concentrations of 0.06 and 0.12 ppm ozone, compared to clean air. Total exercise time decreased as ozone concentration increased, with the reductions in exercise time statistically significant under both the 0.06 and 0.12 ppm conditions,

compared to the air condition. Subjects also had statistically significant reductions in pulmonary function after both ozone experiments, although the changes consequent to both ozone exposures were less than 100 mL, and are within the typical variability of repeated pulmonary function tests and are thus unlikely to be of clinical significance. Subjects also reported a increasing number of symptoms with increasing ozone concentration. It is significant that the subjects in this study only inhaled ozone during the exercise tests, which lasted about 18-23 minutes (depending on atmosphere and gender), in contrast to most of the other studies discussed above, in which the exercise test occurred after ozone exposure.

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

9.6.10.1.1 *<u>Summary</u>*

Significant reduction in exercise performance has been reported at ozone concentrations as low as 0.06 ppm (Linder et al., 1988), although these results have not been confirmed by others using similar protocols with ozone concentrations between 0.06 ppm up to 0.12 ppm (Gong et al., 1986; Schelegle and Adams, 1986). Exercise tolerance and pulmonary function changes are not

always observed in concert (Gong et al., 1986; Foxcroft and Adams, 1986; Schelegle et al., 1987).

9.6.11 Extrapulmonary Effects of Ozone Exposure

9.6.11.1 Animals

Ozone can cause effects in organ systems and tissues outside of the respiratory tract. Due to the highly reactive nature of ozone, it is unlikely that ozone can directly affect extrapulmonary tissues, although ozone reaction products may enter the bloodstream and be transported from the lung to affect other organs and tissues. Potential ozone reaction products have been detected in blood plasma following ozone exposure and have been implicated in extrapulmonary tissue injury (Rahman et al. 1992). Release of cytokines as a result of ozone-induced pulmonary injury has also been proposed as a potential source of extrapulmonary tissue injury (Laskin et al. 1994; Laskin et al. 1998). The immune system, which protects the body from damage by infectious microorganisms and neoplastic cells, can be affected by ozone exposure. ozone exposures as low as 0.2-0.3 ppm have resulted in immunotoxic effects on T-cell lymphocyte function and immune system organs, including the spleen and thymus, but generally continuous or near-continuous multi-day exposures required to achieve an effect (Van Loveren et al. 1990; Van Loveren et al. 1988; Li and Richters 1991; Dziedzic and White 1986; Li and Richters 1991). A long-term study mimicking urban ozone exposures (daily spikes of 0.25 ppm) was negative for immune effects (Selgrade et al. 1990). Recent developmental studies in rodents showed that continuous exposures of 0.6 ppm or greater was required to elicit an effect (Petruzzi et al. 1995; Dell'Omo et al. 1995a). Neurobehavioral developmental effects at equivalent or higher ozone concentrations have yielded ambiguous or negative results (Dell'Omo et al. 1995b; Petruzzi et al. 1999). ozone has been shown to alter bone marrow erythroid progenitor formation (Goodman et al. 1989). However, similar to developmental effects, requires multi-day continuous exposure at high ambient levels (0.5 ppm) are required to elicit an effect. Central nervous system (CNS) and behavioral effects have been recorded at 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 (Umezu et al. 1987; Rivas-Arancibia et al. 1998; Musi et al. 1994; Paz 1997). Cardiac effects, including slowed heart rate and bradyarrythmic episodes were noted in rodents at ozone levels of 0.1 ppm (Arito et al. 1990; Iwasaki et al. 1998). These effects were transient and likely related to the labile thermoregulatory control of the experimental rodent species (Watkinson and Gordon 1993; Watkinson et al. 2001). These ozone-induced thermoregulatory effects have not been reported in humans.

9.6.12 Interaction of Ozone with Other Pollutants

9.6.12.1 Introduction

Typically laboratory investigations of responses to air pollutants have involved exposure to a single pollutant, although some studies have investigated the impacts of exposures to mixtures of two or three pollutants. The reasons for the

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

9.6.12.2 Ozone Plus Sulfur-containing Pollutants

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

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

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

(1987) reached similar conclusions regarding a mixture of ozone and H_2SO_4 aerosol.

More recently, Linn et al. (1994) used the 6.6 hour exposure protocol (see Section 9.6.3) to investigate the pulmonary function and symptom responses of normal, atopic and asthmatic subjects exposed to FA, 0.12 ppm ozone, 100 $\mu g/m^3$ H_2SO_4 aerosol (MMAD = 0.5 μm), and a mixture of the two pollutants. There were no differences between the responses to FA and H_2SO_4. However, both ozone and ozone + H_2SO_4 exposure induced ventilatory symptoms and decrements in pulmonary function, and an increase in bronchial reactivity to methacholine. The authors concluded that ozone is more important than H_2SO_4 aerosol in inducing pulmonary dysfunction in normal, atopic and asthmatic adults. There were, however, several subjects who responded to the combined ozone + H_2SO_4 exposure more strongly than the rest of the subject group, suggesting that there may be some individuals who are more susceptible to joint exposures than to ozone alone.

Utell et al. (1994) investigated the pulmonary function and symptoms responses of healthy and allergic asthmatic subjects exposed for three hour to sodium chloride (NaCl) or H_2SO_4 aerosol (100 $\mu g/m^3$), followed 24 hour later by a three-hour exposure to 0.08, 0.12 or 0.18 ppm ozone. Each subject was exposed to both aerosols at two of the three ozone concentrations (four of the six possible exposure conditions) in an incomplete block design. The statistical analysis indicated that exposure to H_2SO_4 aerosol significantly altered the FVC response to exposure to 0.18 ppm ozone following the H_2SO_4 aerosol preexposure only in the asthmatic but not the healthy subjects. However, although asthmatics reported more symptoms than healthy subjects did, there was no dose-response relationship between ozone concentration and symptom intensity in either subject group. In most cases, the decrements in FVC for both groups fell within the typical reproducibility with repeated pulmonary function tests (±5%). Interpretation of this study is complicated by the fact that each subject was exposed to only four of the six exposure conditions. Thus, differences in the innate responsiveness of the subjects in the different groups could have impacted the results. Furthermore, the responses of both the asthmatic and nonasthmatic subjects were sufficiently variable that it is difficult to draw conclusions as to whether or not there is a difference in the responsiveness of healthy and asthmatic individuals under these exposure conditions. This difficulty is compounded by the fact that there was no FA control exposure, so it is impossible to determine whether the small responses observed in the asthmatic subjects were related to the exposures or to the underlying disease.

Frampton et al. (1995) used the same protocol (described in the preceding paragraph) as Utell et al. (1994) to investigate the responses of healthy and asthmatic subjects who were exposed for three-hour to 100 $\mu g/m^3$ of NaCl or H_2SO_4 aerosol on one day, and 24 hours later to either 0.08, 0.12 or 0.18 ppm ozone. The normal subjects had changes in pulmonary function under these exposure conditions that were very small, and did not follow a dose-response pattern. This led the investigators to conclude that exposure to H_2SO_4 aerosol

had no meaningful effect on their responses to a subsequent ozone exposure. As a group, the asthmatics tended to have larger pulmonary function responses when exposed to 0.18 ppm ozone subsequent to the H_2SO_4 aerosol exposure, although there was large inter-individual variability in the responses. The ozone-associated decrement in FEV1 was about 3% larger after H_2SO_4 versus NaCl exposure. The small reduction in FEV1/FVC indicated that reduced maximal inhalation, and not bronchoconstriction, was the mechanism for the observed responses. Analysis of several subject characteristics, including age, gender, total IgE blood levels, airway responsiveness, and baseline lung function in asthmatics, showed that none predicted ozone responsiveness.

Linn et al. (1997) compared the responses of 41 children (9-12 years) to controlled exposure to FA and a mixture of 0.1 ppm ozone + 0.1 ppm SO_2 + 100 $\mu g/m^3$ of H_2SO_4 aerosol utilizing a four-hour intermittent exercise protocol. Contrary to expectations, there were no significant changes in pulmonary function with either exposure, nor was there a meaningful difference in symptoms between the two exposures. However, the subjects participating in this study resided in areas of the Los Angeles Basin (Downey, Rubidoux, Upland and Torrance) that typically have relatively high air pollution levels. The report does not indicate during which season(s) the subjects were studied, and consequently the possibility of response attenuation (see Section 9.6.9) cannot be ruled out as an effect modifying factor.

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

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

Table 9-20: Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm ozone 0.10 ppm SO_2	1-hour, mouthpiece, IE, 45 min (15 min rest/15 min ex/15 min rest) exposure to air or ozone, followed by 15 min exposure (15 min ex) to ozone or SO_2, (V_E=30 l/min)	Allergic asthmatics, medications withheld for at least 4 hour before exposures, (8 M, 5 F), 12-18 yrs	When the first exposure was to ozone, pulmonary function responses to SO_2 were attenuated. Decrements in FEV1 were 3%, 2%, and 8% for the air/ozone, ozone/ozone, and ozone/SO_2 exposures, respectively.	Koenig et al. 1990
0.08 ppm ozone 0.12 ppm ozone 0.18 ppm ozone 100 µg/m^3 NaCl 100 µg/m^3 H_2SO_4	3 hour exposure to NaCl or H_2SO_4 aerosol, followed 24 hour later by 3 hour exposure to ozone, IE, (10 min ex/20 min rest), (V_E=30-36 l/min for asthmatics; 33-40 l/min for nonasthmatics)	Nonsmoking asthmatics (10 M, 20 F; 18-45 yrs) and nonasthmatics (16 M, 14 F; 21-42 yrs)	No significant changes in symptoms or lung function with any aerosol/ozone combination in the healthy group. In asthmatics, H_2SO_4 preexposure enhanced the small decrements in FVC that occurred after exposure to 0.18 ppm ozone. Asthmatics had no significant changes in FEV1 with any ozone exposure, but did have more symptoms than nonasthmatics. Asthmatic subjects had variable severity of disease, as indicated by medication use. The asthmatic group included 2 subjects who used no regular medications, 16 who used inhaled beta agonists only, 11 who used both inhaled beta agonists and oral theophylline, and 1 who used only oral theophylline.	Frampton et al. 1995 Utell et al. 1994
0.1 ppm ozone 0.1 ppm SO_2 100 µg/m^3 H_2SO_4	4 hour IE (15 min ex/15 min rest), (V_E=22 l/min)	Healthy (N=15), Asthmatic (N=26), 9-12 yrs	Spirometry, PEFR and symptoms score showed no meaningful changes between any of the conditions for the total study population. The symptoms scores reported by a subset of asthmatics/allergics were positively associated with the inhaled concentration of H_2SO_4 ($p<0.01$).	Linn et al. 1997

Table 9-20 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

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

Table 9-20 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.30 ppm ozone (KI) 100 µg/m^3 H_2SO_4	2 hour ozone followed by 4 hour H_2SO_4 15 min ex at 1-hour before end of exposure (V_E=30-35 l/min)	Healthy, (7 M, 5 F), 19-28 yrs, plus one 46 yrs	No significant pulmonary function changes or changes in methacholine reactivity with any exposure. No evidence of synergism or interaction between ozone and H_2SO_4.	Kulle et al. 1982
0.37 ppm ozone (KI) 0.37 ppm SO_2 100 µg/m^3 H_2SO_4	2 hour, IE (15 min rest/15 min ex), (V_E=twice resting)	3 smokers, 7 ex-smokers, 9 never-smokers (14 M, 5 F) 19-54 yrs	Compared responses to the mixture to responses to FA exposure. No ozone alone exposure. Mean decrements in FVC and FEV1 with exposure to the mixture were 2.8% and 3.7%, respectively. This is similar to literature values for a similar exposure to 0.37 ppm ozone alone.	Kleinman et al. 1981
0.40 ppm ozone (KI) 0.4 ppm SO_2	2 hour, IE (15 min rest/15 min ex), (V_E=30 l/min)	Healthy nonsmokers, (8 M), 19-32 yrs	Pulmonary function decrements were similar for the ozone and SO2/ozone conditions. High temperature (35º C) and humidity (85%) did not alter observed responses.	Bedi et al. 1982
0.40 ppm ozone (KI) 0.4 ppm SO_2	2 hour, IE (15 min rest/15 min ex), (V_E=30 l/min)	Healthy (9 M), 2 with history of allergies, 1 had asthma as a child, 18-27 yrs	Exposure to ozone and SO_2/ozone induced similar decrements in FVC, FEV1, and similar types and intensities of symptoms. During the last exercise period, both ozone and SO_2/ozone induced reduction in V_T and a compensatory increase in f_R, with no change in V_E.	Bedi et al. 1979

Table 9-20 (cont.): Responses of Human Subjects to Mixtures of Ozone and Sulfur-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.37 ppm ozone (KI) 0.37 ppm SO_2	2 hour, IE (15 min rest/15 min ex), (V_E=2 to 2.5 times resting), ozone exposure and ozone/SO_2 exposure on consecutive days	Study 1: Los Angeles residents (4 normal, 4 sensitive) Study 2: Montreal residents (N=4), sensitive Los Angeles residents (N=5) Age, gender, smoking history not given	Study 1: No significant changes in lung function with exposure to ozone alone. Small, statistically (but not functionally significant) decrements in FEV1 in the sensitive subjects after the SO_2/ozone exposure. Results probably due to the consecutive day exposure design rather than to an interaction of ozone and SO_2. Study 2: No significant changes in either the Los Angeles or Montreal subjects.	Bell et al. 1977
0.30/0.35 ppm ozone 1.0 ppm SO_2	2 hour, IE, (10 rest/30 min ex), (V_E=38 l/min)	Healthy nonsmokers (21 M), 19-28 yrs	Decrements in FVC and FEV1 were larger following exposure to ozone alone than following exposure to SO_2/ozone exposure. There was no significant change in SR_{aw} with any exposure.	Folinsbee et al. 1985
0.37 ppm ozone (KI) 0.37 ppm SO_2	2 hour IE, (15 min rest/15 min ex), (V_E=twice resting)	Nonsmokers (8 M), 19-25 yrs	SO_2 had no significant effect on pulmonary functions. Decrement in FEF25-75% was larger following SO_2/ozone (~35%) than following exposure to ozone alone (~15%). There was considerable variability among subjects.	Hazucha and Bates 1975
0.15 ppm ozone 0.15 SO_2 0.15 ppm NO_2	2 hour, IE, (15 min ex/15 min rest), (WL=50 W)	Healthy (7 M), 19-23 yrs, 1 smoker	No differences between responses to exposure to ozone alone and exposure to ozone in combination with SO_2 and/or NO_2.	Kagawa 1983

9.6.12.3 Ozone Plus Nitrogen-containing Pollutants

A few early studies (e.g., Folinsbee et al. 1978b, 1981; Hackney et al. 1975) investigated the effects of exposure to mixtures of air pollutants that included relatively high concentrations of nitrogen species as well as ozone, and found that the observed responses could be attributed to the ozone alone. Subsequently, others have investigated whether exposure to ozone and NO_2, either sequentially or concurrently, alters responses to ozone. Adams et al. (1987) reported on the responses of 40 male and female nonsmokers exposed to FA, 0.3 ppm ozone, 0.6 ppm NO_2 and the combination of 0.3 ppm ozone and 0.6 ppm NO_2. The only significant difference between the ozone and NO_2 + ozone exposures was that SR_{aw} was lower following exposure to NO_2 + ozone, compared to that following ozone alone.

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

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

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

Hazucha et al. (1994) investigated the responses of nonsmoking females who underwent a sequential exposure protocol in which they inhaled 0.6 ppm NO_2 or FA for two hour, followed three hour later by a two-hour exposure to 0.3 ppm ozone. Subjects rested in ambient air during the inter-exposure period. There were slightly larger decrements in FEV1 and FEF25-75% following the NO_2/ozone exposure than following the FA/ozone exposure. There were no differences in the changes in SR_{aw} or symptomatology between the two exposure conditions. However, the PD_{10}FEV1 concentration for methacholine following the NO_2/ozone exposure (1.7 mg/mL), was significantly smaller than that following the FA/ozone exposure (5.6 mg/mL), with both significantly smaller than the baseline methacholine PD_{10}FEV1 of 14.3 mg/mL. The results suggest that preexposure to NO_2 potentiated airway responsiveness to the subsequent ozone exposure.

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

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

Table 9-21: Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

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

Table 9-21 (cont.) Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.20 ppm ozone 500µg/m^3 HNO_3 or H_2O fog	5 hour total, IE (50 min ex/10 min rest), (V_E=40 l/min) 2 hour exposure to air, or HNO3 or H_2O fog, followed by 1-hour break, and then by 3 hour exposure to ozone	Healthy nonsmokers, (6 M, 4 F), screened to have a minimum of 10% decrement in FEV1 after 3 hour exposure to 0.20 ppm ozone with 50 min ex/hour	Exposure to HNO_3 or H_2O for followed by ozone induced smaller pulmonary function decrements than air followed by ozone.	Aris et al. 1991
0.25 ppm ozone 0.30 ppm NO_2 30 ppm CO	2 hour, IE, (15 ex/15 rest), (V_E=twice resting)	Healthy, (N=6, gender not given) 22-41 yrs, several ex- and current smokers	No consistent changes attributable to exposure with exposure to ozone alone, or in mixtures. . There was no evidence that inclusion of NO_2 or CO had an additive or potentiating effect on ozone.	Hackney et al. 1975

Table 9-21 (cont.): Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

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

Table 9-21 (cont.): Responses of Human Subjects to Mixtures of Ozone and Nitrogen-Containing Pollutants

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

9.6.12.4 Ozone, Peroxyacetyl Nitrate, and More Complex Mixtures

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

Drechsler-Parks et al. (1989) compared the responses of older men and women (51-76 yrs) with those of young men and women (19-26 yrs) who completed two-hour exposures to FA, 0.45 ppm ozone, and mixtures of 0.45 ppm ozone with 0.60 ppm NO_2 and/or 0.13 ppm PAN. The observed effects could be attributed solely to the ozone in the exposures. In contrast to the studies by (Drechsler-Parks et al. 1984) and (Horvath et al. 1986), there was no evidence for an interaction between PAN and ozone. A likely explanation for this is that the PAN concentration in this study was slightly less than half that used in the earlier studies. Assuming that the PAN-induced augmentation of the ozone response is linear, the expected additional effect (on lung function) of including PAN in the exposure mixture used in this experiment would be expected to be less than 5%, which would be within the variability of the pulmonary function measurements, and therefore not likely to be detectable.

Drechsler-Parks et al. (1987b) investigated the possible influence of PAN on ozone-associated response attenuation in a group of healthy young adults who completed two-hour exposures to 0.45 ppm ozone + 0.3 ppm PAN on five consecutive days. Attenuation and loss of attenuation of pulmonary function and symptoms responses occurred with the same time course and pattern as has been reported for consecutive daily exposures to ozone alone (see Section 9.6.9). The results suggest that PAN does not alter development or loss of ozone-induced attenuation.

In summary, based on the results of a few controlled studies, there is evidence that concurrent exposures to high concentrations of PAN and ozone result in pulmonary function and symptom responses somewhat larger than those observed following exposure to the same concentration of ozone alone. However, typical ambient PAN concentrations are considerably lower than those utilized in any of 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.

Table 9-22: Responses of Human Subjects to Mixtures of Ozone and PeroxyacetylInitrate

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

9.6.12.5 Ozone Plus Particulate Matter

There have been few human exposure studies on mixtures of ozone with particulate matter, with the exception of H_2SO_4 aerosol (discussed above in Section 9.6.12.2). Stacy et al. (1983) published a large study that was designed as a preliminary screening survey intended to direct future research plans. The investigators divided 231 normal male subjects into 20 groups of 9-15 individuals. Each subject participated in only one experiment. Exposure conditions investigated included FA, 0.4 ppm ozone, 0.75 ppm SO_2, 0.5 ppm NO_2, 100 μg/m^3 H_2SO_4, 133 μg/m^3 $(NH_4)_2SO_4$, 116 μg/m^3 NH_4HSO_4, or 80 μg/m^3 NH_4Nozone as single pollutant conditions, and as mixtures of each gas with each of the aerosol pollutants. The mass median diameter of all aerosol particles was 0.55 μ, with extremes of 0.20 to 0.94 μ. Exposures were four hours in duration, and the subjects performed two 15-min periods of moderate exercise during exposure. The groups exposed to ozone or mixtures including ozone demonstrated significant differences in SR_{aw}, FVC and FEF50%. None of the aerosols, SO_2 or NO_2 alone or mixed with aerosols induced significant alterations in any endpoint measured. There was no indication of interaction between any of the pollutants studied, and observed effects could be attributed solely to ozone.

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

Table 9-23: Responses of Human Subjects to Mixtures of Ozone and Particulate Matter

Pollutant Concentrations	Exposure Protocol	Subject Characteristics	Observed Effect(s)	Reference
0.12 ppm ozone 153 μg/m^3 PM2.5	2-2.5 hour resting	Healthy nonsmokers, (15 M, 10 F), 18-50 yrs	Neither systolic nor diastolic pressure was affected by the pollutants, despite a significant constriction of the brachial artery, and a reduction in brachial artery diameter, compared to following FA exposure ($p<0.03$). Flow- and nitroglycerin-mediated brachial artery dilatation were unaffected by exposures to FA or the mixture of ozone+CAPS. There was no exposure to ozone alone.	Brook et al. 2002
FA 0.40 ppm ozone 0.75 ppm SO_2 0.50 ppm NO_2 100 μg/m^3 H_2SO_4 133 μg/m^3 $(NH_4)_2SO_4$ 116 μg/m^3 NH_4HSO_4 80 μg/m^3 NH_4Nozone	4 hour, including two exercise bouts	Healthy, nonsmokers (231 M), 18-40 yrs, divided into 20 groups (N=9-15/group)	Each subject participated in only 1 exposure. Groups exposed to ozone, or gaseous or aerosol mixtures including ozone had decrements in FVC, FEF50, and significant increases in SR_{aw}. None of the aerosols alone, NO_2 or SO_2 alone, or mixtures of aerosols and SO_2 and/or NO_2 induced significant effects.	Stacy et al. 1983

9.6.12.6 Animal Studies

Since most people are exposed to several air pollutants simultaneously or sequentially, animal studies that reproduce these interactions can represent more realistic environmental conditions than studies with ozone alone. Pollutants can interact toxicologically in three basic modes: additive, more than additive (synergistic), or less than additive (antagonistic). Taken together, the studies suggest that the types of interactions produced with ozone and co-occurring pollutants are dependent on many factors. Investigations of sulfuric acid and ozone co-exposures indicate that the type interaction is dependent on the health endpoint, composition of the aerosol, and size of the aerosol. A further complication is that the magnitude of the sulfuric acid/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 (Kimmel et al. 1997) and ozone/sulfuric acid layered on metal particulate (Chen et al. 1991). Interactions of ozone and NO_2 have produced antagonistic or synergistic effects, depending on factors such as exposure concentrations used, animal species tested, and health endpoint examined. One of the more sensitive measures of ozone/NO_2 interactions utilized a bacterial infectivity model in which 15-day exposure to a simulated urban pollutant atmosphere (baseline of 0.5 ppm NO_2 with peaks of 1.0 ppm, and a baseline of 0.05 ppm ozone with peaks of 0.1 ppm) produced a synergistic interaction with regard to death in mice (Graham et al. 1987). 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 (Vincent et al. 1997; Bouthillier et al. 1998; Adamson et al. 1999), and preexposure to ozone followed by toxic particle instillation resulted in a marked retention of the toxic particles in small airways (Pinkerton et al. 1989).

9.6.13 Ozone Plus Allergens: Exacerbation of Allergic Airway Disease

9.6.13.1 Introduction

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

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

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

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

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

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

9.6.13.2 Epidemiological Studies

9.6.13.2.1 Historical Studies Associating Asthma with Air Pollution

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

Another early study in Japan focused on school children from three severely polluted cities and compared these with one unpolluted city (Saku city) (Yoshida

et al. 1974). The sources of pollution included pulp and paper mills (Fuji city), power plants, and petroleum refineries (Chiba prefecture), and urban transportation (Tokyo). The presence of allergic disease was established using peripheral blood eosinophil counts, intradermal skin tests, and serum IgE levels. Increases and/or positive responses in all of these parameters are indicative of an allergic response. Results showed that Fuji city had a prevalence rate of asthma that was 2.19% compared with 0.94% in Saku city. Similarly, the prevalence rate for Tokyo was 2.74%. These data are from the early 1970's.

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

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

9.6.13.2.2 Recent Epidemiological Studies

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

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

9.6.13.2.3 Controlled Human Exposure Studies

Several studies have purposefully exposed human volunteers to various levels of ozone and other pollutants (NO_2 and SO_2) and then challenged them with inhaled allergen while monitoring pulmonary function. Several studies have demonstrated an increased airway response to inhaled allergen following exposure to air pollutants. For example, Molfino et al. (1991) showed that

exposure to 120 ppb ozone for 1-hour increased the bronchial sensitivity of asthmatics to ragweed challenge, as demonstrated by a decrease in the amount of allergen needed to induce a defined reduction in the FEV1. In another study, subjects with mild stable allergic asthma, allergic rhinitis, and normal subjects were exposed to either 250 ppb or air for 3 hours (Jorres et al. 1996). Response to methacholine and response to allergen were measured after 1 and 3 hours respectively. Subjects with mild asthma had decrements in FEV1 averaging 12.5% (+/- 2.2%) after ozone exposure compared with air-exposed subjects ($p<0.001$). Those with allergic rhinitis also showed a significant difference. In contrast to these studies, Ball et al. (1996) demonstrated that pre-exposure to ozone did not alter the amount of allergen necessary to induce a 15% reduction in the FEV1.

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

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

Hiltermann et al. (1999) compared use of induced sputum to bronchoalveolar lavage (BAL) as sampling methods for evaluation of cellular and cytokine responses to ozone in asthmatics. There was a high correlation between eosinophil counts in the sputum and in the BAL fluid. In both samples, the levels

of eosinophils were elevated after exposure to 0.4 ppm ozone for 2 hours. In addition, IL-8 and ECP were elevated and correlated between the two samples.

The synergy of ozone and allergen in increasing the duration of the asthmatic response was demonstrated by Vagaggini et al. (2002) in a study that utilized 12 subjects with mild persistent untreated asthma. After initial allergen challenge tests demonstrated an early and a delayed response to allergen challenge, these patients were exposed to allergen aerosol and then 24 hours after allergen exposure they were exposed to ozone or air for 2 hours. Pulmonary function tests were performed before and after these air or pollutant exposures. Sputum was examined for IL-8 and for cell numbers and types. The results showed that the exposure to ozone potentiated the eosinophilia, but did not alter the production of IL-8.

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

9.6.13.2.4 In Vitro Studies Using Human Tissues and Cells

To examine the mechanisms involved in pollutant enhancement of asthma studies have been performed using both tissues and cells derived from asthmatic and control subjects. For example, to evaluate the effect of ozone and nitrogen dioxide on the permeability of bronchial epithelial cells of asthmatic compared with non-asthmatic subjects, Bayram et al. (2002) exposed cultured bronchial epithelial cells from both types of subjects to either air or pollutant for a period of 6 hours. Cell permeability to ^{14}C-labeled bovine serum albumin (BSA) was measured. The results showed that cells exposed to either 10 to 100 ppb of ozone or 200 to 400 ppb. of NO_2 had increased epithelial permeability, which

was significantly greater in asthmatics than in non-asthmatics. These results pose an interesting possibility that pollutants may indeed facilitate sensitization to inhaled allergens by increasing access to underlying dendritic cells required for antigen processing in the lung.

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

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

9.6.13.2.5 Experimental Models

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

9.6.13.2.6 Guinea Pigs

The first studies on the influence of air pollutants on allergic lung diseases/asthma were performed in ovalbumin sensitized guinea pigs by Matsumura (1970a). At that time the guinea pig was considered the preferred animal model for type 1 hypersensitivity, since they are very sensitive to allergen and display a similar shock organ response as humans, i.e. the lung is the primary target of IgE-mediated mast cell mediator release in both species. Matsumura studied the effects of ozone, nitrogen dioxide, and sulfur dioxide on experimentally induced respiratory allergy using this model, although only the

ozone results will be discussed here. Animals were exposed to ozone at 1, 5, or 10 ppm for 30 minutes, followed by inhalation of either bovine serum albumin or ovalbumin (antigen) for 45 minutes on five to seven separate days. Anaphylactic symptoms were observed after the 5th or 6th exposure. Death in the 10 ppm ozone plus antigen group was 33.3% compared with 0% in the ambient air control group. Skin tests with antigen showed that allergic sensitization had indeed been accomplished through inhalation, rather than parenteral sensitization, the usual means by which animals are sensitized. Results showed that ozone concentrations of 5 ppm and greater were able to enhance sensitization to inhaled antigen.

Matsumura (1970b) further investigated the mechanisms responsible for this anaphylactic sensitization by exposing guinea pigs to aerosolized ^{131}I labeled ovalbumin after a 30 minute exposure to 8 ppm of ozone. After measuring the levels of the labeled ovalbumin in the blood, Matsumura concluded that a single 30 minute exposure to 8 ppm ozone was sufficient to facilitate absorption of egg albumin from guinea pig lungs into the blood, leading to the conclusion that ozone exposure facilitated allergic sensitization.

Twenty years later Sumitomo et al. (1990) built upon Matsumura's early work and showed that exposure to ozone at 1, 3, and 5 ppm decreased the threshold for ovalbumin induced airway hyperresponsiveness. Ozone inhalation also enhanced airway hyperresponsiveness in previously sensitized guinea pigs. Thus, the authors concluded that ozone not only can facilitate sensitization to allergen, but also enhance responsiveness to allergen provocation..

Vargas et al. (1994) examined airway hyper-responsiveness induced by ozone, allergen, and combined ozone and allergen exposure, as measured by the response to histamine (ED50 determination) in guinea pigs. Ovalbumin sensitized guinea pigs responded with airway constriction to a lower concentration of histamine after antigen challenge. This effect was greater in animals that had been exposed for one hour to 3 ppm ozone compared to air control animals.

Schlesinger et al. (2002) investigated the effect of long term ozone exposure on airway hyperresponsiveness in sensitized and non-sensitized guinea pigs. In addition, another group of animals received concurrent ozone and antigen exposure. Two levels of ozone were examined (0.1 ppm and 0.3 ppm) and exposures were for 4 hours per day for 4 days per week for 24 weeks. Non-sensitized animals had no effects with ozone exposure. However, ozone exposure exacerbated airway hyperresponsiveness in response to both specific and non-specific provocation in sensitized animals. This effect lasted for 4 weeks after termination of the exposures. Moreover, airway hyper-responsiveness was significantly correlated with the increase in antibody response to the antigens used in sensitization. .

The guinea pig model has also been used to evaluate the effect of ozone on nasal allergy. Iijima et al. (2001) exposed guinea pigs to filtered air or 0.4 ppm ozone for 5 weeks. Once a week during ozone exposure a 1% solution of

ovalbumin was administered intranasally. By the third week of the protocol, the group of animals exposed to ozone and ovalbumin showed an increased number of sneezes during a 20 minute period after ovalbumin administration, compared to the other groups. By week 5 these animals were sneezing at an average rate of 15 sneezes in 20 minutes. The quantity of nasal secretions was also elevated significantly by the sixth and final ovalbumin administration. Histopathology showed infiltration of eosinophils into the nasal epithelium and subepithelium. In the subepithelium the ozone plus ovalbumin group showed a significant increase in eosinophils compared with the air plus ovalbumin exposed animals. While ozone was shown to enhance the symptoms of nasal allergy in the guinea pig model, the IgE antibody response to ovalbumin did not show a significant difference in ozone compared to air-exposed animals.

9.6.13.2.7 *Mice*

The mouse is currently the model of choice by most immunologists when examining allergic responses. Pioneering studies on the effects of ozone on the development of allergic responses to inhaled ovalbumin in the mouse were performed by Osebold et al. (1980). These investigators examined the effect of cyclic ozone exposure on allergic sensitization in mice exposed to 0.8 ppm ozone for three days in alternating weeks with three cycles of exposure and nonexposure (four days of exposure in the final exposure cycle). Thus, mice were only exposed to ozone for three days every other week, in a protocol intended to mimic acute ozone injury. On the day after each three-day ozone exposure mice inhaled a 1% aerosol of ovalbumin for 30 minutes, after which they remained in ambient air until the next ozone cycle. During the last ovalbumin exposure mice were observed for signs of atopic reactivity. One week after the fourth and final ovalbumin challenge, mice were injected (IV) with ovalbumin, and were observed for development of systemic anaphylactic shock. Results indicated that exposure to ozone enhanced anaphylactic sensitization when compared with similarly sensitized mice housed only in ambient air. One hundred percent of the ovalbumin/ozone exposed mice developed clinical anaphylaxis compared with 74% of ovalbumin/air exposed mice. When the researchers repeated the experiment using a lower ozone concentration (0.5 ppm ozone) 85% of the ozone/ovalbumin group developed anaphylaxis compared to 5% of the ovalbumin/air group (Osebold and Gershwin, 1980; Osebold et al. 1988). The data indicated that the number of IgE producing cells was significantly increased in the lungs of mice exposed to both ozone and ovalbumin aerosol compared to those from animals exposed to air and ovalbumin aerosol The difference in the number of IgE producing cells in ozone exposed mice compared with those in ambient air was highly significant ($p < 0.006$) in the 0.8 ppm experiment previously described (Gershwin et al. 1981). Data from these experiments showed that ozone exposure can enhance sensitization to inhaled antigen.

Exposure to ozone and ovalbumin using a very different protocol showed that the IgE response to parenterally administered ovalbumin was suppressed when the mice had been exposed to 0.8 ppm ozone continuously for 1 to 4 weeks, followed by a single ovalbumin aerosol (6 minutes) one week prior to the

parenteral injection (Ozawa et al. 1985). This protocol is contrived and less relevant to “real life” exposure than others that demonstrate an enhancing effect of ozone on the IgE response.

More recently, others have also studied the effect of ozone on allergic responses using a mouse model. Studies by Neuhaus-Steinmetz et al. (2000) exposed mice for four hours, three times per week, for four weeks to ozone concentrations ranging from 180 to 500 ug/m^3 (0.09 ppm to 0.25 ppm). Some mice also inhaled ovalbumin aerosol five times per week throughout the four week period. Control mice inhaled ovalbumin without ozone exposure. Skin tests were performed 48-hours after the last ozone exposure to evaluate allergic sensitization. Other markers of allergic sensitization studied included: total IgE, ovalbumin specific IgE, IgG1, and IgG2a, Th1 and Th 2 cytokines, and leukotrienes C4, D4, and E4 in bronchoalveolar lung lavage fluid. In BALB/c mice, which are genetically skewed toward Th2 responses (high IgE), ozone exposure induced a dose dependent eosinophil response in BALF after ovalbumin challenge. IL-4 and IL-5 were increased in BALF, whereas interferon gamma remained unchanged. In addition, skin test sensitivity and ovalbumin specific IgG1 levels in serum were elevated in ozone exposed mice. Leukotriene concentrations in BALF were significantly increased in ozone and allergen exposed mice. The low IgE responder mouse strain, C57BL/6, was also evaluated in this protocol. C57BL/6 mice treated with only ovalbumin showed the expected Th1 antibody response profile, while treatment with ozone and antigen was associated with a shift towards a Th2 type response. Thus, ozone appeared to modulate the immune response toward the allergic phenotype in this mouse model. This study is particularly important because it shows that the effect of ozone could potentially induce allergy in the non-atopic population that would not be expected from genetic analysis to develop an IgE response to environmental allergens.

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

9.6.13.2.8 *Rats*

The Brown Norway rat is another rodent model of allergy. . Wagner et al. (2002) used these rats in a recent study that examined the effect of ozone exposure in an ovalbumin-induced model of allergic rhinitis (hayfever). The ovalbumin-sensitized rats were exposed to 0.5 ppm ozone for 8-hours/day on either one or three consecutive days. Following ozone exposure, the rats received either ovalbumin or saline intranasally. Twenty-four hours later the rats were sacrificed and the nasal tissues examined. Results indicated that rats receiving both ozone and allergen had mucus-containing cells in the epithelium, an increased number of epithelial cells, and an increase in intraepithelial mucosubstances in the respiratory cells lining the septum. There was also an increase in the number of eosinophils in the maxilloturbinates of the ovalbumin challenged rats. This study suggests that allergic rhinitis could be exacerbated by f ozone inhalation in non-asthmatic subjects.

9.6.13.2.9 *Dogs*

Several experiments on the interaction of allergen and ozone have been performed using a canine model. The dog is often naturally sensitized to Ascaris antigen due to early infection with the roundworm. This provides a convenient model for aerosol challenge and elicitation of allergic signs. A study by Spannhake (1996), using Ascaris sensitive dogs, exposed for 5 minutes to 1 ppm ozone, compared to dogs breathing only ambient air showed that mast cell mediators (histamine and the arachidonic acid metabolite prostaglandin D_2, PGD_2) were decreased in mast cells obtained by BAL 30 minutes after exposure.. The mast cells were subsequently challenged *in vitro* with Ascaris antigen or an ionophore. The data showed that there was a significant decrease in PGD_2 in ozone compared to filtered air exposed airways. This data may, however, have little relevance to actual *in vivo* allergen exposure.

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

9.6.13.2.10 *Primates*

The primate has been used infrequently as a model for asthma. Yet, the monkey is an ideal model for these studies because both the lung morphology and the immune system are more like that of the human than are other animal models. In 1986 Biagini showed that exposure to 1 ppm ozone enhanced development of

allergy to inhaled platinum (Biagini et al. 1986) using a cynomolgus monkey model. In this study airway response to allergen and methacholine challenge as well as skin test reactivity were significantly affected in the combined allergen and ozone group. The importance of this study is that it is the first primate study to show both functional and immunological enhancement of allergic responsiveness to allergen by ozone.

More recently, a Rhesus monkey model of asthma has been used to examine the influence of ozone exposure on development of both immunological and structural attributes of asthma (Schelegle et al., 2001). The combined exposure to epizootic ozone and house dust mite allergen has been shown to alter both lung development (Evans et al. 2003), and immune responses (Miller et al. 2003). These studies are ongoing and are expected to provide important mechanistic information regarding the interplay of ozone and allergen in the developing infant. This model is particularly relevant because it uses an allergen that is particularly common for asthmatic human subjects (*Dermatophygoides farinae*, the house dust mite). These studies are also the first to provide morphologic, functional, and immunological data supporting the notion of an enhancement effect of ozone on allergic asthma. One study exposed infant rhesus monkeys, starting at 30 days of age, to 11 episodes of either filtered air and house dust mite allergen aerosol, or ozone (0.5 ppm) and house dust mite allergen aerosol. Each cycle consisted of 5 days of ozone exposure followed by 9 days of filtered air exposure. Monkeys that were not allergen-sensitized or were sensitized but only exposed to filtered air had only mild airway effects. However, monkeys that received the combined ozone and allergen treatment had a marked increase in serum IgE, histamine, and airways eosinophilia (Schelegle et al. 2003). These studies use an epizootic and cyclic ozone and allergen exposure that is intended to replicate natural conditions. Moreover, studies using infant monkeys serve as a model for the developing human child (Miller et al. 2003).

9.6.13.2.11 <u>Interactions of Ozone With Other Air Pollutants</u>

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

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

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

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

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

Although these studies investigated the influence of single air pollutant exposures on allergic responses, observations that several of the pollutants investigated appear capable of interacting with allergen suggest the likelihood that enhanced allergic reactivity, and asthma, may be influenced by synergism of multiple air pollutants interacting with environmental allergens.

9.6.13.2.12 Conclusions

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

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

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

There is only limited information on interactive effects between ozone and other ambient co-pollutants. As mentioned above, the reasons for the relative paucity of joint exposure studies include the difficulty of adequately controlling the concentrations of multiple pollutants simultaneously. The large number of

pollutants in ambient air are subject to a practically infinite number of combinations by location and time, making selection of a simplified but still representative exposure atmosphere problematic. In addition, the complexity of atmospheric chemistry increases the difficulty of adequately assessing the exposure mixture as the number of pollutants increases. As opportunities for chemical reactions among various atmospheric chemical species increase, the possibility that observed effects may be related to unknown reaction products increases as well.

The available evidence suggests that ozone is responsible for the observed effects in subjects exposed to mixtures of ozone and other common air pollutants, including NO_2, PAN, H_2SO_4, HNO_3 or SO_2, and several common nitrate and sulfate aerosols, at least within the range of ambient concentrations. However, the sequential exposure studies by Aris et al. (1991), suggested that HNO3 and H_2O fog exposure mitigated pulmonary function effects of a subsequent ozone exposure. Also, Koenig et al. (1990) report that preexposure to ozone induced significant pulmonary function decrements from a subsequent "subthreshold" SO_2 exposure suggests that the initial ozone exposure may have induced airway hyperresponsiveness that may have potentiated the response to SO_2. Together, these studies suggest that some sequential exposures may predispose susceptible individuals to responses that would not have occurred without the initial exposure. Given the dearth of controlled exposure data on pollutant interactions with ozone, there are still many unresolved issues, including the extent to which the results of the studies reviewed in this section apply to endpoints other than lung function.

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

9.7 Summary of the Findings of Controlled Exposure Studies

9.7.1 Pulmonary Function

Collectively, the available literature exploring the responses of human subjects exposed to controlled concentrations of ozone indicates that one to three hour exposures to ozone concentrations as low as 0.12 ppm with moderate to heavy exercise can induce decrements in pulmonary function and increases in ventilatory symptoms for some individual subjects. Statistically significant group mean decrements have not been reported at ozone concentrations less than 0.12 ppm. There are no data on one to three hour exposures to ozone concentrations lower than 0.08 ppm. The data indicate that some individuals exposed to 0.12 ppm for one to three hours while performing moderate to heavy exercise will develop decrements in FEV1 of greater than 20%. Interest in longer averaging times led to studies in healthy adults who performed a protocol simulating a day of active outdoor work or play. These studies demonstrate that statistically significant group mean decrements in FEV1 occur at ozone concentrations as low as 0.08 ppm. Ozone concentrations between 0.04 and

0.08 ppm have not been investigated with multi-hour exposure protocols. Evaluation of the responses of the individual subjects who participated in these studies (decrements as high as -48%) demonstrates that significant decrements in FEV1 can occur in more responsive individuals when they undergo multi-hour exposure to ozone exposure at levels at or below the current federal 8-hour standard, and only marginally above nonanthropogenic ozone background levels.

The literature shows that individual responsiveness to ozone is relatively stable, at least for periods of at least a year or two. The data also indicate that each individual has a characteristic degree of responsiveness to ozone that is related to innate factors that remain to be elucidated.

9.7.2 Symptoms

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

9.7.3 Nonspecific Airway Responsiveness

Increased nonspecific airway responsiveness has been reported with one to 3 hour exposures to 0.40, but not 0.20 ppm ozone at rest. The lowest 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. Available data indicate that exposures to ozone concentrations as low as 0.08 ppm for 6.6 hour can increase non-specific airway hyperresponsiveness.

9.7.4 Airway Inflammation

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

Exposure to 0.08 ppm ozone for 6.6 hour decreases the ability of alveolar macrophages to phagocytize microorganisms via the complement receptor. 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 hour 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.

9.7.5 Exercise Performance

Significant reduction in exercise performance has been reported at ozone concentrations as low as 0.06 ppm (Linder et al., 1988), although these results have not been confirmed by others using similar protocols with ozone concentrations between 0.06 ppm up to 0.12 ppm (Gong et al., 1986; Schelegle and Adams, 1986). Exercise tolerance and pulmonary function changes are not always observed in concert (Gong et al., 1986; Foxcroft and Adams, 1986; Schelegle et al., 1987).

9.7.6 Population Groups Most at Risk

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

It should be kept in mind that the subjects who participated in most studies discussed in this document have been representative of healthy, young and middle-aged adults, although the range of individual responsiveness to ozone in this population group is very broad. 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. The asthmatics studied have typically had changes in symptoms, lung function and inflammation in the same range as nonasthmatics, but larger increases in airway reactivity than healthy, nonasthmatic people. However, since asthmatic individuals often have lower lung function and chronic airway inflammation compared to nonasthmatics, at baseline, even relatively small additional reductions in function or increases in inflammation can be greater cause for concern than similar changes in healthy people. Because of ethical considerations, there are few studies of individuals with cardiovascular disease or COPD, and those few studies involved subjects with relatively mild disease. Similarly, for ethical reasons there are no controlled studies of infants or young children available. However, since seriously impaired individuals are unlikely to spend significant periods of time outdoors working or exercising, the exposure of such individuals is likely to be considerably smaller than that of healthier, more active people, and consequently their overall risk is likely to be less. Therefore, the findings derived from the available literature are likely representative of people who are most at risk of adverse health effects from ozone exposure:

those who are physically able to perform moderate exertion for several hours, and by extension, are likely to experience the greatest ozone exposures.

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

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

9.7.7 Pollutant Mixtures

Although there are isolated findings to the contrary, the available data do not support the likelihood of clinically meaningful interactions in human subjects between ozone and gaseous nitrogen-based air pollutants, SO_2 or H_2SO_4 aerosols at concentrations in the ambient range. Observed responses at the pollutant concentrations studied to date appear to be attributable to the ozone 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 ozone 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_2SO_4 aerosol.

The possibility that ozone impacts asthma in the human population has been investigated in humans and in animal models. However, the mechanisms involved are less clear. For example, the role of ozone in sensitization to allergen, versus its role in exacerbation of the disease remains somewhat ambiguous. Some studies in the mouse model have shown a role for ozone in sensitization to inhaled allergen. Exactly how ozone mediates this effect at the cellular and molecular level, particularly in humans, will require additional study.

9.7.8 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. 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. 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 can be quite symptomatic following exposures at that level. Although children and adolescents do not appear to experience greater adverse responses than adults who complete similar exposures, they are among those most likely to spend significant periods of time outdoors while engaged in exercise, putting them at increased risk of adverse responses.

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

There are insufficient data available to draw a conclusion as to whether there is a difference in the 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 convincingly shown to be linked with responsiveness, both in an inverse direction. This reduced responsiveness in smokers may wane after smoking cessation.

9.7.9 Effective Dose

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. Adams (2003a) provides an illustration of the significance of ozone concentration, compared to V_E and V_T. The subjects in this study completed 6.6-hour exposures to 0.08 ppm ozone (protocol described in Section 9.6.3), and 2-hour exposures to 0.30 ppm with

alternating 15 min periods of rest and exercise. Although the inhaled ozone dose in the 2-hour protocol was only 1.44 times greater than that for the 6.6-hour protocol, the decrements in FEV1 averaged -3.51% following the 6.6-hour protocol (0.08 ppm), and –12.36% following the 2-hour protocol (0.30 ppm). Hazucha et al. (1992) reported similar findings. These results lead to the conclusion that 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 illustrating the need to consider dose-rate in evaluating possible exposure scenarios when selecting concentration and averaging time combinations for ambient air quality standards for ozone.

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

The results of controlled human exposure studies utilizing ozone exposures up to about 8-hour 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. Epidemiology studies may not always provide clear causal relationships because of the presence of confounding factors (i.e., heat, humidity, other pollutants, subject characteristics). However the results can provide associations that may suggest causal relationships or provide hypotheses that can be investigated in animal models, and sometimes in controlled human studies.

Animal toxicology studies are limited by incomplete knowledge of species sensitivity and dosimetry patterns compared to humans, although they can offer controlled experimental conditions for chronic exposures, provide evidence of causal relationships, and also allow investigation of endpoints not possible to study in humans.

However, considering the available evidence collectively, it is reasonable to conclude that repeated episodes of airway inflammation could lead to morphological changes in the lungs, and contribute to long-term respiratory health impacts. Animal studies clearly support this line of reasoning. There is also evidence that children who grow up in high ozone communities have lower lung function values at maturity than children who grow up in low ozone communities (Kunzli et al., 1997; Galizia and Kinney, 1999). This is a significant finding, in that low lung function is a known risk factor for chronic lung disease and premature death.

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10 Epidemiological 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 both short and long-term (i.e., a year or more) effects of ozone in free-living populations. In addition, epidemiologic studies are able to examine a wide range of individuals, behaviors, subgroups, and exposure conditions. Studies of ozone have reported associations with such outcomes as lung function, respiratory symptoms, emergency department visits, hospitalizations and premature death As such, these studies provide compelling evidence of an adverse effect of 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 background 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 which would tend to bias downwards the estimated effect of ozone and make it more difficult to find an association, given that one existed. Secondly, epidemiologic studies may be subject to bias from uncontrolled or poorly controlled confounders such as seasonality, weather and co-pollutants, including other oxidants. 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. 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. However, there is still a possibility that some unmeasured factor is highly correlated with both ozone and the health effect being measured. As ozone effects are observed in more cities under differential coexposure scenarios, there is a diminished likelihood of a common confounder being responsible. 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.

Since many of these limitations are specific to individual study designs, they are reviewed more fully in the sections below describing the different study designs that have been undertaken. Despite these limitations, a large number of epidemiological studies published in the last several years have shown positive associations between ozone levels and several health effects.

10.1 Field Studies Addressing Acute Respiratory Effects of Ozone

10.1.1 Introduction

Numerous field studies carried out over the past decade have tested for, and in many cases observed, acute associations between measures of daily respiratory ill-health and ozone concentrations in small groups of subjects. These studies have often focused on subjects with asthma (Table 10-1). What distinguishes acute field studies from other acute epidemiologic study designs is that field studies recruit and collect data from individual human subjects, as opposed to gathering administrative data on aggregate health outcomes, as in studies of daily death, hospital admissions, or ED visits. Because of the logistical burden associated with direct data collection from individual subjects, field studies tend to be small in both numbers of subjects and in duration of follow up. As a result, statistical power often limits the conclusions that can be drawn from these studies. Epidemiologic studies of asthma in relation to air pollution must also contend with the known peak in asthma exacerbations and admissions which occurs in the fall in many locations, usually around the third week of September (Bates et al., 1990). This peak occurs independently of air pollution, but may interfere with ongoing panel studies by obscuring an association with air pollution during other periods of the year. The peak in asthma during the fall season would be expected, on average, to add noise to epidemiologic studies, but could also bias results if by chance it correlated with a peak or trough in ozone concentrations.

Although personal monitoring using passive diffusion ozone monitors has sometimes been used in the epidemiologic literature, most studies still rely on fixed-site ambient monitoring for characterizing exposures of the population under study. As noted elsewhere, ozone penetrates indoors incompletely, especially where air conditioning is used for indoor temperature control in hot climates, such as inland areas of California. Because ozone levels tend to higher in inland regions, many of the field studies conducted in California have been based in inland communities. In interpreting findings from such studies, it is important to keep in mind that individual exposures to ozone may not be correlated highly with ambient measurements for subjects who spend most of their time indoors. This issue is relevant not only to acute effect studies (including field studies and hospital usage studies) but also to studies of long-term exposures. California studies that may have been influenced by this phenomenon include those by Delfino et al. (1996) conducted in inland San Diego county, those using the Seventh Day Adventist Cohort which is located throughout California (e.g., Glendale, Riverside, Alameda, Santa Barbara), and the Children's Health Study (CHS) (e.g., Gilliland et al., 2001). For example, some of the communities included in the CHS were in hotter inland areas of southern California. Exposure misclassification due to this phenomenon may have partly explained the isolated results in the CHS for the increased risk of asthma onset only among children playing three or more sports in the six out of 12 communities with higher ozone (McConnell et al., 2002; see review below). More outdoor exposure and increased ozone dose may have been a function of the physical activities.

Another issue is the potential for bias in estimated ozone effects related to the inclusion of weather factors into regression models where there is no plausible reason to expect weather factors to influence the outcome. For example, there is no known effect of temperature on lung function at normal ambient conditions. However, it is common to include temperature as a covariate in multiple regression models assessing ozone effects on lung function. Since ambient temperature correlates with ambient ozone, this can lead to instability and possible bias towards the null if temperature picks up some of the ozone effect. A related issue is that ambient temperature may have little relation to the temperatures experienced by study subjects (Delfino et al., 2004).

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

10.1.2 Ozone and Lung Function

A large body of literature from past clinical and field studies has clearly demonstrated reversible decrements in lung function following ozone exposures (US EPA 1996). Studies published over the past decade provide few new insights regarding these effects, with the exception of a study in Fraser Valley, British Columbia that reported unusually large effects on lung function among agricultural workers ages 10-69 yrs exposed over long work-shifts while working outdoors (Brauer et al. 1996). Nine of 11 newer studies that tested for effects of ozone on lung function reported significant associations, although in some cases there are inconsistencies. Four studies carried out in the US are reviewed here first (Delfino et al. 1997b; Neas et al. 1999a; Linn et al. 1996; Ross et al. 2002).

One relatively small study saw no significant effects on morning (AM) and evening (PM) peak expiratory flow rate (PEFR) among 22 asthmatics living in Alpine, California, and ranging in age from 9-46 years, even though daily 12-hour mid-day

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

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

Linn et al. (1996) studied repeated measures of spirometric lung function among 269 school children in three southern California communities (Rubidoux, Upland, and Torrance). Spirometry provides more extensive, precise and reproducible measures of lung function than are available from peak flow meters, including forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1). Lung function was measured over five consecutive days once in each of three seasons over two school years. However, the narrow range of exposures that were experienced within each week limited statistical power. Between-week variability was effectively removed from the analysis by seasonal terms in the model. Furthermore, the study was restricted to the school year, eliminating most of the “high” ozone season from consideration. As a result, ozone levels were quite low, with a 24 hour mean of just 23 ppb, and a range of 3-53 ppb. These factors would tend to compromise the power to detect associations between ozone exposures and outcomes. In a subset of subjects, personal ozone monitoring using passive dosimeters was correlated with central site ozone (r=0.61). The difference between AM (recorded near the beginning of the school day) and PM (recorded following lunch) FEV1 was significantly associated with same day ozone concentration. Other associations (involving individual AM or PM FVC and FEV1 time points) went in the plausible direction but were not statistically significant.

Among international studies, a large study measured spirometric lung function in 895 school children ages 8-13 yrs in three towns in Taiwan (Chen et al. 1999). Unfortunately, lung function was measured only once for each subject. As a result, between-person variability could not be distinguished from within-person variability. This study design results in limited statistical power, compared with a design in which individual subjects provide multiple days of measurements. The

latter study design enables each subject to serve as his/her own control, and provides information about both between and within subject responses. Still, the authors reported statistically significant associations between diminished FVC and FEV1 and the one-day lag of ozone concentration. Effect sizes were typical of those observed in past studies, i.e., 0.5 to 1.0 mL drop in FVC per ppb increase in ozone concentration. Ozone was the only significant air pollutant in multi-pollutant models including SO_2, CO, PM10, and/or NO_2. The ozone associations became non-significant when days with ozone above 60 ppb were excluded from the analysis, implying a practical threshold of around 60 ppb for this individual study.

In Mexico City, two identical studies of asthmatic school children were carried out simultaneously in the northern (Romieu et al. 1996) and south-western sections of the city (Romieu et al. 1997). In the northern study, 71 mildly asthmatic school children, 5-7 years old, were followed over time for daily AM/PM PEFR. In single-pollutant models, ozone at lags 0, 1, and 2 was associated with diminished AM (before breakfast) and PM (bedtime) PEFR, but only the lag 0 AM effect was statistically significant. The ozone effect became non-significant when PM2.5 was added to the model. In the southwestern study, 67 mildly asthmatic children 5-13 years old were followed in both summer and winter for daily AM/PM PEFR. Significant effects of same day and lag one-day ozone were observed on PM PEFR, with effects larger for the one-day lag. Associations involving ozone were stronger than those involving PM2.5. Another study in south-western Mexico City analyzed AM/PM PEFR data collected from 40 school children ages 8-11 years (Gold et al. 1999). Subjects provided measurements upon arrival at, and before departing from, school each day. Diminished PEFR was associated with lag 1 ozone, but the only statistically significant findings were obtained for PEFR regressed on the 10-day moving average of ozone. This may imply a cumulative effect of ozone, or may reflect confounding by other time-varying factors. This is in accord with controlled human exposure studies that have shown a decline in baseline pulmonary function with repeated days of ozone exposure (see Section 9.6.9), and with epidemiologic studies that have assessed lung function over the course of the ozone season (Brauer et al. 1996; Kinney and Lippmann 2000). A similar study carried out in Amsterdam involving 61 school children 7-13 years old, the majority with doctor-diagnosed asthma, reported effects of lag 2 ozone on AM PEFR (Gielen et al. 1997). However, the reported two-day lag effect on AM lung function, in the absence of a same day effect on PM function, seems implausible in light of findings of controlled human exposure studies (see Chapter 9).

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

In a unique study, Brauer et al. (1996) measured spirometric lung function before and after outdoor summer work shifts on a group of 58 berry pickers in Fraser Valley, British Columbia. Subjects ranged form 10 to 69 years old (mean of 44

years). Work shifts averaged 11 hrs in duration. Ozone exposures were measured by personal badges as well as by an ozone monitor very close to the workers. Exertion levels in that study were estimated using portable heart rate monitors carried by a representative subset of subjects for 16 work shift periods of four or more hours. Heart rates were essentially constant over the work shift, averaging 36% higher than resting levels. The authors estimated that minute ventilations might have averaged roughly 30 L/min during work. Post-shift FEV1 and FVC showed large decreases as a function of ozone concentration, and those effects remained significant when PM2.5 was added to the model. Lung function on the morning following exposure also was significantly lower. On a unit ozone basis, the effects observed in this study were larger than have been reported previously. For example, afternoon FVC was 5.4 mL lower per 1 ppb increase in ozone concentrations. Further, ozone effects remained significant when data were restricted to days with 1-hour maximum ozone concentrations less than 40 ppb. These observations suggest that the extended exposure period outdoors, combined with elevated levels of exertion throughout exposure, enhanced response in this group. There is also a possibility of greater responsiveness related to generally less advantaged health status in the study population.

10.1.3 Ozone and Respiratory Symptoms and Medication Use

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

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

Among the 12 studies reporting results for daily symptoms, seven reported associations with ozone concentrations that appear fairly robust (Desqueyroux et al. 2002; Gent et al. 2003; Gold et al. 1999; Hiltermann et al. 1998; Romieu et al. 1997; Romieu et al. 1996; Ross et al. 2002). One of the largest and best-conducted studies was that of Gent and colleagues (2003), where 271 asthmatic children under age 12 living in southern New England were each followed over six months (April through September) for daily symptoms. The data were analyzed

for two separate groups of subjects, 130 who used maintenance asthma medications during the follow-up period, and 141 who did not. The need for regular medication was considered a proxy for more severe asthma. Significant effects of a one-day lag in ozone were observed on a variety of respiratory symptoms in the medication user group only. Both daily maximum 1-hour and 8-hour concentrations were similarly related to symptoms, including chest tightness and shortness of breath. Effects of ozone, but not PM2.5, remained significant and even increased in magnitude, in two-pollutant models. Of particular importance, some of the significant associations were seen at 1-hour maximum ozone levels of 43.2 ppb or higher and at 8-hour average ozone levels of 63.3 ppb or higher. Given the large number of significant associations observed in the medication user subgroup, it is somewhat surprising that no effects were observed among asthmatics not using maintenance medication. It is not clear whether this stratification was determined *a priori* or was chosen following preliminary analyses. The former would indicate a more robust finding. Another uncertain aspect of this study was whether a peak in asthma exacerbations during the fall season was observed during follow-up, and if so how it was handled in the analysis. In terms of person-days of follow-up, this is by far the largest of the currently available studies addressing symptom outcomes in relation to ozone, and provides the strongest evidence for effects of ozone independent of PM2.5.

To put the Gent et al., 2003 study into perspective, we note that the biological mechanism of ozone is in large part related to airway inflammation as discussed above in Section 9.3.3. Therefore, medication that controls airway inflammation such as inhaled corticosteroids would be expected to dampen the effects of ozone. However, finding the opposite in a panel study such as Gent et al. (2003) is not unexpected if use of such medication is largely restricted to more severe asthmatics, who may be more susceptible to ozone. The results contrast findings of Delfino et al. (1998a, discussed below) showing a significantly stronger association between asthma symptom severity and ozone in asthmatic children <u>not</u> taking anti-inflammatory medications, largely inhaled corticosteroids (ICS). Mortimer, et al. (2000, discussed below) compared effects on asthma outcomes by outdoor ozone levels across medication groups based on baseline data for prescribed medication. Associations between incidence of symptoms and an increase of 15 ppb in ozone was largest among those prescribed cromolyn but not ICS (OR 1.46, 95% CI 1.06, 2.01) followed by nonsignificant ORs for those prescribed ß-agonists or xanthines only (1.18), ICS (1.08), and no medication (1.04). The percentage change in PEF was also greatest among those prescribed cromolyn but not ICS (-1.27, 95% CI -2.47, -0.06) followed by nonsignificant PEF changes of around -0.5 for the other groups. So, the issue of effect modification by medication use remains somewhat unclear at present.

Several other US studies reported significant effects of ozone on symptoms (Delfino et al., 1998a, 2003; Mortimer et al., 2000; Ross et al., 2002). Ross and colleagues studied 40 asthmatics ages 5-49 yrs in the East Moline, Illinois area (Ross et al., 2002). There, an undefined four-level symptom score was associated with daily 8-hour max ozone concentrations, controlling for pollen and mold spore levels. Delfino and colleagues (1998a) studied schoolchildren with asthma in

inland San Diego County, and showed significant associations between asthma symptoms (rated as bothersome or interfered with daily activities) and ozone, with similar associations for 1-hour and 8-hour ozone averages. Associations for ozone and PM_{10} were largely independent in models incorporating both pollutants, and outdoor fungal spores did not confound ozone associations. The study also showed significantly stronger associations between asthma symptoms and ozone in a subset of asthmatics not taking anti-inflammatory medications. Threshold analyses suggested effects below 80 ppb 1-hour ozone maximum in this subset, but not among other subjects. This concentration was exceeded 25 times during the three-month study. Delfino et al., (2003) studied Hispanic schoolchildren with asthma in LA, and showed significant associations between asthma symptoms (bothersome or interfered with daily activities) and ambient VOCs, PM_{10} elemental and organic carbon, but not ozone. However, ozone, along with formaldehyde and acetone, were similarly associated with more severe symptoms interfering with daily activities among a subset of children, particularly those on maintenance medication. Odds ratios (OR) for interquartile increases in 1-hour ozone (14 ppb) were identical to 8-hour (11 ppb) (both ORs around 2.0), even though 1-hour ozone never exceeded 52 ppb. Mortimer et al. (2000) reported results of a series of 2-week asthma panels in 846 inner city children with asthma living in low income neighborhoods. Analysis was restricted to diaries completed between June 1 and August 31, avoiding the issue of a possible peak in the fall season asthma exacerbations. They found that ozone was inversely associated with peak flow and positively associated with symptoms, with the strongest associations among children born with low birth weight or prematurely.

Other US studies carried out over the past decade have failed to detect symptom effects associated with ozone. Delfino and colleagues (1996) followed 12 asthmatic ages 9-18 yrs living in San Diego, CA for respiratory symptoms over a two month period, and saw no relationship with central site ambient ozone. Personal ozone exposures measured with passive diffusion monitors were associated with a composite symptom score, but the relationship disappeared when week-day/week-end differences were controlled in the statistical analysis. Study power was likely compromised by the small sample size. The observation of stronger effects based on personal monitoring is intriguing, and suggests that substantial gains in power may be achievable when exposure misclassification is reduced through use of measured personal exposure rather than central site ozone concentration. However, a similar study of 22 asthmatics ages 9-46 yrs in Alpine, CA saw no effects of ozone on symptoms when personal ozone exposure was used as the exposure metric (Delfino et al., 1997). One final US study reported no associations between daily symptoms and ambient ozone concentrations in a cohort of 138 African American children ages 8-13 years old with asthma followed over three months (Aug-Oct) in Central Los Angeles and Pasadena, CA (Ostro et al. 2001). However, use of extra asthma medications was associated with a one-day lag of 1-hour maximum ozone concentrations. Finally, the study by Linn and colleagues (1996) of 269 fourth and fifth grade school children in southern California reported no associations between symptoms and ozone, but as noted earlier, power to test ozone effects was limited due to the low

and narrow range of ozone concentrations observed during the field sampling period.

Several non-US studies have reported significant symptom associations with ozone. Exacerbation of COPD symptoms was significantly associated with ozone concentrations in a group of 39 adult patients (mean age 67 years) followed over a 14 month period in Paris (Desqueyroux et al. 2002). There were no effects of PM10, SO_2, or NO_2 in that study. Interestingly, in contrast to controlled human studies (see Sections 9.6.8.7 and 9.6.6), the ozone effect appeared larger among subjects who smoked and those with more severe COPD. However, the very low ozone concentrations experienced during that study (e.g., summer mean 8-hour max ozone of 21 ppb) raise plausibility questions. In a study of 60 non-smoking asthmatic adults (ages 18-55) in Bilthoven, The Netherlands, Hiltermann and colleagues (1998) reported significant associations between ozone and daily symptoms of shortness of breath and pain upon deep inspiration. The ozone associations were stronger than those of PM10, NO_2, SO_2, and black smoke (BS). No differences in response were evident between subgroups of subjects defined on the basis of steroid use or airway hyper-responsiveness. No effects of ozone were evident with respect to daily use of bronchodilator or steroid inhalers.

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

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

Absence from school was associated with ozone concentrations in a study of 1,933 fourth grade students from 12 southern California communities participating in the Children's Health Study (Gilliland et al. 2001). All school absences that occurred over a six month period, January-June 1996, were followed up with phone calls to determine whether they were illness-related or not. For illness-related absences, further questions assessed whether the illness was respiratory or gastrointestinal, with respiratory including runny nose/sneeze, sore throat, cough, earache, wheezing, or asthma attack. Multiple pollutants were measured at a central site in each of the 12 communities. The statistical analysis controlled for temporal cycles, day of week, and temperature, and expressed exposure as a distributed lag out to 30 days. Some concern exists regarding the possibility of

residual seasonal confounding given the six-month time span of the follow-up period. Significant associations were found between the 30-day distributed lag of ozone (10am to 6pm average) and all absence categories. Bigger effects were seen for respiratory (83% increase) than for non-respiratory (37% increase) causes per 20 ppb rise in 10am to6pm ozone concentrations. Among the respiratory absences, larger effects were seen for lower respiratory diseases with wet cough (174% increase) than for upper respiratory diseases (45% increase) per 20 ppb rise in average ozone concentration for the time period of 10 AM to 6 PM. All effects were summed over the 30 day distributed lag. PM10 was only associated with upper respiratory disease absences. Due to its size and comprehensive characterization of exposures and health outcomes, this study is especially valuable in assessing adverse acute health effects of ozone in children. The study spanned a period, January through June, when ozone levels increase markedly. Thus, a wide range of exposures was captured while staying mostly below the highest levels observed in the summer season. As noted earlier, exposure misclassification for ozone may have varied across study communities due to use of air conditioners and differential rates of penetration of outdoor ozone in warmer regions.

Acute airway inflammation has been shown to occur among adults exposed to 80 ppb ozone over 6.6 hrs with exercise in controlled chamber studies (Devlin et al. 1991). Kopp and colleagues (1999) attempted to document inflammation of the upper airways in response to summer season ozone exposures by following a group of 170 second and third grade school children ages 8-10 yrs in two towns in the German Black Forest from March to October of 1994. To assess inflammation, the investigators collected nasal lavage (NL) samples at 11 time points spanning the follow up period. NL samples were analyzed for markers of inflammation, including eosinophil cationic protein (ECP), albumin, and leukocyte counts. Subjects were excluded who were sensitized to inhaled allergens. When analyzed across the entire follow up period, no statistical association was detectable between upper airway inflammation and ozone concentrations. More detailed analysis showed that the first significant ozone episode of the summer was indeed followed by a rise in ECP levels; however, subsequent and even higher ozone episodes did not affect ECP. These findings suggest an adaptive response in terms of inflammation in the nasal airways that is consistent with controlled human studies (see Section 9.6.9.8), but do not preclude the possibility that other, unmeasured effects including cell damage or lower airway effects, may occur with ongoing summer season exposures. In fact, a study of joggers repeatedly exposed to ozone while exercising over the summer in New York City suggested that cell damage might occur in the absence of ongoing inflammation (Kinney et al.1996).

Table 10-1: Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean ozone levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Delfino et al. 1997b Alpine, CA May 9, 1994 to July 3, 1994	22 asthmatics ages 9-46 followed for respiratory symptoms, AM-PM peak flow rate (PEFR), and B2 agonist inhaler use. Personal ozone measured for 12 hrs/day using passive monitors. GLM mixed model.	1-hour max ozone: Mean 88 SD (25) 12-hour ozone: Mean 64 ppb SD 17 Personal ozone: Mean 18 ppb SD 14	PM10, pollen, fungi	No ozone effects observed.	No quantitative results for ozone.
Delfino et al. 1998a Alpine, California August 1 – October 30, 1995	25 asthmatics ages 9-17 years completed daily diaries of symptoms (n=1,759 person-days. Used general estimating equations (GEE) controlling for autocorrelation, day of week, outdoor fungi and weather.	1-hour max ozone Mean 90 ppb SD 18 ppb 8-hour max Mean 73 ppb SD 15 ppb	PM10, fungi	Results were similar when adjusting for PM10. Largest effects were seen in less frequently symptomatic children not on anti-inflammatory medicine.	OR for asthma symptoms per 0-90th percentile current day ozone: 1-hour max for 58 ppb: 1.54 (1.02 – 2.33) 8-hour max for 46 ppb: 1.42 (1.00 – 2.00)
Delfino et al. 2003 Los Angeles, CA November 4, 1999 – January 23, 2000	Panel study of 22 Hispanic children 10-16 years old living in an area with high traffic density. Subjects filled out daily diaries.	1-hour max Mean (SD) 25.4 ppb (9.6) 8-hour max Mean (SD) 17.1 ppb (7.2)	NO2, SO2, PM10, CO, VOCs, EC	Significant associations between asthma symptoms and VOCs, Pm10 and elemental and organic carbon, but not ozone.	OR for subset of children on medication 2.0 for interquartile increases in 1-hour ozone (14 ppb) 2.0 for 8-hour ozone (11 ppb)

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean ozone levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Gielen et al. 1997 Amsterdam April 26-July, 1995	61 children (age 7-13) from two schools for sick children, followed for twice daily PEFR, symptoms, and medication usage. Majority of cohort had doctor-diagnosed asthma.	8-hour max ozone: Mean (SD) 34 (8) ppb 1-hour max ozone: 39 (8) ppb	PM10, BS, pollen	AM PEFR significantly associated with lag 2 8-hour ozone. BS also associated with PEFR. Among 14 symptom models tested, only one yielded a significant ozone finding (for upper respiratory symptoms). This is likely spurious. PM10 and BS, but not ozone, were related to B2 agonist inhaler use.	Percent change in PEFR for 42.3 ppb change in lag 2 8-hour max ozone: AM data: -1.85% (-0.14 to -3.58), $p<.05$ PM data: -1.88% (0.18 to -3.94), $p<.10$

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean ozone levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Gilliland et al. 2001 12 southern California communities January-June 1996	1,933 4th grad children followed for school absences. Each absence classified as illness-related or not. Among former, further classified into respiratory or gastrointestinal. Respiratory absences further classified into upper or lower. Pollution measured in central site in each town. Analysis of distributed lag effects controlling for time, day of week, and temperature in a Poisson model.	Levels not reported here.	PM10, NO_2	ozone strongly associated with illness-related and respiratory absences. PM10 only associated with upper respiratory absences. Long distributed lag effects for ozone raise questions about adequacy of control for seasonal changes.	Percent change in absences associated with a 20 ppb rise in 8-hour 10-6 ozone: Absences due to: All illness: 62.9% (18.4-124.1) Non-respiratory illnesses: 37.3% (5.7-78.3) Respiratory illnesses: 82.9% (3.9-222.0) Upper respiratory: 45.1% (21.3-73.7) Lower respiratory w/wet cough: 173.9% (91.3-292.3)

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean ozone levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Linn et al. 1996 3 California Towns: Rubidoux, Upland, and Torrance Fall-Spring for 1992-1993 and 1993-1994 school years	269 school children (age unspecified), each followed for AM/PM lung function and symptoms for one week in fall, winter, and spring over two school years. Personal exposure monitoring in a subset. Analyzed PM symptoms vs. same-day pollution and AM symptoms vs. lag 1 pollution.	24-hour avg ozone: Mean (SD) 23 (12) ppb	PM2.5, NO_2	Central site ozone correlated with personal exposures at 0.61. ozone effects observed on lung function but only significant for FEV1 in one analysis. No effects on symptoms. ozone effects were not robust to NO_2 or PM2.5. Power may have been limited by short follow-up within seasons (limiting both person-days and variability in exposures).	Change in lung function (mL) per ppb 24-hour avg ozone (SE). FVC AM: -0.21 (0.22) FVC PM -0.20 (0.29) FVC AM/PM difference: -0.25 (0.25) FEV1 AM: -0.26 (0.25) FEV1 PM: -0.18 (0.26) FEV1 AM/PM difference: -0.58 (0.23)* * $p<0.05$
Mortimer et al. 2000 8 U.S. cities June 1- August 31, 1993	846 inner-city asthmatic children ages 4 – 9 years. Daily diaries compared with ambient ozone levels. Examined effect modification for low birth weight vs. normal birth weight and for medication use.	8-hour avg Mean 48 ppb across 8 urban areas Fewer than 5% of days exceeded 80 ppb.	SO2, NO2, PM10 obtained, but only ozone used in analysis.	Children who had low birth weight or a premature birth had greatest responses to ozone. Nonatopic children also had greater responses to ozone. Type of medication used also affected the associations.	Change in lung function per 15 ppb ozone PEFR for premature or low birth weight: -1.8% PEFR for normal birth weight: 0.3% PEFR for low birth weight and “no medication”: -3.2%

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

Reference, location, years.	Outcomes and Methods	Mean ozone levels (IQR)	Pollutants considered	Findings, Interpretation	Effects
Neas et al. 1999b Philadelphia, PA July 12-September 3, 1993	156 children (age unspecified) at two summer camps followed for twice daily PEFR. Study mainly focused on detecting PM effects.	12-hour 9a-9p ozone: SW Camp: Mean 57.5 ppb IQR 19.8 NE Camp: Mean 55.9 ppb IQR 21.9	H+, SO_4, PM2.5, PM10, PM10-2.5	Some ozone effects detected as well as PM effects. PM effects strongest for SO_4. ozone effects not robust to SO_4 in two-pollutant models, whereas SO_4 relatively robust to ozone.	Change in PEFR (L/min) for 10 ppb increase in 24-hour 9a-9p avg ozone: PM PEFR: -1.10 (-2.83, 0.64) Next AM PEFR: -1.77 (-3.70, 0.17) PM PEFR in relation to 5-day mean ozone: -2.58 (-4.82,-0.35)
Ostro et al., 2001 Central Los Angeles and Pasadena, CA August-October 1993	138 African American children ages 8-13 with doctor diagnosed asthma requiring medications in past year followed for daily respiratory symptoms and medication use. Lags of 0-3 days examined.	1-hour max ozone mean (SD): LA: 59.5 (31.4) ppb Pasadena: 95.8 (49.0) ppb	PM10, NO_2, pollen, mold	PM10 / ozone correlation = 0.35. Significant ozone effect seen for extra medication use (above normal use). No ozone effect on symptoms in expected direction observed. Inverse association seen for cough. PM10 effects seen at lag 3. Time factors not explicitly controlled in analysis; may have led to confounding of ozone effects.	Odds ratio for extra medication use per 40 ppb 1-hour max ozone: 1.15 (1.12-11.19) Odds ratios for respiratory symptoms per 40 ppb lag 3 1-hour max ozone: Shortness of breath: 1.01 (0.92-1.10) Wheeze: 0.94 (0.88-1.00) Cough: 0.93 (0.87-0.99)

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

Table 10-1 (cont.): Effects of Ozone on Lung Function and Respiratory Symptoms in Field Studies

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

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

10.2.1 Introduction: Modeling Issues

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

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

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

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

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

The third method of adjusting for season involves stratification of the full time-series by warm and cold weather months. This technique has been used more for time-series studies of death (see Section 10.4) than disease. However, several of the studies of emergency room visits focus their analysis on the warm season. As a result, these studies may provide a clearer assessment of the effects of ozone.

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

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

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

10.2.2 Hospital Admissions for Respiratory Diseases

Hospital admissions represent a medical response to a serious degree of disease for a particular disease. Hospitalizations are sometimes scheduled in advance when a particular clinical treatment is needed. However, unscheduled admissions are ones that occur in response to unanticipated disease exacerbations and are more likely to be affected by environmental factors, such as air pollution. As such, most of the hospital admissions studies reviewed here focused specifically on unscheduled admissions. Study details and results from 26 hospital admissions studies published over the past decade are summarized in Table 10-2.

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

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

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

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

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

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

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

Using a unique study design, Friedman et al. (2001) examined associations between hospital usage and reductions in air pollution concentrations during the 1996 Summer Olympic Games in Atlanta, GA. The authors compared acute care visits and hospitalizations for asthma during the 17 days of the Olympic Games (July 19–August 4, 1996), when traffic and ozone concentrations dropped due to several public interventions, to a baseline period consisting of the 4 weeks before and 4 weeks after the Olympic Games. During the Games, reductions in hospital usage ranged from 11.1 to 44.1% over four databases. By comparison, changes in nonasthma acute care events ranged from +1.0 to -3.1%. Peak daily ozone concentrations decreased 27.9% from 81.3 ppb during the baseline period to 58.6 ppb during the Olympic games. While it is not possible to uniquely attribute the changes in hospital usage to the changes in ozone concentrations (levels of other pollutants were also likely to have changed), this study provides strong evidence in support of the efficacy of measures to reduce ambient air pollution.

Using a different analytical approach (case-crossover analysis), Lin and colleagues (2003) found no evidence for ozone effects on asthma admissions in 6-12 year olds over the period 1981-1993 in Toronto. There have been few studies utilizing the case-crossover methodology in the air pollution epidemiology literature, making it difficult to interpret these findings.

Moolgavkar and colleagues (1997) reported significant and robust ozone effects on respiratory hospital admissions in adults 65 and older in Minneapolis-St. Paul MN, but not in Birmingham, AL. One may speculate that the absence of effects in the southern city may reflect less penetration of ozone into the indoor environment due to greater use of air conditioning, and thus less correlation between central site ozone monitoring and actual exposures of the urban populace. In a study of over 13,000 hospital admissions in Brisbane Australia over the years 1987-1994, significant ozone effects on all-age and age-stratified asthma and total respiratory hospital admissions were observed (Petroeschevsky et al., 2001). The ozone effects were robust to inclusion of PM (based on light scattering) and SO_2 in co-pollutant regressions. Sulfate levels were very low in this study. Effect sizes appeared consistent in the warm and cool seasons, possibly reflecting the relatively small degree of seasonal variation in ozone levels observed in Brisbane. Significant ozone (and other pollutant) effects on asthma hospitalizations for persons under age 65 were reported for Seattle by Sheppard et al. (1999). Anderson reported significant ozone effects in London during the warm season (1998). Less consistent effects of ozone and other pollutants were seen in Amsterdam and Rotterdam in a study made difficult to interpret due to the large number of statistical tests performed (Schouten et al. 1996). While some inconsistencies are noted across studies, this body of evidence from single or pairs of cities studied over five more years supports the

findings of the multi-city analyses, of significant and robust effects of ozone on various respiratory disease hospitalization outcomes.

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

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

Table 10-2: Effects of Ozone on Daily Hospital Admissions

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Anderson et al., 1996 6 European Cities: (Amsterdam, Barcelona, London, Milan, Paris, Rotterdam) Periods vary	Emergency COPD admissions for all ages. Each city analyzed previously by individual teams. Results combined here via meta-analysis.	Range across cities: 1-hour max ozone: Med 18-39 ppb 8-hour max ozone: Med 10-35 ppb	TSP, SO_2, NO_2, BS	0-5, depending on city.	Poisson GLM using APHEA methodology. Results stratified by season. ozone most consistent and significant predictor of admissions. Warm season effect larger.	Weighted-mean effects across 6 cities: ozone 1-hour max (per 25 ppb): 1.029 (1.011-1.047) ozone 8-hour max (per 25 ppb): 1.043 (1.022-1.065)
Anderson et al. 1998 London 1987-1992	Admissions for asthma in ages 0-14, 15-64, and 65+ age groups	8-hour max ozone: Mean 15.5 IQR 13 1-hour max ozone: Mean 20.6 IQR 16	SO_2, NO_2, BS, pollens	0-5 explored	Poisson GLM using APHEA method; co-adjustment. ozone significantly associated with asthma admissions in the warm for all ages and for the 15-64 age group. Warm season ozone effect robust in 2-pollut models. Inverse associations observed in the cool season for some age groups.	Single pollutant models for lag 1 ozone per 10 ppb for all ages: Whole year: 1.007(0.099-1.010) Warm season: 1.021(1.006-1.038) Cool season: 0.928 (0.946-0.992)
Ballester et al., 2001 Valencia, Spain 1994-1996	Emergency total cardiac admissions for all ages.	8-hour max ozone: Mean 23 ppb Range 5-64	SO_2, NO_2, CO, BS	0-5 explored	Poisson GLM using APHEA methodology. Results stratified by season. No ozone effects.	ozone 8-hour max (per 5 ppb): All cardiac (lag 2): 0.9905 (0.9710-1.0104)
Burnett et al. 1995 168 Hospitals in Ontario, Canada 1983-1988	Cardiac and respiratory admissions for all ages and within age strata. Study focused mainly on testing for sulfate effects.	1-hour max ozone: Mean 36.3 ppb		Lag 1 ozone	GLM with pre-adjustment of outcome variables. Results stratified by season. Authors report that ozone associated with respiratory admission in warm season only.	No quantitative results presented for ozone.

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Burnett et al., 1997b Toronto, Ontario 1992-1994 Summers only	Unscheduled cardiac and respiratory admissions for all ages.	1-hour max ozone: Mean 41.2 IQR 22	PM2.5, PM10, H+, SO_4, SO_2, NO_2, CO, COH	Lags 0-4 explored. The three-day avg. lagged 1 or 2 days chosen as most significant.	Poisson GLM with co-adjustment. Results stratified by season. ozone and COH strongest predictors of outcomes. ozone effects on both outcomes were robust to PM. PM effects were not robust to ozone.	ozone 1-hour max (per 11.5 ppb), controlling for temp and dew point: Respiratory (lag 1, 3-d avg): 1.064 (1.039-1.090) Cardiac: (lag 2, 3-d avg): 1.074 (1.035-1.115)
Burnett et al. 1999 Toronto, Ontario 1980-1994	Cause-specific admissions for all ages. Cause categories included asthma, COPD, respiratory infections, heart failure, ischemic heart disease, and cerebrovascular disease.	24-hour avg ozone: Mean 19.5 ppb IQR 19	Estimated PM2.5, PM10, PM10-2.5, CO, NO_2, SO_2	Single and multi-day averages from 0-2 days explored. Best fit picked for each outcome.	Poisson GAM pre-adjustment. ozone effects seen for respiratory outcomes only. ozone effect robust to PM; not visa versa. No seasonal stratification.	Single-pollutant models for ozone 24-hour average (per 19.5 ppb): Asthma (lag 1, 3-d avg): 1.063 (1.036-1.091) COPD (lag 2, 3-d avg): 1.073 (1.038-1.107) Respiratory Infection (lag 1, 2-d avg): 1.044 (1.024-1.065)
Burnett et al., 2001 Toronto, Ontario 1980-1994	Acute respiratory disease admissions for ages <2 and >18.	1-hour max ozone: Mean 45.2 IQR 25	Estimated PM2.5, PM10, PM10-2.5, CO, NO_2, SO_2	Single and multi-day averages from 0-5 days explored. Best fit picked.	Poisson GLM with pre-adjustment. Sensitivity analyses using co-adjustment. Results stratified by season. ozone effects significant only in warm season. ozone effect robust to PM; not visa versa.	Single-pollutant models for ozone 1-hour max (per 45.2 ppb): Acute respire disease age <2 (lag 0, 5-d avg): 1.348 (1.193-1.523)

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Friedman et al., 2001 Atlanta, Georgia Summer Olympic Games June 21-September 1, 1996	Asthma and nonasthma hospitalizations, emergency department visits, urgent care visits for ages 1-16 yrs	1-hour max ozone Olympic period Mean 58.6 ppb Baseline period Mean 81.3 ppb	PM10, CO, NO_2, SO_2, Mold	Same day; 2-day and 3-day cumulative exposure.	Compared events during Olympic games (July 19-Aug 4) with baseline period 4 weeks before and after games. Also used time series Poisson regression for all 73 days, with single ozone variable and with low, moderate and high ozone as independent variables.	During games, reductions in hospital usage ranged from 11.1% to 44.1% over 4 databases. In time series, with 3-day cumulative ozone, RR of >90 ppb vs. <60 ppb ranged from 1.03 to 1.88. RR for 50 ppb change ranged from 1.0 – 1.4 (1.01-1.94)
Gouveia and Fletcher 2000a Sao Paulo, Brazil Nov 1992-Sep 1994	Total respiratory, pneumonia, and asthma admissions for ages <5.	1-hour max ozone: Mean 32 ppb IQR 25	PM10, SO_2, NO_2, CO	0-2 explored. Best fit picked.	Poisson GLM with co-adjustment using sine/cosine waves. Significant ozone effects on total respiratory and pneumonia admissions. ozone effects fairly robust to NO_2 and PM10.	Single-pollutant models for ozone 1-hour max (per 60.9 ppb): Total respiratory (lag 0): 1.054 (1.003-1.107) Pneumonia (lag 0): 1.076 (1.014-1.142) Asthma (lag 2): 1.011 (0.899-1.136)
Gwynn et al. 2000 Buffalo, NY May 1988-Oct 1990	Total respiratory admissions for all ages.	24-hour avg ozone: Mean 26 ppb IQR 14.8	PM10, SO4, H+, COH, CO, NO_2, SO_2	0-3 explored. Best fit picked.	Poisson GAM control of temperature; moving average control for time. ozone significant predictor of outcome. No 2-pollutant models reported.	ozone 24-hour avg (per 14.8 ppb): Total respiratory (lag 1): 1.029 (1.013-1.045)

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Hagen et al. 2000 Dramen, Sweden Nov 1994-Dec 1997	Total and respiratory admissions for all ages.	24-hour avg ozone: Mean 22.6 ppb IQR 13.4	PM10, NO_2, SO_2, benzene, toluene, HCHO	0	Poisson GAM co-adjustment. Single and multi-pollutant models evaluated. No ozone effects. ozone levels very low and cycles may not have been adequately controlled.	Single-pollutant model for ozone 24-hour avg (per 13.4 ppb): Total respiratory (lag 0): 0.964 (0.899-1.033)
Lin et al. 2003 Toronto, Ontario 1981-1993	Asthma admission for 6-12 year olds. Case-crossover design.	1-hour max ozone: Mean 30 ppb IQR 20	CO, SO_2, NO_2	Explored 4-7 day averages ending on admit day	Case-crossover analysis. No ozone effects observed.	No significant effects.
Linn et al. 2000 Los Angeles, CA 1992-1995	Total respiratory and total cardiac admissions for age >64.	24-hour avg ozone: Mean (SD): Winter 14 (7) Spring 32 (10) Summer 36 (8) Fall 15 (9)	PM10, CO, NO_2	0	Poisson GLM; co-adjustment. Only significant ozone effects observed were inverse associations with total cardiac admission in full-year and winter season, suggesting residual confounding. No significant effects of ozone on respiratory disease admits.	No significant effects.
Mann et al. 2002 Los Angeles basin 1988-1995	Ischemic heart disease admissions for adults 40 and over.	8-hour max ozone: Mean 50.3 ppb IQR 39.6	PM10, CO, NO_2	0-5 single and multi-day averages explored.	Poisson GAM with co-adjustment. No significant ozone effects observed overall or in warm season. CO and NO_2 significant in full-year analyses. P	No significant effects.

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Moolgavkar et al. 1997 Minneapolis/St. Paul, MN and Birmingham, AL 1986-1991	Pneumonia and COPD admissions for adults >64.	Metric not specified: Mean ppb(IQR): MN 26 (15) AL 25 (13)	PM10, SO_2, NO_2	Explored lags 0-3. Best fit picked.	Poisson GLM with co-adjustment. Both ozone and PM10 significant in MN; not in AL. ozone, but not PM10, effects were robust to NO_2 and SO_2.	Single-pollutant models (20 df smoother) for ozone (per 15 ppb) in MN: Pneumonia (lag 1): 1.066 (1.034-1.098) COPD (lag 0): 1.045 (0.995-1.067)
Nauenberg and Basu, 1999 Los Angeles, CA 1991-1994	Unscheduled asthma admissions for all ages.	14-hour average ozone: Mean 19.9 ppb SD 11.1	PM10	0	Poisson GLM with pre-adjustment. No significant effects of ozone. No warm season results presented.	No significant effects.
Nauenberg and Basu, 1999 Los Angeles, CA 1991-1994	Unscheduled asthma admissions for all ages.	14-hour average ozone: Mean 19.9 ppb SD 11.1	PM10	0	Poisson GLM with pre-adjustment. No significant effects of ozone. No warm season results presented.	No significant effects.
Petroeschevsky et al. 2001 Brisbane, Australia 1987-1994	Unscheduled asthma, total respiratory and total cardiac admissions in several age strata: 0-4, 5-14, 15-64, 65+, all.	1-hour max ozone: Mean 25.3 ppb Range 2.5-107.3 8-hour 10-6 ozone: Mean 19 ppb Range 1.7-64.7	B_{scat}, SO_2, NO_2	0-4 single and multi-day averages.	Poisson GLM using APHEA co-adjustment methodology. Results stratified by season. ozone significantly related to asthma and total respiratory admissions, not for cardiac. Effects varied by age group. ozone effects robust to co-pollutants	Single-pollutant models for ozone 8-hour 10am-6pm (per 10 ppb): All-age respiratory (lag 2): 1.023 (1.003-1.043 All-age asthma (5-d avg): 1.084 (1.037-1.133)

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

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

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

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

Table 10-2 (cont.): Effects of Ozone on Daily Hospital Admissions

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

10.2.3 Emergency Department Visits for Respiratory Diseases

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

Several studies have been published in the past decade examining the temporal associations between ED visits for asthma or other respiratory diseases and ozone exposures (Table 10-3). As a group, these ED studies tend to be smaller in terms of geographic and temporal coverage than those reviewed above for hospital admissions. Results regarding associations between ED visits and ozone levels also tend to be somewhat less consistent, with effects apparent only in selected sub-groups and at certain lags. Among 20 studies with adequate controls for seasonal patterns, 16 reported at least one significant positive association involving ozone: Cassino et al. (1999) for asthma, Delfino et al. (1998a,b; 1997b) for respiratory complaints, Hernandez-Guarduno (1997) for respiratory complaints, Jaffe et al. (2003) for asthma, Jones et al. (1995) for respiratory complaints, Lin et al. (1999) for respiratory complaints, Martins et al. (2002) for chronic lower-respiratory complaints, Stieb et al. (1996) for asthma, Tenias et al. (1998, 2002) for asthma and COPD, Tobias et al. (1999) for asthma, Tolbert et al. (2000) for asthma, White et al. (1994) for asthma, Romieu et al. (1995) for asthma, and Weisel et al. (1995) for asthma.

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

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

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

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

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

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

particular concern. For example, as reported above, Stieb et al. (1996) found an increased risk for emergency room visits among adults exposed to concentrations above 75 ppb (with a maximum concentration of 160 ppb). The analysis of emergency room visits for childhood asthma in summertime Atlanta by White et al. (1994) indicated a significantly elevated risk when 1-hour ozone exceeded 110 ppb (maximum 160 ppb). Weisel et al (1995) analyzed the relationship between summertime ozone and emergency room visits for asthma. They reported statistically significant effects for 1-hour ozone concentrations above 60 ppb (maximum 100 ppb). Romieu et al. (1995) reported an association between emergency room visits for childhood asthma and ozone in Mexico City. Particularly large risks were observed after consecutive days when the 1-hour maximum ozone concentration exceeded 110 ppb. As reported above, Tolbert et al. (2000) reported an association between pediatric emergency room visits for asthma and ozone in Atlanta. Elevated risks were apparent at concentrations greater than 8-hour average concentrations of 70 ppb, with effects becoming statistically significant when 8-hour average ozone concentrations were between 100 and 113 ppb. Although a similar analysis was not reported for 1-hour ozone concentrations, the related concentrations for elevated risk would be approximately 95 ppb (using a 1- to 8-hour ratio of 1.33), with statistical significance at approximately 133 ppb.

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

10.2.4 Which Diseases Have Been Most Consistently Associated with Ozone

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

asthma. In the case of ED studies, asthma has been studied most often, with variable results.

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

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

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

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

given concentration range are not directly comparable across metrics, unless the exposure increment is a relative measure, e.g., based on the interquartile range of concentrations. Although results from the epidemiologic studies reviewed here do not make it possible to choose an optimal exposure metric, when taken together with information from controlled chamber exposure studies, it would appear that the daily 8-hour maximum ozone concentration represents an optimal exposure metric for health risk assessment.

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

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

Table 10-3 : Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

Reference, location, years.	Outcomes and Design	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method, Findings, Interpretation	Effects (RR and 95% CI)
Jones et al., 1995 Baton Rouge, LA June-August 1990	Daily ED visits for respiratory complaints over a three-month period in pediatric (0-17), adult (18-60), and geriatric (>60) subgroups.	24-hour avg ozone: Mean 28.2 ppb; SD 11.7 1-hour max ozone: Mean 69.1 ppb; SD 28.7	No co-pollutants. Mold pollen data included	None specified	Relatively simple statistical approach using ordinary least squares regression to model effects of ozone by itself and of ozone along with pollen counts, mold counts, temperature, and relative humidity. No direct control of cycles but authors reported non-significant auto-correlations among model residuals. Data restriction to 3-month period may have reduced any cyclic behavior. Significant associations between respiratory ED visits and ozone observed for adult age group only in multiple regression models.	24-hour avg ozone (per 20 ppb): Pediatric:0.87 (0.65 to 1.09) Adult: 1.20 (1.01 to 1.39) Geriatric: 1.27 (0.93 to 1.61)
Lin et al. 1999 Sao Paulo, Brazil May 1991-April 1993	Daily pediatric (no age range specified) respiratory ED visits	1-hour max ozone: Mean 34 ppb SD 22	SO_2, CO, PM10, NO_2	0-6 examined; settled on 1-5 day moving avg.	Seasonal control using month dummy variables. Also controlled day of week, temperature. Both ozone and PM10 associated with outcome alone and together.	1-hour max ozone (per 5 ppb): Respiratory ED visits with ozone alone: 1-5 d MA: 1.022 (1.016-1.028) With PM10 in model: 1-5 d MA: 1.015 (1.009-1.021)

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

Table 10-3 (cont.): Effects of Ozone on Daily Emergency Department Visits

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

10.3 Studies Addressing Respiratory Effects of Long-Term Ozone Exposures

10.3.1 Introduction

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

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

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

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

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

Here we review studies published from 1996 onward in which health effects were tested in relation to ozone exposures extending from several weeks to many years (Table 10-4). The available literature falls in five general categories: studies addressing respiratory inflammation in high vs. low ozone exposure groups or time periods; studies looking at seasonal changes in lung function and respiratory symptoms as a function of seasonal average ozone exposures; studies examining long-term death risks; studies addressing growth or decline of lung function over many years in relation to long-term ozone exposures; and finally, studies addressing cross-sectional associations between asthma prevalence and long-term ozone exposures.

10.3.2 Respiratory Inflammation

As noted (see Section 9.6.3.4 and Tables 9-7, 9-10, and 9-14), human chamber studies have clearly demonstrated that acute (2-7.6 hour) ozone exposures, with light to moderate exercise, result in inflammation in the lung, including the alveolar region where gas exchange takes place. This acute effect is potentially important for chronic effects because it is established that repeated inflammation could result in the release of substances from inflammatory cells that can damage the sensitive cells lining the lung. Over extended periods, this could lead to permanent damage to and re-structuring of the small airways and alveoli. In addition, since inflammation is a fundamental feature of asthma there has been concern that ozone-induced inflammation could exacerbate existing asthma or perhaps promote the development of asthma among genetically pre-disposed individuals.

In a group of 19 adult joggers living and working on an island in New York harbor, there was little evidence for acute inflammation in bronchoalveolar lavage (BAL) fluids collected during the summer of 1992 compared with BAL fluids collected from the same subjects in winter (Kinney et al. 1996). However, there was evidence of greater levels of cell damage, measured by lactate dehydrogenase (LDH) in the fluids. The three-month mean of the daily 1-hour ozone maxima was 58 ppb in the summer and 32 ppb in winter. PM10 and NO_2 concentrations were similar across the two seasons. These results are consistent with attenuation of acute inflammatory effects (which has been demonstrated in multi-day chamber exposures, see Section 9.6.9), but ongoing cellular damage due to repeated ozone exposures over the course of a summer.

A series of interesting studies of children living in Mexico City and a rural area of Mexico have demonstrated inflammation of and genetic damage to cells in the nasal passages of the urban compared to the rural children (Calderon-Garciduenas et al. 1997; Calderon-Garciduenas et al. 1995; Calderon-Garciduenas et al. 1999). Pollution effects in the nose can be viewed as surrogates for effects that might occur in the lungs, although doses to nasal tissues are usually higher for a given ambient pollutant concentration. In the 1997 study, 129 children living in Mexico City were compared to 19 children living in a

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

10.3.3 Seasonal Changes in Lung Function

While it has been well documented in both chamber and field studies that daily, multi-hour exposures to ozone result in transient declines in lung function, much less is known about the effects of repeated exposures to ozone on lung function (see Section 9.6.9 for discussion of controlled studies on this topic). Two studies reported over the past decade have examined lung function changes over seasonal time periods with differing levels of ozone exposures (Frischer et al. 1999; Kinney and Lippmann 2000). In the larger of the two studies, Frischer and colleagues (1999) collected repeated lung function measurements on 1,150 Austrian school children (mean age 7.8 years) from nine towns that differed in mean ozone levels. Lung function was measured in spring and fall over a three-year period, yielding six measurements per child. The seasonal change in lung function was analyzed in relation to seasonal mean ozone exposures, controlling for baseline function, atopy (assessed by skin prick testing), sex, study site, ETS, season, temperature, and height change. Mean ozone exposures in each town were significantly associated with decrements in lung function. FVC declined by between 10 and 33 mL for each ppb increase in mean seasonal 24 hour ozone concentrations. FEV1 declined by between 24 and 34 mL. Other pollutants (PM10, SO_2, and NO_2) had less consistent associations with lung function changes. These results suggest that seasonal ozone exposures result in decrements in lung function. Left unanswered was whether these effects would resolve following exposure to lower ozone exposures over the winter months.

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

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

10.3.4 Death Risk

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

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

10.3.5 Long-term Effects on Lung Function

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

Several studies published over the past decade have examined the relationship between lung function and long-term ozone exposure. The most extensive and robust recent study of respiratory effects, including lung function, in relation to long-term air pollution exposures among children has been the Children's Health Study carried out in 12 communities of southern California starting in 1993 (Gauderman et al. 2000; Peters et al. 1999a). An initial report examined the relationship between lung function at enrollment and levels of air pollution in the community (Peters et al. 1999a), and found evidence for declines in FVC, FEV1, PEFR and FEF25-75% (the latter two being statistically significant) among females in relation to elevated annual mean daily 1-hour maximum ozone levels. For male children, there were declines in all four lung function variables only in a subset of subjects who reported spending more time outdoors; these associations were significant only for FVC and FEV1. There were no significant associations between long-term ozone exposures and self-reports of respiratory symptoms or asthma (Peters et al., 1999b). In longitudinal analyses of lung function growth in the fourth grade cohort, decrements in lung function growth were associated with particulate matter and NO_2, but not with ozone (Gauderman et al. 2000; 2004). Exposure misclassification related to incomplete penetration of ozone indoors may have impacted these negative findings for ozone.

Evidence for a relationship between long-term ozone exposures and decrements in maximally attained lung function was observed in a nationwide cohort of 520 first year students at Yale College in New Haven, CT (Galizia and Kinney 1999; Kinney et al. 1998). Students performed one lung function test in the spring of their first year at college. Ozone exposures were estimated by linking 10-year mean summer-season 1-hour maximum ozone levels at the nearest monitoring station to the residential locations reported each year from birth to the time of measurement. Students who had lived four or more years in areas with long-term

summer ozone levels above 80 ppb had significantly lower FEV1 (-3.0 percent; 95% CI -0.22 to -5.92 percent) and FEF25-75% (-8.11 percent; 95% CI -2.32 to -13.90 percent) compared to their classmates with lower long-term exposures, controlling for age, sex, race, height, parental education and maternal smoking. Stratified by sex, males had larger effect estimates than females, which might reflect higher outdoor activity levels during childhood. A similar study of 130 first year college freshmen at the University of California at Berkeley also reported significant effects of lifetime mean of daily 10 AM to 6 PM ozone averages on lung function (Kunzli et al. 1997; Tager et al. 1998). Enrollment was limited to students from either the San Francisco or Los Angeles metropolitan areas. After controlling for city of origin, FEF25-75% and FEF75% were significantly lower in relation to long-term exposures. No effects were seen for PM10 and NO_2. In another California-based study, there was no relationship between acute responsiveness to 400 ppb ozone over two hours, measured with exercise in a controlled chamber environment, and long-term changes in lung function in a group of 45 adults (Gong et al. 1998) drawn from a longitudinal cohort study that had previously reported faster lung function decline in persons living in high ozone communities (Detels et al. 1987).

10.3.6 Risk of Asthma Development

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

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

A similar sub-study of incident asthma cases in relation to long-term ozone exposure was carried out on the Children's Health Study cohort (McConnell et al. 2002; Peters et al. 1999b). Here, annual surveys of 3535 initially non-asthmatic children (ages 9-16 at enrollment) enabled identification of new-onset asthma cases through 1998. Asthma risk was not higher for residents of the six high ozone communities (four year mean of January-December 10am-6pm ozone was 60 ppb, May to September mean of 8-hour maximum ozone concentrations was 84 ppb) vs. residents of the six low ozone communities (4-year January-December mean of 10am-6pm ozone concentrations was 40 ppb, May to September mean of 8-hour maximum ozone concentrations was 49 ppb). However, within the six high ozone communities, asthma risk was elevated by 3.3-fold for children who played three or more sports as compared with children who played no sports. This association was absent in the low ozone communities. No associations with asthma were seen when communities were stratified by PM10, PM2.5, NO_2, or inorganic acid vapors. These results are consistent with the interpretation that effects on asthma are seen only for individuals who are most exposed to ozone by virtue of their spending time outdoors engaged in physical activity. Replication of these findings in other cohorts would lend greater weight to a causal interpretation.

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

Table 10-4: Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

Table 10-4 (cont.): Respiratory Effects of Long Term Ozone Exposures

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

10.4 Ozone and Acute Death

10.4.1 Introduction

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

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

10.4.2 Interpretational Issues

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

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

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

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

A related, more technical, issue is the choice of degrees of freedom for the smoothing function of time. By using more degrees of freedom, a tighter fit to the temporal variations in death can be achieved. The question is, how much is enough but not too much? Table 10-5 lists some examples of the models used to adjust for temporal trends and weather effects in recent studies that examined ozone. There appears to be no consensus as to what number of degrees of freedom (per year) is appropriate in adjusting for seasonal trends. Statistical diagnostics such as AIC or residual autocorrelation or dispersion of the regression model are often used to choose the degrees of freedom for temporal trend, but these diagnostics do not provide epidemiological justification for, or interpretation of the fitted model. Clearly, using more degrees of freedom in temporal trend fitting means ascribing more details (i.e., shorter temporal fluctuations) of daily death to unmeasured potential confounders and possibly taking away the weather and air pollution effects. Therefore, it is not surprising that we often observe smaller pollution effect estimates when more degrees of freedom are used to adjust for temporal trends. More work is needed in this area to reduce the uncertainty involved in the interpretation of ozone death effect estimates.

Table 10-5: Examples of model specifications used in recent studies to adjust fortemporal trends and weather in recent death studies that analyzed ozone.

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

Table 10-5 (cont.): Examples of model specifications used in recent studies to adjust for temporal trends and weather in recent death studies that analyzed ozone.

Study	Adjustment for temporal trend	Adjustment for weather
Hoek et al., 2000 The Netherlands	ns (days, df=10/yr) + influenza-of-past-3 wks	Warm: 0 for temp <= 14°C; temp for temp > 14 °C. Cold: 0 for temp >= 14°C; temp for temp < 14 °C.
Fairly 2003 Santa Clara County, CA	ns (days, df=7 for 7yrs) + ns (day-of-year, df=12)	ns (min temp, df=3) + ns (max. temp, df=2)
Lippmann et al, 2000 Ito K 2003 Detroit, MI	ns (days, df=12/yr)	ns (temp, df=2) + lo (lag1-3temp, df=2) + hot-and-humid indicator

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

As shown in Table 10-5, the weather models used in recent death time-series analyses can be classified as using: (1) quantile (e.g., quartile, quintile, etc.) indicators of temperature; (2) a parametric functional form, such as V- or U-shape functions, of temperature; and, (3) parametric (e.g., natural splines) or non-parametric (e.g., LOESS) smoothing function of temperature. More recent studies tend to use the smoothing function of temperature variables. While these methods provide flexible ways to fit death as a function of temperature and other weather variables, there are two major issues that need to be resolved before death effects of ozone can be meaningfully interpreted.

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

As can be seen in Table 10-5, the use of (parametric or non-parametric) smoothers has become popular in recent years, and is the currently the most commonly used approach to adjust for temporal trends in death time-series studies. However, this flexible fitting approach still cannot adjust for the relationships among variables that change across seasons. This is particularly important for ozone, which appears to change its relationship with temperature (and with other pollutants) across seasons. In some urban locations during the winter, ozone comes mainly from the free troposphere and can be considered a tracer for relatively clean air. The clean air during the winter is associated with high-pressure systems (i.e., cold, clean air coming down from upper atmosphere). Thus, sunny clear winter days in the urban environment are the days when ozone levels may be relatively high (compared with other winter days) but when air pollution levels from primary emissions (e.g., NO, which also "quenches" ozone), SO_2 and PM from local sources) are low. This leads to negative correlation between ozone and the primary pollutants.

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

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

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

Anderson et al. (1996) examined the relationship between air pollution (ozone, NO_2, black smoke, and SO_2) and daily death (all cause, cardiovascular, and respiratory) in London, England for study period 1987-1992 using the Poisson GLM model. They examined the associations for all seasons, as well as the warm season (April to September) and the cool season (October to March) separately. Their results indicate that the estimated ozone relative risks were larger for the warm season than for the cool season for all cause death. For example, the percent excess risk estimated per 10th-to-90th percentile ppb increment of 8-hour (9am to 5pm) average ozone was 3.48% (1.73, 5.26) for the warm season (7-36 ppb), 0.77% (-0.88, 2.44) for the cool season (2-22 ppb), and 2.43% (95%CI: 1.11, 3.76) for the full year (3-29 ppb). A similar pattern was seen for cardiovascular death, but the estimated risk was negative (not significantly) for the cool season. For respiratory death, the estimated excess risks were similar between the cool and warm seasons. In the Air Pollution and Health: A European Approach (APHEA2) study of 23 European cities from 1990-1997, Gryparis et al. (2004) found no significant effects of ozone in the cold half of the year. For the warm season, a 10 $\mu g/m^3$ increase in the 1-hour ozone concentration was associated with a 0.33% (0.17, 0.52) increase in total daily deaths, 0.45% (0.22, 0.69) in the number of cardiovascular deaths and 1.33% (0.62, 1.48) in the number of respiratory deaths. The associations with total death were independent of SO_2 and PM10, but somewhat confounded by NO_2 and CO.

The largest meta-analysis on ozone effects on death are those from the National Death and Disease Air Pollution Study (NMMAPS) reported originally by Samet and colleagues (2000) and reanalyzed using non-GAM methods by Dominici et al. (2003) and Bell et al. (2004). This study tested acute air pollution effects on death in the largest 90 cities in the US. The focus of the study was PM10, but

ozone and other gaseous pollutants were also analyzed in single and multi-pollutant models. In the original analysis (using default GAM convergence criteria), the combined excess risk estimate for ozone at lag 0 day was essentially zero for all seasons combined, but positive for summer (June, July, and August) and negative for winter (December, January, and February) in the same magnitude (~0.5% and ~-0.5% excess death per 10ppb 24-hour average ozone, respectively). In the re-analysis, the ozone effect estimate was positive and significant for summer (0.4% excess death per 10 ppb 24-hour average ozone), and negative and significant for winter (-0.5%) (Dominici et al., 2003). These results are consistent with the results reported by Moolgavkar and co-investigators (1995), and raise a serious concern regarding the interpretation of ozone death risk estimates from analyses of year-round data. It would be of interest to examine the effects of ozone in multiple cities that, for example, have very low correlation between ozone and particulate matter, for which significant effects on death have been demonstrated.

In a comprehensive reanalysis of NMMAPS specifically directed at ozone effects, using additional years of data and cities, Bell et al. (2004) estimated a national average relative rate of death associated with short-term exposure to ambient ozone for 95 large US urban communities from 1987-2000. Distributed-lag models were used to estimate community-specific death effects adjusted for time-varying confounders (particulate matter, weather, seasonality, and long-term trends). Community-specific effects were then combined into a national average relative rate using hierarchical models. A 10-ppb increase in the previous week's ozone was associated with a 0.52% increase in daily death (95% posterior interval [PI], 0.27% - 0.77%) and a 0.64% increase in cardiovascular and respiratory death (95% PI, 0.31%-0.98%). Effect estimates for aggregate ozone during the previous week were larger than for models considering only a single day's exposure. Additional analyses of these data indicated: (1) associations with cardiopulmonary death were slightly greater than those for all-cause death; (2) similar effects were obtained in analysis of summers only versus the full years of data; (3) inclusion of PM10 in the model did not impact the estimated ozone coefficient; (4) similar relative risk estimates were observed among ages less than 65, 65 to 74 and 75 and above; and (5) the results were not sensitive the exclusion of high temperature (and possibly confounding) days. Overall, these results provide the strongest evidence to-date for acute impacts of ozone on death.

There are other studies that also reported larger excess death risks in the warm (or summer) season than in the cool (or winter) season. These include the analyses of: the Netherlands data (Hoek et al. 2000; re-analysis by Hoek G 2003), Brisbane, Australia (Simpson et al. 1997), Montreal, Canada (Goldberg and Burnett 2003), and Vancouver, Canada (Vedal et al. 2003). These studies showed either smaller than summer or slightly negative (but not significant) risk estimates for the cool season. Of the studies that analyzed data by season, only one study in Pittsburgh, PA (Chock et al. 2000) showed negative risk estimates in summer. The results that show more positive and significant associations between ozone and death in warm seasons are consistent with the expectation

that ozone, if harmful, should have a positive association with health outcomes in summer when the levels are substantially higher than during the winter. However, the negative ozone-death associations seen for winter in the 90 cities study and Philadelphia data suggest that further examination of this issue is required. Specifically, if ozone in winter at low levels is shown to be negatively associated with the factors (e.g., PM) that are positively associated with death, then these potentially spurious negative ozone-death associations can be explained. In other words, if days with very low winter ozone levels often have elevated PM concentrations, then elevated death due to PM might be spuriously associated with low ozone. Alternatively, the negative winter findings may reflect uncontrolled confounding by seasonal cycles. Thus, ozone-death effect estimates computed for year-round data need to be interpreted with caution.

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

10.4.3 Acute Death Risk Estimates for Ozone

This section reviews available ozone-death excess risk estimates in the current literature. For simplicity, this review excludes the (20+) studies that used GAM with default convergence criteria that have not been reanalyzed (with more than one non-parametric smoothing term) and focuses on the subset of studies listed on Table 10-6 for which risk estimates were available. Two parameters that can complicate the comparison of the risk estimates from different time-series studies include the time lag between exposure and death, and the ozone exposure index used. An examination of the "most significant" lags extracted from the studies on

Table 10-6 also suggests that the majority of the single-day associations were immediate (0-day lag: 21 studies; 1-day lag: 8 studies; 2-day lag: 3 studies; and, longer lag days: 3 studies). It should be noted that, when associations are found at multiple days (as in the case of ozone risk estimates in the U.S. 90 cities analysis by Dominici et al. 2003), choosing only a single-day's lag results would also underestimate the multi-day effects. On the other hand, testing several lags and choosing the largest effect would tend to produce a positive bias. Thus, using a single lag day's risk estimate can result in bias in either direction.

With respect to exposure indices, the three used most often are the daily 1-hour maximum (1-hour max.), the daily maximum 8-hour running average (8-h max.), and the 24-hour average (24-hour avg.) concentrations. Of the 47 studies listed in Table 10.6, 29 studies used 1-hour max, 12 used 8-hour max, 17 used 24-hour avg. concentration, and 2 used alternative ozone exposure indices in addition to commonly used indices. Thus, the 1-hour max. ozone concentration is the most frequently used index. Eight studies used two or more indices. An examination of these eight studies suggest that, in general, the significance of associations was similar for these indices, and for the same distributional increment (e.g., inter-quartile range), the excess death risks computed were also similar. This is expected because, for most urban locales, the daily fluctuations of these three indices are highly correlated ($r > 0.9$). However, in comparing relative risk estimates based on these alternative indices, it is important to be aware of the differences in relevant exposure increments. The exposure increment is the arbitrarily chosen exposure range for which the relative risk (i.e., percent change in death) is computed. Because day to day changes in concentrations of short term ozone indices like the 1-hour max are much more variable than those of longer term indices like the 24-hour mean, it makes sense to scale the exposure increments to account for these differences. Using the nationwide data for ozone monitors in MSA (Langstaff and Pinto, personal communication, 2003), the difference between the mean and the 95th percentile concentrations (that is, "average" to "high" ozone increment) were approximately 40, 30, and 20 ppb, for 1-hour max, 8-hour max, and 24-hour avg., respectively. This, to facilitate comparisons across studies in the following sections, we express ozone death relative risks using an increment of 40 ppb if the original risk estimate was computed using the 1-hour max; we use an increment of 30 ppb for the 8-hour max, and we use an increment of 20 ppb for the 24-hour average in the tables and discussion for this section.

As listed in Table 10-6, there are over 40 studies that reported short-term death risk estimates for ozone. Table 10-6 does not include the additional 20+ studies that used GAM with default convergence criteria (with more than one non-parametric smoothing terms) that have not been reanalyzed. Among the reanalyzed GAM studies that presented ozone risk estimates, there was a tendency to find similar or slightly lower ozone effect estimates using GAM than alternative analysis methods. For example, in the analysis of Santa Clara County, CA data (Fairley 1999; re-analysis Fairley D 2003), the ozone non-accidental death risk estimate increased slightly from 2.7% (default GAM) to 2.8% (stringent GAM), and to 3.0% (GLM) per 30 ppb increase in 8-hour max

ozone. In Hoek et al.'s analysis of the Netherlands data (Hoek et al. 1997; re-analysis, Hoek G 2003), the ozone non-accidental death risk estimates increased from 1.8% (default GAM) to 2.1% (stringent GAM), and to 2.2% (GLM) per 40 ppb increase in 1-hour max ozone. In the analysis of the largest 90 U.S. cities (Samet, et al, 2000; re-analysis, Dominici et al. 2003), the year-round combined estimate of ozone non-accidental death risk changed from a non-significant negative (~ -0.1% per 20 ppb change in 24-hour avg. ozone) to a significantly positive value (0.4%), while the estimates for winter (~ -1%) and summer (~ 1%) did not change appreciably. This limited evidence suggests that use of GAM tends to underestimate effects of ozone on acute death.

Figure 10-1 shows ozone non-accidental death risk estimates for all ages and for all season data in single pollutant models from 33 studies listed in Table 10-6. The majority of the estimates are positive with a few exceptions. Two multi-city studies (the U.S. 90 cities study and the APHEA European 4 cities results) both showed positive and significant estimates, but the APHEA estimate was larger (4.5% per 40 ppb increase in 1-hour max ozone) than that for the 90 cities study (0.9%). Alternative analyses of data from the same city sometimes resulted in differing results. For example, Anderson et al's. (1996) analysis of London, England data showed a larger ozone death risk estimate (3.3% per 40 ppb increase in 1-hour max ozone) than Bremner et al. (1999) estimate (-0.8%). Another example is the Amsterdam data analyses for which Verhoeff et al.'s (1996) estimate (3.8%) was larger than that by Roemer and van Wijnen's (2001) estimate (-0.2%). Clearly, differences in analytical approaches can produce differing results. These results do suggest that more sensitivity analyses using alternative model specifications should be conducted. Despite these discrepancies, overall, the range of estimates were relatively narrow, with 27 out of the 33 estimates being between 0 and 6.6% per 40 ppb increase in 1-hour max ozone (median = 2.9%) concentration. A new meta-analysis of time-series studies has been undertaken by WHO (Anderson et al. 2004). Using results from only European cities, this study reports an effect estimate of about 0.6% per 40 ppb change in 8-hour ozone concentration.

As discussed above, the ozone risk estimates computed for year-round data may be misleading. A smaller number of studies conducted season specific analyses. Figure 10-2 shows the studies that reported ozone risk estimates by season. For those studies that calculated ozone risk estimates for each of the four seasons, only summer and winter results are shown. The estimates for year-round data analyses, when available, are also shown for comparisons. In all the studies, except the Brisbane data (for which the estimates are essentially the same for summer, winter, and year-round subsets), the ozone risk estimates are larger for summer than for winter, with the year-around estimates in between the summer and winter estimates. It is not surprising that the summer and winter estimates were similar in Brisbane, as the ozone levels do not differ greatly between summer and winter (22 ppb and 27 ppb, respectively, for 1-hour max ozone concentration). These results, showing that the ozone risk estimates are in fact larger in summer when ozone is higher, are consistent with causal association,

and also question the value of interpreting ozone risk estimates obtained from year-round data.

Confounding between “winter type” pollution (i.e., CO, SO_2, and NO_2) and ozone is not a great concern because the peaks of these pollutants do not strongly coincide. The main confounders of interest for ozone, especially for the northeast US, are “summer haze” type pollutants such as acid aerosols and sulfate. Since few studies had these chemical measurements, PM (especially PM2.5), may serve as a surrogate. However, due to the expected high correlation among the constituents of the “summer haze mix”, multi-pollutant models including these pollutants may result in unstable coefficients, and therefore, interpretation of such results requires some caution. Only one study, which was conducted in Philadelphia, PA, (Lipfert et al. 2000) reported ozone risk estimates with and without sulfate (as well as PM2.5). The ozone risk estimate (2.8% per 45 ppb increase in avg. of lag 0 and 1 day 1-hour max ozone concentration in the single pollutant model) in this study was not substantially affected by the addition of sulfate (3.4%, an increase). Figure 10-3 shows the ozone risk estimates with and without PM indices from 16 studies (note: these results are for year-round data). In general, the ozone death risk estimates were not affected by addition of PM indices, with the exception of the Los Angeles data with PM10 and Mexico City data with TSP.

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

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

Few studies have examined sub-categories of cause-specific death, and most of those that have had limited statistical power to detect associations in small daily counts databases. The results from a study of a relatively large data set from the Netherlands suggest that certain cardiovascular sub-categories had larger relative risk estimates than that for all cardiovascular categories combined, but

the same pattern was also seen for other pollutants. This remains an issue requiring further study.

10.4.4 Conclusions

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

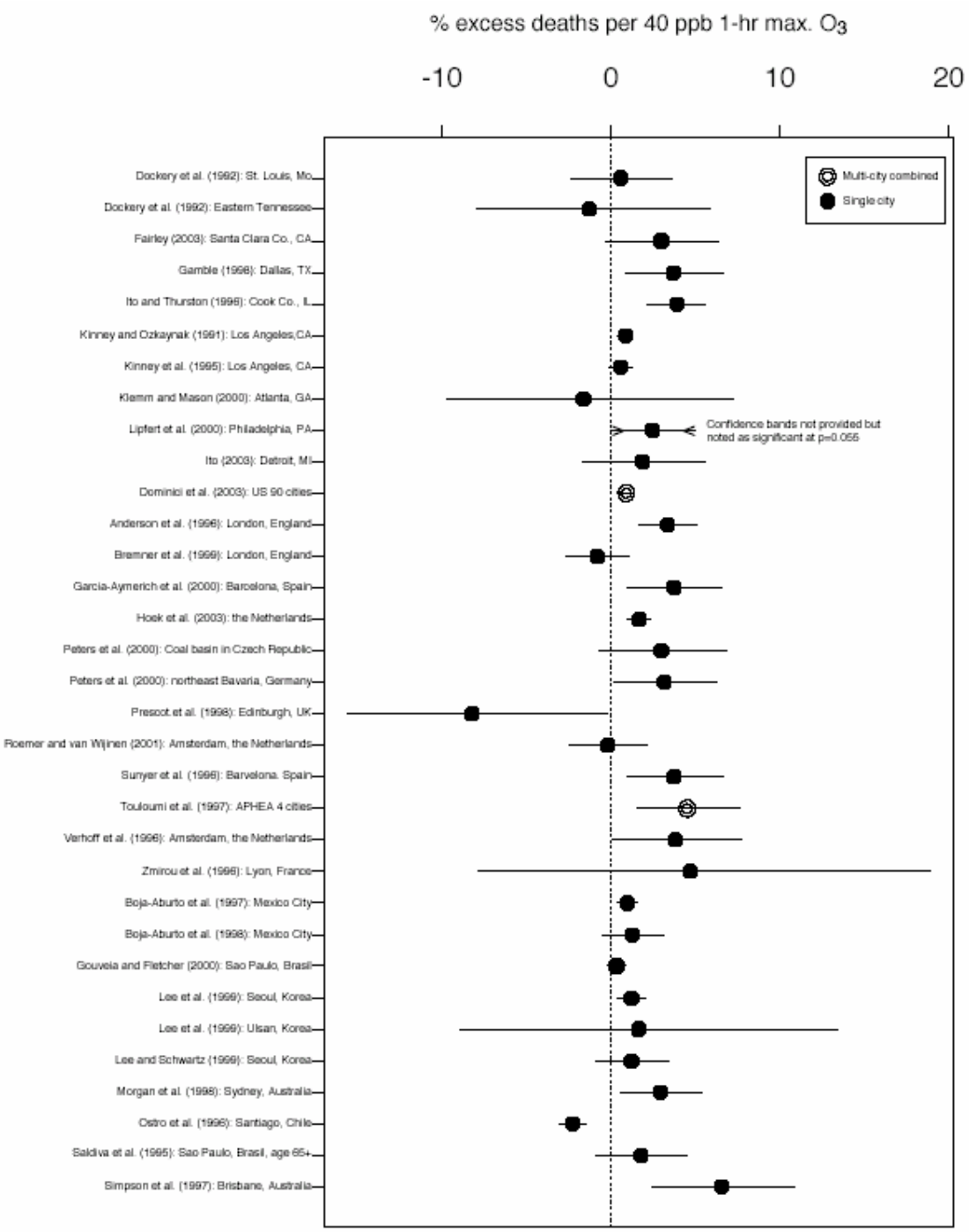

Figure 10-1: Ozone excess non-accidental death risk estimates <u>for all season data</u>. All age, unless otherwise noted.

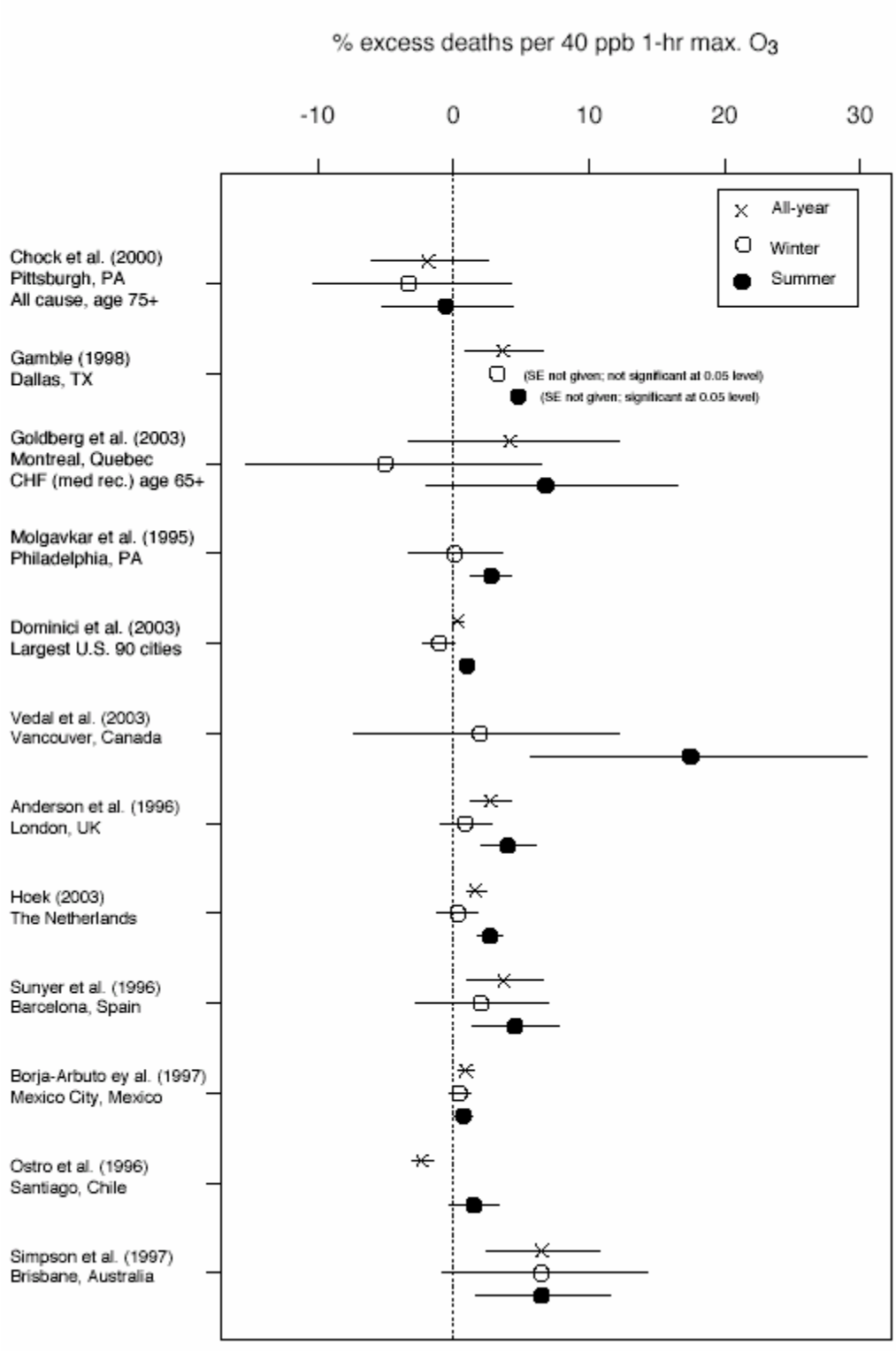

Figure 10-2: Ozone death risk estimates <u>by season</u>. All age non-accidental death, unless otherwise noted.

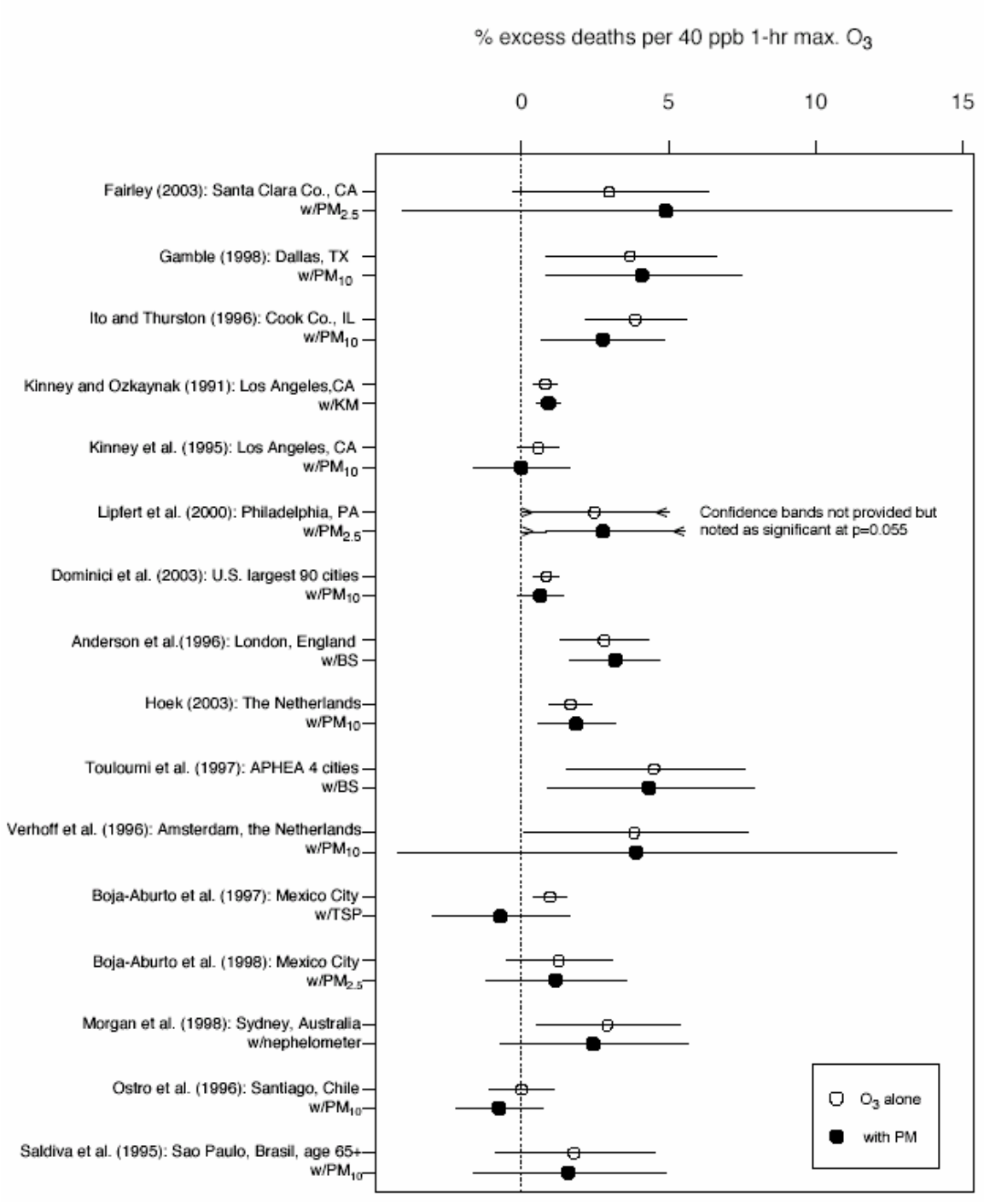

Figure 10-3: Ozone death risk estimates <u>with and without PM index</u>. All age nonaccidental death, unless otherwise noted.

Table 10-6: Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Anderson et al. 1996 Greater London, UK, 1987-1992	All; respiratory; cardiovascular	1-hour max = 20.6; 8-hour avg. =15.5 (mean)	BS, NO_2, SO_2; also 2-poll model	Lag 0	Poisson GLM	8-hour avg.; 2.43% (1.11-3.76) per 26 ppb, all cause, all year; 1-hour max: 2.59% (1.30-3.89) per 31 ppb, all cause, all year
Bell et al. 2004 95 U.S. cities 1987-2000	All; cardiovascular and respiratory combined; ages<65, 65-74, and $\geq$75	24-hour avg. = 26 across 95 communities	PM10	Single day lags up to 3 days Distributed lag up to 6 days before	2 stage model; Poisson GAM with strict convergence criteria	0.52% (0.27-0.77) per 10 ppb previous week's ozone for total death ; 0.64% (0.31-0.98) for cardiovascular and respiratory combined
Borja-Aburto et al. 1997b Mexico City, 1990-1992	All (all age); ages < 5yr; ages > 65yr	1-hour max = 155; 24-hour avg. = 54; 8am-6pm avg. = 87; 8-hour moving avg. = 94 (median)	TSP, SO_2, CO; also 2-poll model	Lag 0	Poisson IWFLS	2.4% (1.1-3.9) per 100ppb daily 1-hour max.
Borja-Aburto et al. 1998 Mexico City, 1993-1995	All; age > 65yr; Cardiovascular; respiratory; other.	1-hour max = 163; 24-hour avg. = 44 (median)	PM2.5, NO_2, SO_2; also 2-poll model	Avg. of lag 1 and 2	Poisson GAM but with only smoother	0.63% (-0.27, 1.53) per 10 ppb increase for total; 1.76% (0.07,3.46) for cardiovascular; 0.82%(-0.39, 2.03) for age > 65yr; -0.74% (-3.58, 2.10) for respiratory; 0.30 (-0.85, 1.44) for others

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Bremner et al. 1999 London, UK	All cause; respiratory; cardiovascular; and age specific (0-64; 65+; 65-74; 75+) of these causes; all cancer; all others.	1-hour max = 22.6; 8-hour avg. = 17.5 (median)	BS, PM10, NO_2, SO_2, CO; also 2-poll model	Lag 2 for all age group all cause, respiratory, and cardiovascular; lag varied for age-specific and cause	Poisson GLM	8-hour avg.; -0.7% (-2.3-0.9) per 26 ppb, all cause, all year; 3.5% (0.5-6.7) per 26 ppb, CV, all year;
Chock et al. 2000 Pittsburgh, PA	All cause by two age groups (0-74 and 75+ yr)	1-hour max (distribution statistics not reported)	PM10, NO_2, SO_2, CO; also 2-, 5-, and 6-poll model	Lag 0	Poisson GLM	For older age (> 75): beta = -0.459 (t=-0.82) per PPM in 1-pollutant model; even more negative with 2- and 5-pollutant models.
Dab et al. 1996 Paris, 1987-1992	Respiratory deaths	1-hour max = 23.2; 8-hour avg. = 11.5 $\mu g/m^3$ (median)	BS, PM_{13}, NO_2, SO_2, CO	Lag 0	Poisson autoregressive	Beta = 0.000715 (se = 0.000713) for 8-hour avg.; Beta = 0.000390 (se = 0.000545) for 8-hour avg. (NOTE: $\mu g/m^3$)
De Leon et al. 2003 New York City, 1985-1994	Circulatory and cancer with and without contributing respiratory	24-hour avg. = 22	PM10, NO_2, SO_2, CO; 2-pollutant models also	Lag 0 or 1	Poisson GAM/GLM	For underlying circulatory, those with contributing respiratory had larger estimates than those without. For underlying cancer, opposite
Diaz et al. 1999 Madrid, Spain, 1990-1992	All cause; respiratory; cardiovascular	24-hour avg. (distribution statistics not reported)	TSP, NO_2, SO_2, CO	Lag 1, 4, 10	autoregressive linear	U-shaped ozone-death relationship

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Dockery et al. 1992 St. Louis, MO; Eastern Tennessee, 1985-1986	All cause	24-hour avg. = 22.5 in St. Louis, 23.0 in Eastern Tennessee (mean)	PM10, PM2.5, $SO_4^{=}$, H^+, NO_2, SO_2	Lag 1	Poisson, GEE	Beta= 0.00029 (se = 0.00076) in St. Louis; beta = -0.00065 (se = 0.00178) in Eastern Tennessee
Fairley 1999; Fairley D 2003 Santa Clara County, CA	All cause; respiratory; cardiovascular	8-hour avg. = 29; 24-hour avg. = 16; ozone ppb-hrs greater than 60 ppb (mean)	PM10, PM2.5, PM10-2.5, COH, $SO_4^{=}$, NO_3, NO_2, SO_2; 2-pollutant models	Lag 0	GAM (re-analyzed with stringent convergence criteria), GLM	3.3% (-0.3-7.0) per 33 ppb 8-hour max
Gamble 1998 Dallas, TX, 1990-1994	All cause; respiratory; cardiovascular; cancer; other	24-hour avg. = 22 (mean)	PM10, NO_2, SO_2, CO; 2-pollutant models also	Avg. of lag 1 and 2	Poisson GLM	2.7% (0.6-4.8) per 14.7ppb for total death. (larger for summer)
Garcia-Aymerich et al. 2000 Barcelona, Spain	All cause; respiratory; CVD; general population and patients with COPD	1-hour max (distribution not reported)	BS, NO_2, SO_2,	Lag 5 for general population; lag 3 for COPD cohort	Poisson GLM	2.4% (0.6, 4.2) per 50µg/m^3 4.0% (-4.7, 13.4)
Goldberg et al. 2003 Montreal, Quebec, 1984-1993	Underlying congestive heart failure (CHF) vs. those classified as having CHF from medical records	24-hour avg. = 15 (mean)	TSP, COH, PM10, sulfate, SO_2, NO_2, CO	Avg. of lag 0, 1, and 2.	Poisson GLM	Underlying CHF: 4.5% (-5.6, 15.8) per 11 ppb avg. of lag 0-2; those classified as having CHF from medical records: 2.3% (-1.8, 6.6)

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Gouveia and Fletcher 2000b Sao Paulo, Brazil	All cause; age under 5 (all cause, respiratory, pneumonia); age 65+ (all cause, respiratory, CVD)	1-hour max = 67.9 (mean)	PM10, NO_2, SO_2, CO	Lag 0 for	Poisson	0.8% (-0.11-2.7) per 106ppb for all cause and ages; 2.3% (0-4.6) per 106ppb for all cause, age 65+.
Gryparis et al. 2004 23 European cities, 1990-1997	Total natural deaths, cardiovascular, respiratory	For 23 cities, Summer: 1-hour max=53–117 8-hour max =30-99 Winter: 1-hour max = 11-57 8-hour max = 8-49 (median)	SO_2, NO_2, PM10, CO	Avg. of lags 0, 1	City specific models: Poisson GAM, strict convergence criteria Second-stage fixed effect pooled regression;	Warm season only: for 1-hour ozone 0.33%(0.17-0.52) increase per 10µg/m^3 for total deaths; 0.45%(0.22-0.69) for cardiovascular deaths; 1.13%(0.62-1.48) for respiratory deaths
Hoek et al., 2000; Hoek G 2003 the Netherlands (entire country, 4 urban areas), 1986-1994	All cause; CVD, COPD, pneumonia	8-hour avg. = 24 (47 µg/m^3) (median)	PM10, BS, $SO_4^=$, NO_3, NO_2, SO_2, CO; also 2-poll models	Lag 1 or avg, of lag 0 through 6	Poisson GAM re-analyzed; GLM	4.0% (2.5-5.4) per 77 ppb at lag 1 day; 4.2% (2.6-5.7) per 61 ppb avg. of 0-6 days for all cause.
Hoek et al. 2001; Hoek G 2003 the Netherlands, 1986-1994	Total CVD, MI, arrhythmia (ARH), heart failure (HF), cerebrovascular disease (CRB), thrombosis (TRM).	Same as above	PM10, NO_2, SO_2, CO	Lag 1	Poisson GAM re-analyzed; GLM	CVD: 6.2% (3.3, 9.2); MI: 4.3%(0.1, 8.6); ARH: 11.4% (-1.2, 25.5); HF: 10.2% (1.2, 19.9); CRB: 9.1% (2.9, 15.7); TRM: 16.6% (2.8, 32.2)

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Ito and Thurston 1996 Cook County, IL	All cause, circulatory, respiratory, cancer; race/gender sub-categories	1-hour max. = 38 (mean)	PM10, NO_2, SO_2, CO	Avg, of 0 and 1 lag days	Poisson GLM	10% (6.0, 15.0) per 100ppb (avg. of 0 and 1 days).
Kinney and Ozkaynak 1991 Los Angeles, 1970-1979	All cause, circulatory, respiratory.	Total oxidants (O_x) 1-hour max. = 75 ppb (mean)	KM, B_{ext}, NO_2, SO_2, CO; multiple poll. model	Lag 1	OLS on high-pass filtered variables.	beta=0.03 (se=0.009)
Kinney et al. 1995 Los Angeles	All cause	1-hour max. = 70 (mean)	PM10, NO_2, SO_2, CO; also 2-poll, models	Lag 1	linear, log-linear, and Poisson	2.0% (0.0, 5.0) per 143ppb.
Klemm and Mason 2000 Atlanta, GA	All cause; age 65+; separate counties.	8-hour avg. = 46 (mean)	PM10, PM2.5, PM10-2.5, EC, OC, etc.; NO_2, SO_2, CO	Lag 0	Poisson GLM	beta=-0.00041 (se=0.00110)
Lee et al. 1999 Seoul and Ulsan, Korea, 1991-1995	All cause	1-hour max. = 32 in Seoul; 26 in Ulsan (mean)	TSP, SO_2	Lag 0	Poisson with GEE	Seoul: 1.5% (0.5, 2.5) per 50 ppb; Ulsan: 2.0% (-11.1, 17.0) per 50 ppb.
Lee and Schwartz 1999 Seoul, Korea, 1991-1995	All cause	1-hour max. = 32 in Seoul; 26 in Ulsan (mean)	TSP, SO_2	Lag 0	conditional logistic (case crossover)	1.5% (-1.2, 4.2) per 50ppbusing plus/minus 1 wk controls; 2.3% (-0.1, 4.8) per 50ppb using plus/minus 2 wks controls
Lipfert et al. 2000 Philadelphia, PA, 1991-1995	All cause; CVD, respiratory, age 65+, age <65; various sub-regional boundaries	1-hour max. = 45; 24-hour avg. = 23 (mean)	PM10, PM2.5, PM10-2.5, $SO_4^{=}$, NO_3, and other PM indices; NO_2, SO_2, CO;	Avg. of lag 0 and 1	linear, with Shumway filters, SPSS	2.8% per 45 ppb 1-hour max. (avg. of 0 and 1 day), significant at $p < 0.055$, but no SE or confidence bands provided.

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Lippmann et al, 2000; Ito K 2003 Detroit, MI, 1992-1994	All cause; CVD, respiratory	24-hour avg. = 25 (mean)	PM10, PM2.5, PM10-2.5, $SO_4^{=}$, H^+; NO_2, SO_2, CO; also 2-poll. model	Lag 0	Poisson, GAM, GLM	2.6% (-2.4, 7.8) per 28 ppb for GLM
Moolgavkar et al. 1995 Philadelphia, 1973-1988	All causes	1-hour max. = 26 in spring; 36 in summer; 16 in fall; 12 in winter (mean)	TSP, SO_2		Poisson, also GEE and nonparametric bootstrap methods	Spring: 2.0% (-6.7-11.5); Summer: 14.9% (6.8-23.6); Fall: -4.5% (-13.9-5.9); Winter: 0.4% (-15.6%-19.4) per 100ppb daily 24-hour avg.
Morgan et al. 1998 Sydney, Australia, 1989-1993	All cause, cardiovascular, respiratory.	1-hour max. = 24 (mean)	B_{scat}, NO_2	Lag 0	Poisson with GEE in SAS	All cause: 2.04% (0.37, 3.73) per 28ppb
Ostro 1995 2 Southern California counties	All cause, cardiovascular, respiratory, but ozone results were reported only for all cause	1-hour max. = 14 (mean)	PM2.5	Lag 0	Autoregressive linear; Poisson, other in sensitivity	2.0% (0.0, 5.0) per 100ppb. Summer quarters only.
Ostro et al. 1996 Santiago, Chile, 1989-1991	All cause, cardiovascular, respiratory	1-hour max. = 53 (mean)	PM10, NO_2, SO_2	Lag 1	OLS, several other methods	All season: -3.0% (-4.0, -2.0) per 53ppb; summer: 4.0% (0, 9.0) per 100ppb

Table 10-6 (cont.): Ozone Effects on Acute Death

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

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Saez et al. 1999 Barcelona, Spain, 1986-1989	Asthma death	1-hour max., distribution not reported.	BS, NO_2, SO_2,	Avg. of lags 0 through 2	Poisson with GEE	beta = 0.021 (se=0.011) for 0-2 day average, for asthma death.
Saldiva et al. 1994 Sao Paulo, Brazil, 1990-1991	Respiratory death in children age under 5	24-hour avg. = 12	PM10, NO_2, SO_2, CO; all in the model	Avg. of lag 0, 1, and 2	OLS of transformed data	Beta = 0.01048 (se=0.02481)
Saldiva et al. 1995 Sao Paulo, Brazil, 1990-1991	All cause for age 65+	1-hour max. = 38; 24-hour avg. = 13 (mean)	PM10, NO_2, SO_2, CO; also 2-poll. model	Avg. of lag 0 and 1	OLS/autoregressive, Poisson/GEE	Beta = 0.0093 (se=0.0813) for 24-hour ozone; beta = 0.0280 (se=0.0213) for 1-hour max. ozone
Samet et al., 2000; Dominici et al. 2003 90 U.S. cities, 1987-1994	All cause, cardiorespiratory	24-hour avg. = ranged from 12 in Des Moines to 36 in San Bernardino (mean)	PM10, NO_2, SO_2, CO; also 2-poll. model	Lag 0	Poisson GAM (re-analyzed with stringent convergence criteria); GLM	All seasons: ~ 0.2%; Summer: ~ 0.5%; Winter: ~ -0.5% per 10ppb.
Sartor et al. 1995 Heat wave in Belgium, 1994	All cause in two age groups: 0-64 and 65+	24-hour avg. = 37 during heat wave; 27 and 20 before and after heat wave	TSP, NO, NO_2, SO_2	Lag 0 and 1	Data stratification, log-linear regression; correlation	No individual coefficient for ozone alone; interaction with temperature suggested.
Simpson et al. 1997 Brisbane, Australia, 1987-1993	All cause, CVD, and respiratory in all ages and 65+	1-hour max. = 24; 8-hour avg. = 18 (mean)	PM10, nephelometry, NO_2, SO_2, CO	Lags 0	(method follows APHEA approach) Poisson with GEE	Daily 1-hour max: 1.6% (0.6, 2.6) per 10ppb; 8-hour average: 2.4% (0.8, 4.0) per 10ppb.

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Sunyer et al. 1996 Barcelona, 1985-1991	All cause (all age and 70+), CVD, respiratory	1-hour max. = 28 in winter 44 in summer	BS, SO_2, NO_2	Lag 0	(APHEA) autoregressive Poisson	4.8% (1.2, 8.6) per 51 ppb for all year; 2.6% (-3.5, 9.1) in winter; 5.8% (1.7, 10.1) in summer
Sunyer and Basagana 2001 Barcelona, 1990-1995	Death in a cohort of patients with COPD	1-hour max. mean not reported; IQR = 11	PM10, NO_2, CO	Lag 0	Conditional logistic (case-crossover)	-2.1% (-8.1-6.5) per 11 ppb
Sunyer et al. 1996 Barcelona, 1985-1995	Death in a cohort of patients with severe asthma	1-hour max. = 35; 8-hour avg. = 28 (median)	PM10, BS, SO_2, NO_2, CO, pollen	Avg. of lag 0, 1, and 2	Conditional logistic (case-crossover)	Patients with only 1 admission with asthma: 9.6% (-18.0, 46.6); patients with more than 1 admissions with asthma: 68.8% (-2.2, 264.3) per 24 ppb 1-hour max. ozone
Tellez-Rojo et al. 2000 Mexico City,1994	Respiratory death, COPD death; within medical unit and out of medical unit	1-hour max. = 24	PM10, NO_2, SO_2	Avg. of lag 1 through 5 days	Poisson, IWFLS	Respiratory death (outside medical unit): 14.0% (4.1-24.9) per 40ppb 5-day average; COPD death (outside medical unit): 15.6% (4.0-28.4) per 40 ppb 5-day average.
Touloumi et al. 1997 APHEA1 (subset: four cities: London, Athens, Barcelona, and Paris)	All cause	1-hour max. = 48 in Athens; 37 in Barcelona; 21 in London; 24 in Paris (mean)	BS, NO_2; 2-poll. model also considered.	Single-day best lag chosen in each city; average of lag 2 through 5	Poisson autoregressive	Combined across 4 cities: 2.9% (1.0, 4.9) per 25.5 ppb in random effects; 2.3% (1.4, 3.3) in fixed effects.

Table 10-6 (cont.): Ozone Effects on Acute Death

Reference, location, years.	Outcome measure	Mean ozone levels (IQR)	Pollutants considered	Lag structure	Method	Effect estimates
Vedal et al. 2003 Vancouver, BC, 1994-1996	All cause, CVD, respiratory	1-hour max. = 27	PM10, NO_2, SO_2, CO	Lag 0	GAM, stringent with new SE methods	Summer: 4.2% (1.4, 7.0) per 10.2 ppb; Winter: 0.5% (-1.9, 3.0) per 10.2 ppb.
Verhoeff et al., 1996 Amsterdam	All cause, age 65+	1-hour max. = 43	PM10, NO_2, SO_2, CO	Lag 2	Poisson	4.9% (0.1-10.0) per 51 ppb
Zmirou et al. 1996 Lyon, France, 1985-199	All cause, CVD, respiratory, digestive	1-hour max. = 15; 24-hour avg. = 10 (mean)	PM_{13}, SO_2, NO_2	Lag 0 for all cause; lag 1 for CVD; lag 2 for respiratory	Poisson GLM	All cause: 3.0% (-5.0, 12.0) per 26 ppb.
Zmirou et al. 1998 APHEA (four cities: London, Lyon, Barcelona, and Paris)	CVD, respiratory	8-hour avg. (9am-5pm), for cold/warm seasons= 11/21 in London; 6/22 in Paris; 5/6 in Lyon; 27/46 in Barcelona (mean)	BS, SO_2, NO_2	Each city chose best 1-day lag fits	Poisson GLM	Combined CVD estimate: 2% (1, 3) per 25.5 ppb 1-hour max.; 2% (0, 3)

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11 Basis for Staff Recommendation to Revise the Ozone Standard

This chapter presents the staff recommendation for the ozone ambient air quality standard (AAQS) for California, based on the recommendation from the Office of Environmental Health Hazard Assessment (Health and Safety Code Section 39606(a)(2). The chapter begins with a general discussion of the elements and factors to be considered for setting ambient air quality standards. Following is a summary of the scientific evidence for health effects from ozone exposure taken from Chapter 9, Controlled Ozone Exposure Studies, Chapter 10, Epidemiological Studies, contained in Volume II of the Staff Report, and Appendix E, Review of Animal Toxicological Studies on the Health Effects of Ozone, contained in Volume III of the Staff Report. The chapter continues with recommendations for pollutant definition, averaging times, and concentrations adequate to protect public health.

11.1 Introduction

State law (Health and Safety Code section 39014) defines an ambient air quality standard in terms of a concentration and an averaging time, which reflect the relationship between air pollution and undesirable effects. H&SC section 39606(d)(2) also requires that standards incorporate an adequate margin of safety. Thus, an ambient air quality standard defines a maximum exposure (concentration and averaging time) estimated to be without adverse effects for the general population that undergoes the exposure defined by the standard. Further, in California, ambient air quality standards are based solely on health considerations, and do not consider such factors as cost of attainability, or control measures.

The recommended ozone standards are based on scientific information about the health impacts associated with ozone exposure, recognizing the uncertainties in these data. The definition of California ambient air quality standards assumes a threshold below which effects do not occur. The controlled human exposure literature suggests that for any given averaging time a threshold ozone concentration can likely be identified on an individual subject level. However, in practice, the extremely wide range of individual responsiveness to ozone makes identification of a threshold on a population level somewhat problematic. Consequently, it is difficult to identify a “bright line” or threshold ozone concentration for a given averaging time below which health effects would not occur in at least some individuals.

In addition, the Children’s Environmental Health Protection Act (Senate Bill 25, Escutia; Stats. 1999, Ch. 731, specifically Health and 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, Health and Safety Code section 39606(b)(1) called for the following information to be assessed, to the extent that information is available, in development of ambient air quality standards:

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 (Health and 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. 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 protect vulnerable subpopulations, there may be exquisitely sensitive individuals remaining outside the ambit of protection. Although both the Health and 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 Chapter 9, data from controlled exposure studies demonstrates that some individuals experience ozone-associated toxicity at relatively low concentrations. Several epidemiologic studies suggest a fairly linear relationship 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 recommended standards that, when attained, should protect nearly all of the California population, including infants and children, against ozone-associated effects throughout the year.

11.2 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 in assessing the significance of air pollutant-associated physiological, biological or pathological changes.

It is important to acknowledge the difference 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, death, 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 increased 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. *Death:* Increased death 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.

Sherwin (1983) has defined an adverse health effect as "the causation, promotion, facilitation and/or exacerbation of a structural and/or functional abnormality, the implication that the abnormality produced has the potential of lowering the quality of life, contributing to a disabling illness, or leading to a premature death." Sherwin also argues that ambient air quality setting should consider as adverse effects that are subclinical so as to prevent early subclinical changes. Effects of this nature would primarily be subtle tissue changes that may lead or contribute to future lung disease.

Based on these recommendations, many health outcomes found to be associated with ozone could be considered adverse. Many of these effects, 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 death, are related to acute ozone exposures. In addition, outcomes such as increased airway reactivity and inflammation may be considered adverse if they signify increases in the potential risk of the population profile for disease exacerbation or initiation. Animal studies showing tissue changes with repeated or chronic ozone exposures raise concern as to possible subclinical effects of repeated and long-term exposure to elevated ozone concentrations.

11.3 Summary of the Scientific Evidence

This section provides a summary of the health effects of ozone exposure presented in chapters 9 and 10, and Appendix G.

11.3.1 Summary of Findings from Chamber Studies

11.3.1.1 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, pointing to the importance of the dose-rate. For example, observed effects are typically larger following a short exposure to a high ozone concentration, compared to a longer, lower concentration exposure, even if the total inhaled dose of ozone is equivalent. 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.

Ozone concentrations are highest outdoors and the proportion of outdoor ozone penetrating indoors is variable (20-80%). There are few indoor sources of ozone. 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.

The protocols used in controlled human exposure studies have been largely standardized over the past 30 years. There are three basic protocols: one-hour continuous exercise, two- to four-hour intermittent exercise, and six- to eight-hour quasi-continuous exercise. Each of these protocols was designed to simulate a common outdoor exposure pattern, and the ventilation rates used are representative of activities common to the exposure durations.

The one-hour continuous exercise protocol examines responses to ozone in subjects performing moderate to heavy exercise continuously for one hour, with endpoint measurements before and after exposure. A variation on this protocol for studies primarily investigating the impact of ozone exposure on exercise performance adds a sprint to exhaustion at the end of the hour exposure. This protocol simulates short-term, moderate to heavy exercise exposures, and is representative of the activity patterns of people engaged in athletics, personal exercise programs, and after-school endurance sports. This protocol investigates responses to short, peak concentration exposures.

The two- to four-hour intermittent exercise protocol was designed to simulate somewhat longer exposures during which the activity level was of moderate, noncontinuous intensity. It was designed to simulate longer-term, less intense activity patterns, such as personal exercise programs and athletic training where activity occurs in alternating periods with rest, and outdoor home maintenance and yardwork. Endpoints are typically measured during the rest periods, as well as before and after exposure, except for those measured by bronchoscopy.

Recognition that some areas of the country had a wider peak concentration pattern of ozone concentrations led to development of the 6.6 hour quasi-continuous exercise protocol. Several recent studies have extended this

protocol to eight hours. This protocol simulates a day of outdoor work, and the exercise level, at 50 minutes per hour, is representative of that which can be maintained for a full day of work. As such, this protocol is a simulation of outdoor labor, such as construction, landscaping, or highway work. It is also representative of weekend and vacation exposure of children and adults. Typically, endpoints, except those measured through bronchoscopy, are measures before, and after exposure, as well as during the 10 minute rest periods each hour.

Participants in most chamber studies have been healthy, exercising young adults (ages approximately 18 to 35), although there are a few studies on children, older adults, asthmatics and people with COPD. 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 mild to moderate asthmatics studied have typically had changes in symptoms and lung function in the same ranges as nonasthmatics. However, some, but not all, 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 can 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 criteria, and suggests that asthmatics may represent a sensitive subpopulation for ozone. Asthma is a health outcome that disproportionately impacts children. Children have higher prevalence rates and children under age five have higher rates of hospitalization rates for asthma than any other age group. Lost school days also impact children's educational progress.

Because of ethical and major logistical considerations, there are few studies of individuals with cardiovascular disease or COPD. 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 chamber studies. Epidemiological studies, however, are likely to include individuals with these diseases. 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.

11.3.1.2 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-hour 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 and/or ventilatory 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 FEV1 after a 1-hour exposure to 0.10 ppm (as part of a multi-hour exposure). Group mean responses with short exposures to 0.12 ppm ozone have been relatively small – about a 3 to 5 percent decrement in FEV1. 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 FEV1 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.

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 FEV1 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 FEV1 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 60 subjects had FEV1 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 on 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 FEV1 or symptoms were not statistically different relative to clean air.

However, five of the 30 subjects had FEV1 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.

11.3.1.3 Symptoms

Significantly increased symptoms of respiratory irritation have been reported with 1 to 3 hour 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 (Adams 2002) were reported.

11.3.1.4 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- hour 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 filtered air exposure. Exposures to ozone concentrations as low as 0.08 ppm for 6.6 hour can increase nonspecific airway hyperresponsiveness.

11.3.1.5 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 hour 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 hour exposures at ozone concentrations lower than 0.20 ppm. Analysis of bronchoalveolar lavage fluid (BALF) after 6.6-hour 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 at concentrations lower than 0.08 ppm for 6.6 hour or longer have not been investigated.

Exposure to 0.08 ppm ozone for 6.6 hours decreases the ability of alveolar macrophages to phagocytize 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 increased fibronectin and protein recovered following 6.6 hour 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.

11.3.1.6 Pollutant Mixtures

Although there are a few 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, such as NO_2 and PAN, SO_2 or H_2SO_4 aerosols at concentrations in the ambient range. Observed responses at the pollutant concentrations studied to date appear to be attributable to the ozone 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 ozone alone. However, typical ambient PAN concentrations are considerably lower than those utilized in these studies. There have been few human exposure studies on mixtures of ozone with particulate matter, with the exception of H_2SO_4 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 inhalation of higher effective doses (ozone concentrations above 0.20 ppm, with moderate exercise and a duration of 3 hours or more) can result in allergic asthmatics' requiring a lower dose of allergen to produce a given degree of airway hyperresponsiveness.

11.3.1.7 Effect Modifiers

Overall, the currently available literature suggests that young adult females might be somewhat more sensitive to ozone if the comparison is made with the same absolute (same ozone concentration, ventilation rate, and exposure duration) inhaled dose as young adult males, but not if the comparison is made at the same relative dose (same ozone concentration and exposure duration, but with ventilation rate the same <u>percent</u> of maximal). Since in most cases females exercise at a similar percentage of maximal as males, their relative doses are comparable, and no differences would be expected.

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). It is unknown whether children really do not have symptoms or are unwilling or unable to articulate them. 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 shown to impact responses to ozone exposure, both in an inverse direction. The reduced responsiveness in smokers may wane after smoking cessation (Emmons and Foster 1991).

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

The results of controlled human exposure studies utilizing ozone exposures up to about eight hours have clearly established that ozone can induce 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 for 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 regarded 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.

11.3.1.9 Concentrations at which 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. Adverse outcomes, including reduced pulmonary function and increased respiratory and ventilatory symptoms, are demonstrated among exercising individuals exposed for 1-hour to an ozone concentration of 0.12 ppm, and for 6.6 to 8-hours to an ozone concentration of 0.08 ppm, with a wide range of individual responsiveness under both exposure protocols. Multi-hour

exposures to 0.08 ppm also induced increased airways reactivity and airways inflammation, which may signify an increase in the potential risk profile for the population. For asthmatics, frequent decreases in FEV1 of 20 to 30% could necessitate medical intervention (National Asthma Education and Prevention Program 2002), which clearly qualifies as an adverse effect.

11.3.2 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-hour/d for 6 to 90 days showed significant distal airway remodeling, with 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 daily 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 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 (HDMA) could amplify the immune response to allergens in sensitized infant monkeys, 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 study of changes in airway epithelial innervation induced in developing rhesus monkeys by exposure to ozone, and 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 concluded 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, present difficulties for developing a quantitative relationship for chronic effects.

11.3.3 Summary of Findings from Epidemiologic Studies

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 either short- and long-term effects of ozone on lung function and ventilatory and respiratory symptoms, hospitalization, emergency department usage, and premature death in free-living populations. As such, epidemiologic studies are able to examine a wide range of individuals, behaviors, subgroups, and exposure conditions.

There are some limitations to epidemiologic studies. First, it is not possible to characterize exposure in a precise manner similar to that of a chamber study. Most epidemiologic studies rely on regional air pollution monitors, which may not reflect the true exposures of the study subjects. For ozone and other gases this may result in 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 errors in measuring exposure from fixed site monitors can be significant.

Second, epidemiologic studies may be subject to bias from uncontrolled or poorly controlled confounders such as seasonality, weather and co-pollutants. 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 in this regard because elevated ozone concentrations are seasonal in nature, and are highly correlated with

temperature. More recent studies appear to be successful in addressing some of these potential problems. Third, the epidemiologic studies in this review used different ozone concentration averaging times for their exposure measurements. Many used a 1-hour maximum, while others reported results based on 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 all-cause and cardiopulmonary death, hospitalization, emergency room visits for asthma, restrictions in activity, respiratory symptoms and decreased lung function. The findings of these studies are fairly consistent and evidence of a suite of adverse outcomes suggest coherence (Bates, 1992) Many of the findings are observed or studied only in the summer season, when ozone is often highest. The findings in many cases have biological plausibility based on human and animal studies. In addition, many epidemiologic studies provide evidence for a concentration-response relationship. However, it is difficult to use these studies to determine a low or no effects level useful for standard setting, although they contribute to consideration of the margin of safety and to the calculations of potential benefits of controlling ozone. While any given epidemiologic study may have some limitations, taken together these studies provide a compelling 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.

11.3.3.1 Field Studies Addressing Acute Respiratory Effects of Ozone

Nine of the 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 a 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, who wore personal ozone monitors during the study period, 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 result in decrements in pulmonary function in response to ozone exposure. 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 PM2.5, 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.

11.3.3.2 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.

11.3.3.3 Short-term Exposure and Death

Though limited in some ways, a large and growing body of data now exists examining the association between daily death and ozone concentrations. These data support a preliminary conclusion that ozone concentrations represent an independent risk factor for premature death, controlling for weather effects and other air pollutants. The effects are observed more consistently and appear larger during the warm season. The largest multi-city analysis of ozone effects on death are derived from the National Death and Disease Air Pollution Study (NMMAPS), a study of death in the largest 90 cities in the U.S. which was reanalyzed in 2003 using non-GAM methods (Dominici, 2003). The ozone-related analyses of the NMMAPS study were extended and the results confirmed by Bell et al. (2004). The latter included additional years and cities and indicated effects of ozone on death for both the full year and summer only. In addition, the estimated effects were not attenuated by inclusion of PM10 in the model specification, or by the elimination of days with high temperature from the analysis. In addition, several meta-analyses report associations between ozone and premature death (Levy et al., 2001; Thurston and Ito, 2001).

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, Gryparis et al., 2004), reported larger excess death risks in the warm (or summer) season than in the cool (or winter) season. Gryparis et al. (2004) included 29 European cities in their analysis. While there is a real potential for the occurrence of these outcomes, based on the inflammatory response and other effects 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.

11.3.3.4 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 death 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 FEV1 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 ozone effects. 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_2, 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 concentrations (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). In the latter, children who were both exposed to higher ozone concentrations and involved in three or more outdoor sports activities exhibited higher rates of asthma induction.

Finally, there is inconsistent and inconclusive evidence for a relationship between long-term ozone exposure and increased death 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 death and July-September daily 1-hour maximum ozone was positive and nearly significant.

11.4 Consideration of People With Chronic Diseases

Controlled exposure and epidemiological studies involving individuals with COPD, asthma and allergic rhinitis indicate that ozone may exacerbate disease in at least some patients. The largest body of data on people with chronic disease concerns asthmatics. Epidemiological studies associate ozone exposure with hospitalization and emergency room visits for asthma, and also with increased asthma-like symptoms. Our review of the controlled exposures studies suggest that asthmatics and individuals with COPD do not appear to have an effect greater than that observed in healthy individuals. However, these individuals' baseline health status is typically already compromised, on an absolute basis, and thus ozone-associated effects would likely carry more significant clinical implications for these groups. In addition, the chamber studies suggest that there are several mechanisms by which ozone may potentiate the effects of allergen exposure in allergic asthmatics. Therefore, asthmatics and individuals with other chronic respiratory conditions may constitute a particularly sensitive subgroup when subjected to elevated exposure to ozone. In addition, the results from the study of Gong et al. (1998) on subjects with stable hypertension suggest that individuals with preexisting cardiovascular disease

may also constitute a sensitive subgroup. Additional research on this question, however, is necessary.

11.5 Consideration of Infants and Children

As noted earlier, SB25 specifically asks that staff 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 an association between elevated long-term ozone concentrations and new-onset asthma in children playing three or more 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.

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, it should be noted in considering epidemiological studies (including field studies), that exposures to highly correlated chemicals in the ambient air are inherently included in the evaluation. In addition, notwithstanding a few 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 exposure to ozone alone.

1. 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 at current ambient concentrations other criteria air pollutants have little or no modifying influence on 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.

11.6 Conclusions and Recommendations

11.6.1 Recommended Pollutant to Be Addressed

Staff recommends that ozone continue to be the pollutant addressed by the standard. As discussed in chapter 2, the state standard was originally set in 1959 for total oxidants because the monitoring method in use at that time could not distinguish ozone from other oxidants such as peroxyacetyl nitrate (PAN). However, even the earliest available human exposure studies were based on exposure to ozone, rather than to a mixture of oxidants. Adoption of ultraviolet photometry in 1974 as the monitoring method for the ozone standard changed the monitored species to ozone. Although it is possible that ambient oxidants other than ozone can induce adverse health effects, the available controlled human studies health effects literature, which formed the basis for the standard, is based on ozone, not total oxidants. Few data are available on responses to other oxidants at ambient concentrations, although several papers from the Children's Health Study suggest that ambient acids may contribute to responses to oxidant air pollutants (Gauderman et al., 2000; 2002).

In addition, it is generally recognized that control of ambient ozone levels provides the most effective means of controlling other potentially harmful photochemical oxidants. Furthermore, the limited 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, staff recommends that ozone, among all oxidants, remain as the pollutant to be regulated by the proposed standard.

11.6.2 Recommended Averaging Times

11.6.2.1 One-Hour Averaging Time

The current California ambient air quality standard for ozone has a 1-hour averaging time. Selection of this averaging period in 1987 was based on a likely exposure duration, evidence of health effects associated with short-term exposures, typical ozone exposure patterns in the South Coast Air Basin. In addition, continuation of the 1-hour standard was important for historical tracking, since this averaging time had been used since the original State standard was

set in 1959. When the ozone standard was last reviewed in 1987 it was recognized 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 understood that a stringent 1-hour 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 hour. The studies on which the 1-hour standard was based indicated that exposures to ozone as low as 0.12 ppm for one- or two- hour induced decrements in lung function and increased symptoms in exercising subjects. Chamber studies have also shown increased airway resistance at 0.18 ppm, and airway inflammation at 0.20 ppm, but neither endpoint has been studied at lower concentrations in one- to three-hour protocols.

Epidemiological studies also demonstrate an association between one-hour daily maximum concentrations of ozone and a wide range of adverse health effects, including premature death, 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 due to multi-hour exposures to ozone, which are highly correlated with one-hour averages, rather than a long term average. Thus, it is difficult to use epidemiological studies to ascribe measured health effects solely to one-hour ambient peak concentrations rather than longer-term exposures. However, short-term exposures are of concern given the nature of some of the health effects reported (i.e., cardiovascular death among the elderly and emergency room visits for infants). It is possible that at least some of the important exposures may be related to relatively short-term exposures (i.e., less than 2 hours), since these subgroups are unlikely to be engaged in multi-hour periods of moderate or heavy exercise.

In addition, key studies by Hazucha et al. (1992) and Adams (2003) comparing responses to square and triangular wave exposure patterns support the need for protection against short, peak concentration exposures. With the variable concentration protocol, Hazucha et al. (1992) reported that the response over the first three hours was minimal, followed by a mean decrease in FEV1 over hours 4 through 6 that peaked at approximately 10%. The maximal response lagged behind the peak ozone concentration by about two hours, since the maximal ozone concentration occurred at hour 4, yet the maximal FEV1 response occurred at hour 6. FEV1 improved during the last two hours of the exposure, and by the end of the exposure the FEV1 decrement was nearly identical to that following the constant concentration exposure (-5%). Adams (2003) compared responses of healthy young adults exposed to two ozone concentration profiles: (1) a constant ozone concentration of 0.08 ppm, and (2) a triangular ozone profile where the ozone concentration increased from 0.03 ppm to 0.15 ppm over four hours, and then decreased to 0.03 over the next 2.6 hours (mean ozone concentration = 0.08 ppm). The total inhaled dose of ozone was equivalent for both protocols. The maximal FEV1 decrement occurred at the time of the peak

ozone concentration with the triangular profile (hour 4), but after six hours in the constant concentration exposure.

The results of these two studies emphasize the importance of dose rate. They also indicate that a short exposure to a relatively high ozone concentration can result in larger functional decrements than a larger total inhaled effective dose comprised of a lower ozone concentration inhaled over a longer exposure time.

Therefore, staff recommends that the one-hour standard be retained to protect against short, peak exposures, based on the findings from controlled human exposure studies. Further, staff recommends that a substantial margin of safety be included in the 1-hour standard to account for the possibility of additional significant adverse health effects, as suggested by the epidemiologic studies.

11.6.2.2 Eight-hour Averaging Time

Since the 1987 review of the California AAQS for ozone, it has become clear that prolonged exposure to a low ozone concentration can also lead to adverse effects. A series of controlled human exposure studies addressing this type of ozone concentration profile has appeared that used a 6.6 to 8-hour protocol, in simulation of a full day of outdoor work, recreation or play. These studies were undertaken in response to observations, primarily in the eastern US, that many areas had a broad, ozone peak that lasted from six to eight hours. The results of these multi-hour studies indicate that 6.6 to 8-hour exposures to ozone concentrations as low as 0.08 ppm can induce statistically significant decrements in group mean lung function, and increases in ventilatory and respiratory symptoms, airway hyperreactivity, and airway inflammation. However, the magnitude of the functional decrements is typically smaller than observed with shorter, higher concentration exposures. Further, the group mean changes in the endpoints studied with either 6.6 or 8-hour exposures were similar. The results of these studies, in concert with observations of broad, peaks in ozone concentration profiles in much of the US led the US EPA to select an averaging time of 8-hours for its ozone standard recommendation in 1996.

In California, different regions of the State exhibit varying relationships between the 1- and 8-hour average ozone concentrations. Some areas exhibit narrow, high peaks (and relatively high ratios of 1 to 8-hour averages) while others exhibit a wide afternoon peak concentration and a relatively low ratio of 1- to 8-hour averages. Since many areas in the state experience these broad peaks in ozone, and since a large number of California residents spend multi-hour periods outdoors working or exercising, staff recommends the adoption of an 8-hour averaging time for ozone, in addition to the one-hour standard. A case could be made for a 6 hour average standard given that many of the relevant studies used a 6.6 hour exposure time. However, due to the similarity of the measured responses with 6.6 and 8-hour exposures, staff recommends that the multi-hour averaging time be 8-hours, which corresponds to a typical work day.

As noted at the outset of this chapter, State law (Health and Safety Code section 39014) defines an ambient air quality standard in terms of a concentration and an averaging time, which reflect the relationship between air pollution and

undesirable effects. Thus, an ambient air quality standard defines a maximum exposure (concentration and averaging time) estimated to be without adverse effects for most individuals who undergo the exposure defined by the standard. The recommended averaging times, one and eight hours, are based on common exposure patterns, and both are needed to address the non-linear aspects of the relationship between health and ozone concentration, ventilation rate and exposure duration. As such, these averaging times address the influence of both dose-rate and exposure duration on induced responses. staff recognizes that in some areas of the State one of the two recommended standards may be more controlling than the other. However, State law requires that ambient air quality standards be based on protection of public health through standards that set maximum acceptable exposures (concentrations and averaging times).

11.6.2.3 Not to be Exceeded

California ambient air quality standards are typically “not to be exceeded”. Staff recommends that the recommended ozone standards continue to be designated as “not to be exceeded.”

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 one to two 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 concern that there were likely adverse effects related to multihour exposures to relatively low concentrations of ozone and repeated exposures, but there were no human exposure studies addressing these concerns available. Experimental evidence in animals indicated that repeated or prolonged ozone exposure could induce adverse effects. Thus, DHS recommended a 1-hour standard of 0.08 ppm to provide protection from possible effects related to multihour or repeated exposures. This was the only averaging time for which a standard was recommended.

This recommendation is based on several studies that suggest long-term, and possibly permanent, effects related to frequent exposures to elevated concentrations of ozone. Two studies provide evidence for 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 have been above 0.10 ppm. 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 (McConnell et al. 2002). 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. Based on these results, and supported by animal toxicological studies reporting morphological changes with repeated ozone exposures, staff concludes that repeated exposures to elevated ozone concentrations are of

concern, and therefore recommends that both the one and eight hour average ozone standards continue to be designated as “not to be exceeded.”

11.6.3 Recommended Concentrations

11.6.3.1 Considerations for the Margin of Safety

Both the Health and 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, Ch,731, sec. 3; Health and 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.

Consequently, our approach was to identify the lowest ozone concentrations for selected averaging times for which statistically significant group mean decrements in lung functions and increases in symptoms were observed in the chamber studies. These studies have been given primary focus since both the dose and response are well characterized. Because the subjects studied in the chamber studies have been mainly relatively healthy young adults, and thus may not be representative of the wider population, a margin of safety was developed. The margin was as based on the available scientific data describing population variability, on epidemiological data examining endpoints and subgroups that cannot be studied in chambers, and on toxicological evidence of biological mechanisms. These considerations included: (1) chamber studies indicating wide variability in human response and the existence of particularly large individual responses; (2) chamber studies indicating both bronchial responsiveness and pulmonary inflammation; (3) animal toxicology studies supporting these findings and also suggesting the possibility of decreases in lung defense mechanisms; and (4) epidemiologic studies reporting associations between ambient ozone and a suite of adverse outcomes including premature death, hospitalization, emergency room visits, school loss, respiratory symptoms and changes in lung function.

While it is difficult to determine the precise dose in these latter studies, for the most part they involve ozone concentrations that are below those studied in the chamber studies. Together these epidemiological studies provide compelling evidence of adverse effects that have not been studied in a controlled chamber setting. This body of evidence cannot be ignored and needs to be reflected in the

margin of safety to ensure protection of other potentially sensitive groups from particularly adverse outcomes. Nevertheless, even with standards tailored to protect vulnerable populations, there may be exquisitely sensitive individuals who still have adverse responses. Thus, this standard should not be viewed as an absolute no effects level. Below, we provide the scientific rationale for the recommended one- and eight-hour average ozone concentrations.

11.6.3.2 Concentration for a One-hour Average

We recommend that the current 1-hour 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 staff recommendation is based on several factors.

First, In several studies that exposed subjects to 0.12 ppm ozone for one to tow hours, 10 - 25% of the subjects experienced a decline of 10% of 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 and adults 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 potentially vulnerable populations (e.g., people with moderate to severe asthma, COPD, and heart disease) who are included in the epidemiologic studies.

Second, chamber studies indicate that bronchial responsiveness and pulmonary inflammation occur with 1-hour exposure to 0.18 to 0.20 ppm. Bronchial responsiveness can aggravate 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 mechanisms.

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 death, 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 death (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 work or 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 death 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 death demonstrate effects only in the warmer months when ozone concentrations are highest. This suggests the importance of outdoor exposure, the possibility of thresholds (i.e., non-linear concentration-response functions), or both.

Additional uncertainties with these studies exist due to issues related to errors in exposure assessment and biological mechanisms. Concerning exposure assessment, 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. Therefore, in some regions, ozone measured at an outdoor fixed-site monitor may not be highly correlated with personal doses of ozone, rendering it more difficult to find an effect if one exists. In addition, it is difficult to reconcile some of the epidemiological studies with the admittedly limited number of chamber studies to date. The latter 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, while epidemiology studies report some positive effect estimates for these subgroups, implying increased sensitivity. Therefore, these effects should be viewed as suggestive until additional epidemiologic studies are undertaken that carefully control for factors such as seasonality, weather and potential confounding by co-pollutants, most importantly, particulate matter. In addition, in the panel studies of asthmatics which demonstrate both positive and negative findings, the role of preventive versus “as needed” medication needs to be addressed. Additional research on potential biological mechanisms is warranted, 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-linearity and/or a threshold. We have reviewed these studies and attempted to determine the likely interval of concentrations in each study where associations are clearly demonstrable (Figure 11-1). Taken together these studies suggest that the low end of the interval ranged from 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 PM10, PM2.5 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 one-hour ozone. We also can make some inferences about no effects levels from negative studies, which rarely have values above one-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 one-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 death (Pope et al. 2002). Also, long-term exposure to ozone, particularly prior to age 6 has been associated with impairment of small airways function (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 cites 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.

Viewing all of the evidence, staff recommends 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.

11.6.3.3 Concentration for an Eight-hour Standard

We recommend establishing an 8-hour average 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 past 15 years, supported by the important health outcomes reported in many of the epidemiologic studies. With exposure for 6.6 to 8-hours to an ozone concentration of 0.08 ppm, several studies have reported statistically significant group effects on lung function changes, ventilatory and respiratory symptoms, airway hyperresponsiveness, and airways inflammation in healthy, exercising individuals. A substantial fraction of subjects in these studies exhibited particularly marked responses in lung function and symptoms. Consequently, a concentration of 0.08 ppm ozone for an 8-hour averaging time should not be considered adequately protective of public health, and does not include any margin of safety, based on the definitions put

forth in State law. 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 individual subjects had changes in FEV1 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, staff has recommended an 8-hour concentration of 0.070 ppm, not to be exceeded.

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 the 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 findings must be factored into the margin of safety for the 8-hour average.

Studies of emergency room visits for asthma provide some limited evidence for the possibility of a population response threshold (see Figure 11-1). The one study of emergency room visits using exposure intervals that examined 8-hour average ozone concentration (Tolbert et al. 2000) reported an elevated risk within the interval of 0.070 to 0.13 ppm. This study reported more consistent responses in the interval from 0.09 to 0.10 ppm and statistical significance attained for the interval between 0.10 and 0.113 ppm for an 8-hour average ozone concentration. In addition, if we convert the 0.11 level of concern from studies using a one hour exposure, this relates to a concentration of 0.083 (using a ratio of one hour to eight hour concentrations of 1.33). Consideration of the results of the chamber studies reporting statistically significant group mean effects at 0.08 ppm, and suggestions of a diminution of effect from epidemiological analyses of emergency room visits, leads staff to recommend that the 8-hour average ozone concentration be set at 0.070.

It should be noted that the recommended 8-hour average concentration has three, rather than two, decimal places. Staff considered selection of 0.07 ppm. However, rounding conventions applied to air quality data (see Section 7.1.4) are such that any measured value up to and including 0.074 ppm would round down to 0.07 ppm when attainment designations are evaluated. Staff assessment of the available data suggested that selection of 0.07 ppm would not include an adequate margin of safety, as required by State law. The one available controlled exposure study at 0.06 ppm did not find a group mean effect. Therefore, staff is recommending that the 8-hour average standard have three decimal places, 0.070 ppm, to ensure an adequate margin of safety. Section 6.3 discusses issues related to precision and accuracy of the monitored data.

11.6.3.4 Monitoring Method

Staff recommends retention of the current monitoring method for ozone which uses the ultraviolet (UV) absorption method for determining compliance with the state Ambient Air Quality Standard for ozone. Further, staff recommends that all federally approved UV methods (listed at http://www.epa.gov/ttn/amtic/criteria.html) be incorporated by reference as California Approved Samplers. This will result in no change in current air monitoring practices, but will align state monitoring requirements with federal requirements.

11.6.4 Consideration of Infants and Children

As noted above, children have a higher ventilation rate relative to body weight at rest and during activity than adults. Children also tend to be outside more and more active than adults. Thus, by virtue of physiology and behavior, they are likely to be more highly exposed to ozone than the general population. However, the chamber studies of exercising children suggest that they have responses generally similar to adults, pointing to a similar degree of responsiveness. Epidemiologic studies that have examined 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 that may be associated with ozone exposure. . Collectively, this body of evidence suggests that although children appear to be similarly responsive to a given dose of ozone as adults, they are at greater risk than adults of experiencing adverse responses to ozone by virtue of their higher level of outdoor activity, and consequently greater total exposure. In addition, asthma disproportionately impacts children and thus both induction and exacerbation of asthma are important endpoints considered in evaluating the ozone literature.

11.6.5 Further Research Needs

There is a large body of research on the health effects of ozone, including controlled human, epidemiological and animal toxicological studies, which has formed the basis for the staff recommendation presented in this report. While staff is confident that the recommendations presented in this document are adequately supported by the available data, we nevertheless recommend that the Board consider funding (or encourage other agencies to fund) studies that investigate the following topics for which the data are currently inadequate to allow conclusions.

1. Multiple controlled studies have investigated the responses of human subjects to ozone concentrations as low as 0.08 ppm for up to 6.6 hours, and these studies consistently indicate that a substantial portion of the population is likely to experience adverse responses with similar exposure. However, there is a critical need for multi-hour controlled human studies at ozone concentrations lower than 0.08 ppm. We have utilized the two extant multi-hour controlled human exposure studies, one published and one unpublished, at ozone concentrations below 0.08 ppm in developing this recommendation. Epidemiologic and toxicologic results supplement these two studies in forming the basis for our recommendation for an 8-hour average ozone standard concentration of 0.070 ppm, although uncertainties in exposure assessment and animal to human extrapolation, respectively, add a degree of uncertainty to the findings from these bodies of literature. Thus, one critical research topic is investigation of responses to multi hour exposures to ozone concentrations between 0.04 and 0.08 ppm in human subjects.
2. Investigation into acute toxicity mechanisms in sensitive populations (i.e. individuals with chronic respiratory and heart diseases) to allow adequate assessment of the risk to these populations associated with ozone exposure.
3. Investigation into long-term effects of *in utero* and early infant exposure to ozone on the cardiorespiratory system, the nervous system, and the developing organism is needed to more adequately assess the risk to children and infants associated with ozone exposure is needed.
4. Investigation into mechanisms of ozone exposure effects on cardiopulmonary function at concentrations below 0.08 ppm are needed to follow up on recent data suggesting possible cardiopulmonary effects associated with ozone exposure.
5. Investigation into possible interactions of ozone with organic vapors to form secondary organic aerosols (the toxicity of these compounds is nearly unknown) is needed.

11.7 Summary of Staff Recommendations:

1. Retain ozone as the pollutant to be adressed by the standard.
2. Retain the existing 1-hour average standard of 0.09 ppm, not to be exceeded.
3. Establish an 8-hour average standard of 0.070 ppm, not to be exceeded.

4. These recommendations are based on the following findings:

a. Reduced lung function, and increased respiratory and ventilatory symptoms following 1-hour exposure to 0.12 ppm ozone with moderate to heavy exercise.

b. Increased airway hyperreactivity following 2 hour exposure to 0.18 ppm in exercising subjects.

c. Airways inflammation following 2 hour exposure to 0.20 ppm ozone in exercising subjects

d. Reduced lung function, increased respiratory and ventilatory symptoms, increased airway hyperreactivity, and increased airways inflammation following 6.6 to 8-hour exposure to 0.08 ppm ozone.

e. Evidence from epidemiological studies of several health endpoints including premature death, hospitalization, respiratory symptoms, and restrictions in activity and lung function.

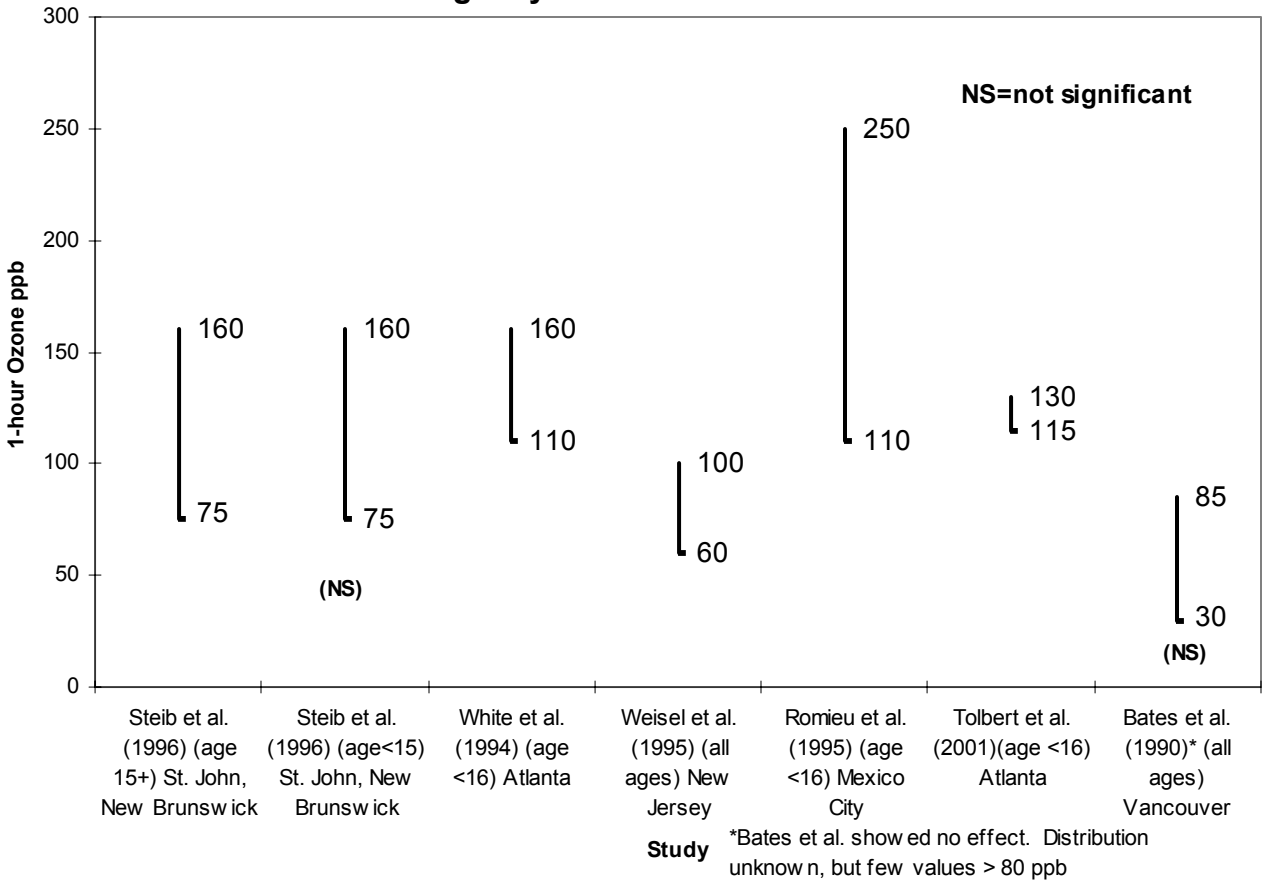
f. Evidence from epidemiological studies of emergency room visits for asthma suggesting a possible threshold concentration between 0.075 and 0.11 ppm from analyses based on a 1-hour averaging time, and a possible threshold concentration between 0.070 and 0.10 ppm from analyses based on an 8-hour averaging time.

5. Ozone Monitoring Method – retain the current monitoring method for ozone which uses the ultraviolet (UV) absorption method for determining compliance with the state Ambient Air Quality Standard for ozone. Incorporate all federally approved UV methods (listed at http://www.epa.gov/ttn/amtic/criteria.html) as California Approved Samplers for ozone. This will not change current air monitoring practices, but will align state monitoring requirements with federal requirements.

6. Fund additional research investigating the responses of human subjects to multi-hour exposures to ozone concentrations between 0.04 and 0.08 ppm.

7. Revisit the standards within five years, in order to re-evaluate the evidence regarding the health effects associated with ozone.

Figure 11-1 Intervals of 1-hr Ozone Indicating Likely Effect Levels for Emergency Room Visits for Asthma



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State of California

Air Resources Board

Governor Arnold Schwarzenegger

October 2005 Revision

Review of the California Ambient Air Quality Standard For Ozone

Volume IV of IV
Appendices B-G

Staff Report
Initial Statement of Reasons for Proposed Rulemaking

October 27, 2005

California Environmental Protection Agency

Air Resources Board

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California Environmental Protection Agency

Alan C. Lloyd, Ph.D., Secretary

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Appendix B

Quantifying the Health Impacts of Ozone Exposure

Quantifying the Health Impacts of Ozone Exposure

Numerous epidemiologic studies conducted in the United States and other countries point to adverse health effects from exposure to ozone. The effects from short-term exposure include, but are not limited to: hospital admissions for respiratory causes, emergency-room visits for asthma, minor restricted activity days, acute respiratory symptoms, exacerbation of asthma, and premature mortality (National Research Council, 2002; U.S. Environmental Protection Agency, 2004). In addition, there is more limited evidence that long-term exposure to ozone may result in new cases of asthma and premature mortality.

The objective of this appendix is to quantify a subset of adverse health effects attributable to current ozone levels in California to illustrate some of the health impacts of continued ozone exposures. This health impacts assessment was not used to select the appropriate levels of health-based ambient air quality or to perform a cost-benefit analysis. Rather, the results from this assessment provide information to the public on some of the health impacts of current ozone levels. While this section presents the results of our analysis as the impacts of exposure to current levels of ozone, the results can also be viewed as the public health benefits expected to accrue with attainment of the proposed standards. Specifically, we present results of an analysis of the impacts of the current ozone levels compared to attaining both the proposed State 8-hour standard and the federal 8-hour standard. In addition, we compare the benefits from attaining the proposed State 8-hour standard to the state 1-hour standard.

There have been several recent published efforts to estimate the impacts of ozone on public health, and the health benefits likely to be associated with reducing population exposures to ozone (U.S. Environmental Protection Agency 1999; Levy et al. 2000, Hubbell et al. 2005). This appendix presents a specific analysis of the health impacts of ozone exposure. It describes the methods, data, results and uncertainties involved with estimating the health impacts associated with exposures to current ambient concentrations of ozone.

The reader should note that health impacts estimates were made for only a small number of known effects of ozone exposure, and consequently this analysis is an underestimate of the total public health impact of current ozone levels.

Health Effects Estimation Approach

Section 812 of the federal Clean Air Act requires the U.S. EPA to periodically conduct an analysis of the health benefits of current federal air pollution regulations, which resulted in a report to the U.S. Congress (U.S. EPA, 1999). These efforts have undergone years of public review and comment as well as full peer review by the U.S. EPA's independent Science Advisory Board and by the National Research Council (2002). We have, therefore, drawn considerably from these prior efforts at the federal

level, particularly in the development of concentration-response functions. We have also added new studies from around the world that have appeared since publication of the most recent U.S. EPA report. Selection of the studies and functions included in our analysis has undergone review by several independent experts on the subject of air pollution and health.

Estimating the health impacts associated with current levels of ambient ozone involves four elements:

1. Estimates of the changes in ozone concentrations due to a hypothetical ozone reduction control strategy that achieves attainment of a standard.
2. Estimates of the number of people exposed to ozone.
3. Baseline incidence of the adverse health outcomes associated with ozone.
4. Concentration-response (CR) functions that link changes in ozone concentrations with changes in the incidence of adverse health effects. These functions come from epidemiological studies and are expressed in terms of a beta coefficient, indicating the percent reduction in a given health outcome due to a unit change in ozone.

Health effects results from epidemiological studies are based on various ozone averaging times: 24-hour, 8-hour and 1-hour. As a result, we converted the 8-hour and 24-hour epidemiological results into equivalent values on a 1-hour scale to allow direct comparison of the different studies.

Ultimately, the product of the above four elements generates estimates that represent the current impact of ozone on public health, compared to attainment of the proposed 8-hour standard, since that is the more stringent of the two standards proposed. Each of these elements is discussed below. Our methods make use of U.S. EPA's Environmental Benefits Mapping and Analysis Program (BenMAP), with modifications where appropriate to reflect application to California. In addition, we incorporated several recent studies that were not available when the most recent version of BenMAP was released (Bell et al., 2004; Gryparis et al., 2004). All methods and results presented herein are consistent with those methods used by U.S. EPA in their health benefits assessment (Hubbell et al., 2005). In addition, we have derived substantial material from other previous health impact studies including the U.S. EPA estimates of health benefits of the Clean Air Act (U.S. EPA, 1999), the World Health Organization (WHO) meta-analysis of ozone health effects (Anderson et al. 2004), and the Levy et al. (2001) analysis of the public health impacts of current ozone concentrations.

Exposure Estimation and Assumptions

The estimation of ozone exposure involves two key elements: assessing changes in ozone concentrations, and estimating the population exposed to these changes in ozone levels.

To assess the changes in the current ozone concentrations necessary to achieve the

proposed standards, we first determined the design value, the benchmark used for attainment status. The design value is the Expected Peak Day Concentration, a value that reflects the highest concentration expected to occur, once per year on average, based on the past three years of air quality monitoring data. The use of three years of data reduces the effect of an anomalous year. Details on how the design values are calculated are presented in Chapter 7. Because the designations of the air quality standards are generally made at the air basin level, the design value for the basin was used for all counties within the basin.

Monitoring data for 2001 to 2003 were used from all monitors in the State meeting quality assurance criteria for valid data, and were extracted from the ARB ADAM database (ARB, 2004). Chapter 7 provides detailed analyses of exposure to ozone in California.

To calculate changes in exposure to ozone that reflect a hypothetical attainment of the proposed ambient air quality standards, a proportional linear rollback procedure was used. Under real-world conditions, control strategies will likely have some impact on days with low and moderate levels of ozone, as well as on days with high levels. Our rollback procedure reflects this observation. Details on the changes in the distribution of ozone concentrations over time are provided in the Supplement to this appendix.

Design Value Rollback Method

Rollback factors from the 1-hour and 8-hour ozone design values to the applicable standard were calculated for each air basin to assess the daily reductions in current ozone concentrations estimated to result at all monitoring sites when the standards are achieved. Rollback factors were based on design values for 2003. The design values reflect measured air quality for three years, 2001 through 2003. The design value was determined for each monitoring site according to the relevant regulatory specifications. For example, the Federal 8-hour design value is the three-year average of the annual 4th highest 8-hour ozone measurements. The design values for the state 1-hour and 8-hour standards are Expected Peak Day Concentrations (the statistically derived value expected to be exceeded once per year, on average – details are in Section 7.1.2 of Chapter 7). Design values for basins are simply the highest design value at any site within the basin. The basin design values typically determine attainment of each ozone standard. An uncontrollable ozone concentration of 0.04 ppm (see Chapter 4) was factored into the calculation of the rollback factor (see below). This represents the average daily one-hour maximum background ozone concentration. The rollback factor was assumed to apply to each site in the air basin for every day in a given year.
Our methodology assumed that under the hypothetical attainment condition all ozone observations within an air basin were subjected to the same percentage rollback factor based on the basin's three-year high value. To investigate the plausibility of this assumption, we examined the trends in the annual distributions of the 1-hour and 8-hour concentrations of ozone in the South Coast Air Basin (SoCAB). Due to its population and current ozone levels, a significant proportion of current statewide health impacts occur in the SoCAB. For this region, the estimated downward trend was consistent for

both 1-hour and 8-hour concentration reductions from the 1980s to the current period. The maximum, the 90th, 80th, 70th, 60th, 50th and 40th percentiles from the annual distribution of the basin's daily high concentrations, as well as the individual site's daily highs, show a consistent downward trend since the 1980s. More importantly, when we examined the rate of change in the concentrations above background since the 1980s, it was similar among the percentiles. This analysis supports our application of a constant percentage rollback to all sites within each air basin. Results for several representative sites used in this analysis of ozone trends can be found in the Supplement to this appendix.

Roll-Back Procedure

For each air basin in the State, the rollback factor necessary to move the basin-high design value to the proposed standard was calculated for both the 1- and 8-hour averages. We assumed that only the portion of each ozone value above a background of 40 ppb will decrease as progress toward attainment takes place. For example, in the table of hypothetical values below (Table B-1), suppose the basin-high design value would need to be reduced by 50% for the portion above 40 ppb to achieve the standard. Thus, an ozone measurement of 100 ppb today would face a reduction of (100 – 40) x 50% = 30 ppb. Hence the projected value at attainment would be 30 ppb + 40 ppb = 70 ppb. The effective rollback rate of moving from 100 ppb to 70 ppb is 30%. Similarly, a value close to 40 ppb like 50 ppb would face a reduction of (50 – 40) x 50% = 5 ppb. With the projected value at attainment of 5 ppb + 40 ppb = 45 ppb, the effective rollback rate is 10%, calculated from 50 ppb today. In summary, while both measurements in this hypothetical example (100 ppb and 50 ppb) are subject to the same rollback rate of 50%, the effective rollback rate differs due to the rollback procedure taking into account the background of 40 ppb

.

Table B-1: Example of Proportional Roll-Back Procedure

Current ozone		Rollback Rate for Portion Above 40 ppb	Future ozone		Effective Rollback Rate
Measured (ppb)	Above 40 ppb		Above 40 ppb	Projected	
100	60	50%	30	70	30%
50	10	50%	5	45	10%

For the 1-hour standard, rollback factors based on 1-hour design values were used to project 1-hour observations assuming a scenario where the 1-hour standard of 0.09 ppm was attained. Specifically, the rollback factor for an air basin was: (0.094 ppm – 0.04 ppm) / (1-hour Design Value – 0.04 ppm). The concentration of 0.094 was used since this is the highest value considered in attainment (after being rounded to 0.09), based on rounding conventions. This procedure produced basin-specific rollback

factors, which were applied to daily maximum 1-hour ozone values at all sites in the applicable basin, considering the background of 0.04 ppm. Basin-specific ratios were used for this purpose since the ratios can be significantly different from one basin to another. For example, in the South Coast Air Basin, the 1-hour design value was 0.178 ppm, so the rollback factor was (0.094 – 0.04) / (0.178 – 0.04) = 39%.

For the 8-hour standard, we used basin-specific 1-hour and 8-hour design values to convert the 1-hour data into a form useable for rollback calculations for the 8-hour standard. One-hour ozone concentrations are highly correlated with 8-hour concentrations. Therefore, we calculated the 1-hour value when the 8-hour standard is attained in each air basin and defined this as the "equivalent design value" for the 8-hour standard in that basin. The target for the 8-hour standard (0.070 ppm) was converted to a basin-specific 1-hour equivalent so that the CR functions that were all converted to 1-hour averaging times could be utilized. The conversion was based on the assumption that the future ratio between 1-hour and 8-hour design values will be similar to its current value. For example, in the South Coast, the 1-hour and 8-hour design values are 0.178 and 0.146 ppm, respectively. To attain the 8-hour standard of 0.070 ppm, the equivalent 1-hour target was projected to be 0.070 * (0.178/0.146) = 0.086 ppm. Therefore, 0.086 is the equivalent 1-hour target for attainment of the proposed State 8-hour standard in the South Coast.

Rollback factors for attainment of the 8-hour standard are calculated as: (Equivalent Target – 0.040 ppm) / (1-hour Design Value – 0.04 ppm). So, for the South Coast Air Basin, the rollback factor was (0.086 – 0.04) / (0.178 – 0.040) = 33%.

The roll-back procedure toward a 0.040 ppm background reflects currently observed rates of change in all parts of the ozone concentration distribution. The rollback factors were applied on a site–by-site basis to the ozone monitoring data for each day. The difference between the observed value and the rolled-back value was calculated for each day of the year in terms of 1-hour maximum ozone (for both standards), thus avoiding the uncertainty associated with converting CR functions into an 8-hour maximum ozone scale.

Similar calculations were made for the federal 8-hour standard. The concentration of 0.084 ppm was used in the rollback since this is the highest value considered in attainment (after being rounded to 0.08), based on rounding conventions.

Health impacts were then estimated for each day in a given year, summed across sites over the year, and then averaged over the three years of data. We also ensured that no impacts would be calculated for any day with an average concentration at or below the assumed background ozone level of 0.04 ppm. For the technical reader, the mathematical formulae for our rollback procedure and evidence supporting the rollback assumptions are provided in the Supplement to this appendix.

Estimation of Exposed Population

To estimate the number of people exposed to the ozone changes observed at each monitoring site, the county population was divided by the number of monitoring sites in a given county. This assumes that the population is equally distributed around each monitoring site within a county. We used county population data from the year 2000 census. For further details, see the Supplement to this appendix. We also examined the sensitivity of this assumption by considering two alternative methods for estimating exposure to ozone: census tract interpolation and county averaging of monitored concentrations. Details of these sensitivity analyses are provided below.

Estimates of the Baseline Incidence of Adverse Health Outcomes

The health effect baseline incidences are the number of health events per year per unit population. In this analysis, all baseline incidence rates except those for school absenteeism were taken from U.S. EPA's BenMAP software program.

Mortality incidence rates were obtained from the U.S. Centers for Disease Control (CDC), as derived from U.S. death records and the U.S. Census Bureau. Regional hospitalization counts were obtained from the National Center for Health Statistics (NCHS) National Hospital Discharge Survey (NHDS). Per capita hospitalizations were calculated by dividing these counts by the estimated county population estimates derived from the U.S. Census Bureau and the population projections used by NHDS. Hospitalization rates for "all respiratory causes" included ICD-9 codes 460-519. Similarly, regional asthma emergency room visit counts were obtained from the National Ambulatory Medical Care Survey (NHAMCS), combined with population estimates from the 2000 U.S. Census to obtain rates. Illness-related school loss baseline incidence rates were based on Hall et al. (2003). Ostro and Rothschild (1989) provided the estimated rate for minor restricted activity days.

The assumed incidence rates are summarized in Table B-19 in the Supplement to this appendix. All counties and sites within each county were assumed to have the same incidence rate for a given population age group.

Concentration-Response Functions

Concentration-response (CR) functions are equations developed from epidemiologic studies that relate the change in the number of adverse health effect incidences in a population to a change in pollutant concentration experienced by that population. As reviewed in Chapter 9 (Controlled Exposure Studies) and Chapter 10 (Epidemiologic Studies), a wide range of adverse health effects has been associated with exposure to current ambient concentrations of ozone. However, we only used CR functions derived from epidemiologic studies in this analysis. Developing concentration-response functions from this vast and not fully consistent literature is a difficult task and ultimately involves subjective evaluations. In this section, we aim to provide a fair and accurate

reflection of the current scientific literature. We also aim to provide enough detail so that others may fully evaluate our assumptions and methodology. Below, we provide CR functions for effects of short-term ozone exposure on premature mortality, hospital admissions for respiratory disease, emergency room visits for asthma, school absenteeism, and minor restrictions in activity. Although epidemiologic studies also report other adverse effects associated with ozone exposure – such as asthma exacerbations, respiratory symptoms, hospital admissions for cardiovascular disease with short-term ozone exposures, and mortality and asthma onset associated with long-term exposure (i.e., several years) – we determined that the existing evidence was either insufficient or too uncertain to serve as a basis for making quantitative impacts estimates. A good example is asthma exacerbations for which several studies have reported associations with ozone. However, different subgroups of asthmatics and different outcome measures were used, making it difficult to develop consensus estimates.

In this appendix, the primary studies used in the health impacts assessment are epidemiological. There are a number of reasons for using epidemiological studies. While human chamber studies have the merit of being able to carefully control for dose and response, they usually involve small sample sizes that may not include the most sensitive subpopulations, and cannot capture severe outcomes like hospitalization or premature death. Lagged or cumulative effects are similarly omitted, and only a limited range of exposures is examined. In short, human chamber studies are helpful to support causality and to determine effects of short-term exposure on measures like lung function and airways inflammation, but they do not necessarily provide information on general population responses to exposure to ozone. For the latter purpose, epidemiological studies which incorporate varying subgroups, exposure scenarios, behaviors, and health outcomes will best serve to estimate the overall potential human health impact of air pollutants and be the source of CR functions used for quantitative health impact assessment.

Besides the primary studies, some CR functions were developed from previous estimates of the health impacts of ozone exposures. Sources for these studies include the U.S. EPA estimates of the health effects associated with the Clean Air Act under Section 812 (U.S. EPA, 1999), the World Health Organization meta-analyses on ozone (Anderson et al., 2004), and the Levy et al. (2001) analysis of the public health benefits of reducing ozone.

This section discusses some factors that impact health effect estimates and outlines the epidemiological studies that were used for the basis of the CR functions.

Conversions for Ozone Measurements of Various Averaging Times

Most health studies considered in our analysis were conducted with ozone levels measured as 1-hour maximum or 8-hour maximum. However, there were some studies that measured ozone averaged over other time increments. Since these studies were conducted throughout the United States and other parts of the world, national average

adjustment factors were used to convert all measurements to 1-hour and 8-hour averages (Schwartz 1997). The 1-hour maximum was assumed to be 2.5 times the 24-hour average, and 1.33 times the 8-hour average concentration. These conversion factors have been used in previous meta-analyses in the ozone epidemiological literature (Levy et al., 2001; Thurston and Ito 2001). Our examination of California monitoring data for 2001-2003 in the San Francisco Bay Area and South Coast indicates that the ratios in California are similar. To reduce the uncertainty associated with converting the results into both the 1-hour and 8-hour time scales, we converted the epidemiological results into a common 1-hour scale only. Because the majority of studies report findings in term of ppb, CR functions were calculated per ppb, and air quality measurements were converted from ppm to ppb accordingly in the calculation of health effects.

Thresholds

Assumptions regarding the appropriateness of applying thresholds, and at what level, can have a major effect on health impacts estimates. One important issue in estimating ozone-related health impacts is whether it is valid to apply the CR functions throughout the range of predicted changes in ambient concentrations, even changes occurring at levels approaching the natural background concentration (without any human activity).
As reviewed in Chapter 10, most of the epidemiologic studies include very low ozone concentrations in their analysis and no clear threshold for effects has been reported, although the issue has not been fully investigated except with reference to ER visits for asthma. These latter studies, reviewed in Section 10.2.3 suggest a population threshold in the range of 0.075 to 0.110 ppm for 1-hour exposures, and 0.056 to 0.084 ppm (using a ratio of 1.33) for 8-hour exposures (see pg. 8-14; figure 8-1). In our approach of applying a constant percent change rollback to all of the basin-wide monitors, many of the reductions in ozone concentrations will occur below the proposed standard. Thus, for some days, our estimate of impacts of ozone exposure will be based on ozone concentrations that are within the range of the original epidemiologic studies, but below the proposed standards. In our base-case model, we assumed that there was no effect threshold concentration, and therefore we used the background level of 0.04 ppm as the no effects level. For the sensitivity analysis, we assumed several no effects levels but adjusted the remaining slope to account for application of a threshold to the concentration-response function. This is described in greater detail below.

Developing the Concentration-Response Function

Most of the epidemiologic studies used in our estimates have used a log-linear model to describe the relationship between ozone exposure and the health endpoint. In this case, the relationship between ozone levels and the natural logarithm of the health effect is estimated by a linear regression. This regression model generates a beta coefficient that relates the percent change in the health outcome to a unit change in ozone. Existing studies have reported either a beta coefficient for a unit change in exposure or a relative risk (RR) for a specified change in ozone concentrations, such as 10 ppb in 1-hour maximum. The RR is defined as the ratio of the health effect predicted from the

higher exposure relative to some baseline exposure. Health effect estimates presented in a given study as RR for a specified change in ozone, ΔO_3, were converted into an estimated beta using the equation:

$$\beta = \ln(RR) / \Delta O_3$$

The daily change in ozone at each monitoring site i.e., the difference between current ozone and the standard (= ΔO_3) was used to calculate RR:

$$RR = \exp(\beta \Delta O_3)$$

Then, the RR estimates were used to determine the population attributable risk (PAR), which represents the proportion of the health effects in the whole population that may be prevented if the cause (ozone pollution in our case) is reduced by a given amount. Specifically,

$$PAR = (RR - 1) / RR$$

Ultimately, the estimated impact on the health outcome is calculated as follows:

$$\Delta y = PAR \times y_0 \times pop$$

where:

Δy = changes in the incidence of a health endpoint corresponding to a particular change in ozone,
y_0 = baseline incidence rate/person within a defined at-risk subgroup, and
pop = population size of the group exposed.

The parameters in the functions differ depending on the study. For example, some studies considered only members of a particular subgroup of the population, such as individuals 65 and older or children, while other studies considered the entire population in the study location. When using a CR function from an epidemiological study to estimate changes in the incidence of a health endpoint corresponding to a particular change in ozone in a location, it is important to use the appropriate parameters for the CR function. That is, the ozone averaging time, the subgroup studied, and the health endpoint should be the same as, or as close as possible to, those used in the study that estimated the CR function.

In some cases, results from several studies of the same health endpoint were combined to estimate the health effect. An inverse-variance weighting scheme was used to pool results from these studies, allowing studies with greater statistical power to receive more weight in the pooled assessment. This approach implicitly assumes that all studies are equally valid and representative of the population in question, and is the standard approach applied in many impact analysis settings.

Mortality from Short-Term Exposure

Chapter 10 concludes that there is sufficient evidence for an effect of daily exposure to ozone (possibly with a lag response of a day or two) on premature mortality. These effects are based on daily time-series studies of counts of daily all-cause mortality within a given city reviewed over several years. The studies control for most other factors that may impact daily mortality such as weather, time trends, seasonality, day of week, and other pollutants. In addition, the studies have been undertaken over a wide range of weather conditions, seasonal patterns, covarying pollutants, baseline population characteristics. Chapter 10 reviews the uncertainties inherent in these studies. The U.S. EPA has funded several meta-analyses investigating the association between ozone and mortality, but the results of these analyses are not yet available. Therefore, below we present the effect estimates from the available literature and develop our rationale for a central estimate and probable bounds that reflect the observed range of effect estimates. Figure 1 summarizes the most relevant meta-analytic studies to date. Additional information about these studies is provided in Chapter 10.

The World Health Organization (Anderson et al., 2004) conducted a meta-analysis of the 15 cities in Europe (Anderson et al. 2004). Their meta-estimates indicate a relative risk of 1.003 (95% CI = 1.001 – 1.004) for a 10 $\mu g/m^3$ change in 8-hour ozone. For standard pressure (1 atmosphere) and temperature (25º C), 1 ppb ozone equals 1.96 $\mu g/m^3$. We have assumed the ratio between 1-hour and 8-hour ozone of 1.33 and between 1-hour and 24-hour of 2.5 (Schwartz 1997). Making the conversions, the WHO estimate implies a 1.13% change (95% CI = 0.38 - 1.51) in daily mortality per 10 ppb change in 24-hour average ozone. The WHO also provided an estimate correcting for possible publication bias using a trim and fill technique. Under an assumption that bias was present, the adjusted estimate is 0.75 % (95% CI = 0.19 – 1.32) per 10-ppb change in 24-hour average ozone.

This estimate is very similar to that produced by Levy et al. (2001). In their meta-analysis they began with 50 time-series analyses from 39 published articles. A set of very strict inclusion criteria was applied, which eliminated all but four studies. Reasons for exclusion included: studies outside the US, use of linear temperature terms (versus non-linear and better modeled temperature), lack of quantitative estimates, and failure to include particulate matter (PM) in the regression models. Ultimately, their analysis generated an estimate of 0.98% (95% CI = 0.59 – 1.38) per 10 ppb change in 24-hour average ozone. If the criteria are loosened to include eleven more studies, the pooled estimate decreases to 0.80 (0.60 – 1.00). Stieb et al. (2002) also reported a similar effect estimate based on 109 previous studies (including those with single- and multi-pollutant models) of 1.12 (0.32 – 1.92). Thurston and Ito (2001) reviewed studies published prior to the year 2000. When the authors focused on seven studies that more carefully specified the effect of a possible confounder, daily temperature, by using non-linear functional forms, the resulting meta-estimate was 1.37% (95% CI = 0.78 – 1.96). Relaxing this constraint to include all 19 available studies, the resulting risk estimate was 0.89% (95% CI = 0.56 – 1.22) per 10-ppb change in 24-hour ozone.

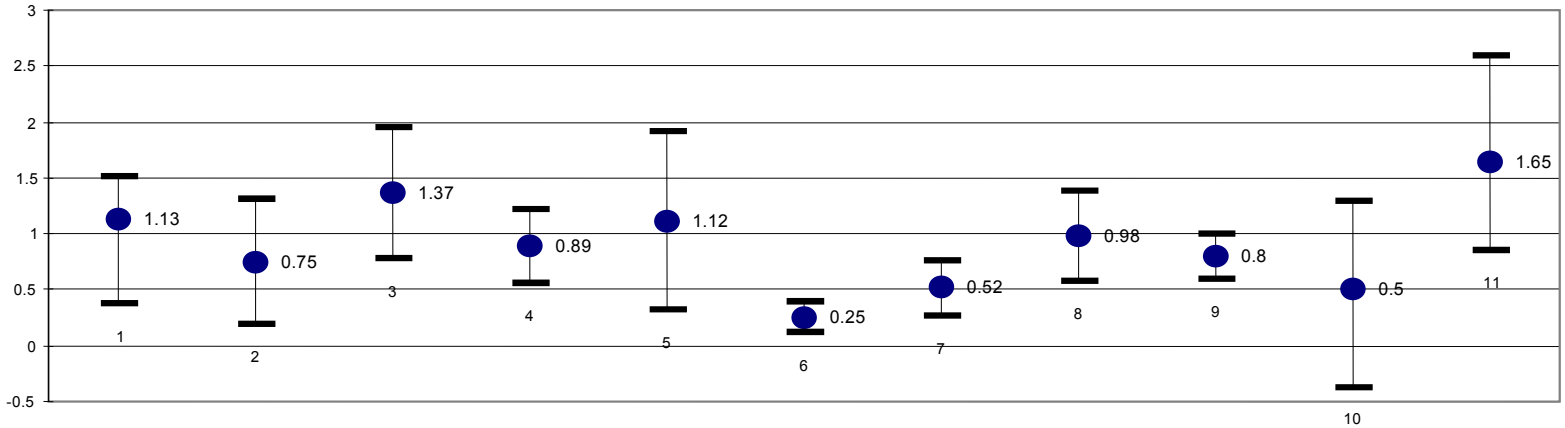
Two more recent meta-analyses have been published that provide lower effect estimates. Gryparis et al. (2004) is an analysis of 23 European cities from the APEAH2 study. The study controlled for potential confounders by including average daily temperature and humidity, respiratory epidemics, day of week in the regression model. The overall full-year estimate was 0.5% (95% CI = -0.38 – 1.30) per 10-ppb change in 24-hour average ozone. A meta-analysis was also conducted using summer-only data. Presumably this estimate will be less confounded by seasonality and also represent a time when the population would be spending more time outdoors. The summer-only estimate was 1.65% (95% CI = 0.85 – 2.60) per 10-ppb change in 24-hour average ozone. This summer-specific estimate might be particularly relevant for California due to its climate. A meta-analysis of the 95 largest U.S. cities from the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) data base provided estimates using a similar natural spline model for every city (Bell et al., 2004). Ultimately, the model suggested an effect estimate of 0.25% (95% CI = 0.12 – 0.39) per 10-ppb change in 24-hour average ozone. The NMMAPS study may generate an underestimate of the impact of mortality due to the modeling methodology used to control weather factors. Specifically, this effort included four different controls for temperature and dewpoint, where most other times-series analyses used only two or modeled extreme weather events more carefully and used city-specific models to ensure the best fits. In comparing the results for particulate matter (PM) for a given city with studies of individual cities by other researchers, the NMMAPS results are usually lower (Samet al et al, 2000). This estimate was based on a lag consisting of today's and yesterday's ozone concentrations. When a longer period 7-day lag was used the estimate increased to 0.52% (95% CI = 0.27 – 0.77) per 10-ppb change in 24-hour ozone.

Our estimates for the effects of ozone on mortality reflect the range provided in the above-cited studies. Table B-2 summarizes the effect estimates reported in these studies. Figure B-1 provides a graphical summary of the range of effect estimates and our suggested central, low and high estimates. A low estimate of 0.5% per 10 ppb, 24-hour average ozone, corresponds to estimates from the NMMAPS study (using a one-week cumulative lag) and the APEAH2 European study, but is below most of the other central estimates. The central estimate of 1% per 10 ppb is very similar to the central estimate generated by WHO (Anderson et al., 2004), Levy et al. (2001), and Stieb (2003). Finally, as a high estimate, we use 1.5% per 10 ppb, which reflects the central estimates of Thurston and Ito (using non-linear functions for temperature) and the summer-only estimates of Gryparis et al. (2004). Our range of estimates is applied to all age groups.

On the 1-hour scale, a 1% change per 10 ppb of 24-hour ozone is about 0.4% per 10 ppb change in 1-hour daily maximum ozone based on an assumed the ratio between 1-hour and 8-hour ozone of 1.33 and between 1-hour and 24-hour of 2.5 (Schwartz 1997). Specifically,

$$\frac{1\%}{10\,ppb24hr} = \frac{1\%}{10\,ppb24hr}\,x\,\frac{1ppb24hr}{2.5\,ppb1hr} = \frac{0.40\%}{10\,ppb1hr}$$

Figure B-1: Percent Change in Mortality Associated with Ozone (per 10 ppb 24-hour average)



Study #	Author	# of studies	Comment
1	Anderson (2004)	15	European
2	Anderson (2004)	20	Euro, corrected for possible publication bias
3	Thurston+Ito (2001)	7	Studies using non-linear temperature
4	Thurston+Ito (2001)	19	All studies
5	Stieb et al. (2003)	109	All studies
6	Bell et. al. (2004)	95	NMMAPS, lag(01)
7	Bell et. al. (2004)	95	NMMAPS, lag(06)
8	Levy et al. (2001)	4	Strict criteria
9	Levy et al. (2001)	15	Less strict criteria
10	Gryparis et al. (2004)	23	All year Europe
11	Gryparis et al. (2004)	23	Summer Europe

Hospital Admissions for Respiratory Diseases

Studies of a possible ozone-hospitalization relationship have been conducted for a number of locations in the United States, including California. These studies use a daily time-series design and focus on hospitalizations with a first-listed discharge diagnosis attributed to diseases of the circulatory system (ICD9-CM codes 390-459) or diseases associated with the respiratory system (ICD9-CM codes 460-519). Various age groups are also considered, which vary across studies. For our estimate, we relied on the meta-analysis by Thurston and Ito (1999). These authors used a random effects model based on three studies from North America. The studies were Burnett et al. (1994), Thurston et al. (1994), and Burnett et al. (1997). The category of all respiratory admissions for all ages yielded an estimate of relative risk of 1.18 (95% CI= 1.10 – 1.26) per 100 ppb change in daily 1-hour maximum ozone. This category includes hospital admissions for asthma and bronchitis, so separate estimates of these outcomes are not necessary. The estimate converts to a 1.65% change in hospital admissions (95% CI = 0.95 – 2.31%) per 10 ppb change in 1-hour daily maximum ozone. This estimate was applied to all age groups. Additional studies of respiratory admissions for specific diseases or subpopulations provide additional support for the above relationship, but are not quantified to avoid double counting. For example, Anderson et al. (1997) reported a relative risk of 1.04 (95% CI= 1.02-1.07) for hospital admissions for COPD for all ages for a 50 $\mu g/m^3$ change in 24-hour ozone. This converts to 0.63% per 10 ppb change in 1-hour maximum ozone. Burnett et al. (2001) investigated respiratory hospitalizations in children under age 2, and reported a 7.8% increase in hospital admissions per 10 ppb change in five-day moving average of 1-hour daily maximum ozone concentrations.

Emergency Room Visits for Asthma

Some studies have examined the relationship between air pollution and emergency room (ER) visits for pediatric asthma. Because most ER visits do not result in an admission to the hospital, we evaluated hospital admissions and ER visits separately, taking into account the fraction of ER patients that were admitted to the hospital. Our estimate is based on five studies which provide CR functions across the full range of ozone concentrations: Tolbert et al. (2000), Friedman et al. (2001), Jaffe et al. (2003), Romieu et al. (1995), and Stieb et al. (1996). Tolbert et al. (2000) report an association between pediatric emergency room visits (age < 16) for asthma and ozone in Atlanta during the summers of 1993-1995. The authors report a relative risk of 1.04 (95% CI = 1.008 – 1.074) per 20 ppb change in 8-hour ozone. Friedman et al. (2001) reported an association between daily counts for asthma in two pediatric emergency departments (age 1 to 16) and ozone in Atlanta during the summer of 1996. They report a RR of 1.2 (95% CI = 0.99 – 1.56) per 50 ppb change in 1-hour maximum ozone. This model included PM10 as a co-pollutant. Jaffe et al. (2003) reported an association between ozone and emergency room visits for asthma (ages 5 to 34) among Medicaid recipients in three cities in Ohio for the summer months from 1991- 1996. Estimates for the combined three cities indicate a RR of 1.03 (1.00 – 1.06) for a 10 ppb change in the 8-hour average of ozone. Romieu et al. (1995) reported results for emergency visits for asthma (age < 16) in Mexico City from January to June, 1990. A RR of 1.43 (95% CI=

1.24 – 1.66) was obtained for a 50 ppb change in 1-hour maximum ozone. Finally, Stieb et al. (1996) reported a beta of 0.0035 (95% CI = 0.00 –0.0070) for a 1 ppb change in 1 hour maximum ozone for ER visits for asthma in Saint John, New Brunswick, Canada.
Using an inverse variance weight for these five studies, we obtained a meta-analytic result of 2.4% per 10 ppb in daily 1-hour maximum ozone with a 95% CI = 1.46 to 3.34%. This estimate was applied over the entire range of ozone concentrations to children under 18. Several studies on ER visits for asthma report a non-linear response consistent with an effect threshold (see Section 8.3.3.2 and Figure 8-1, and Section 10.2.5). The threshold level appears to be somewhere between 0.075 and 0.110 ppm for a 1-hour average (or, using a ratio of 1.33, an 8-hour average of 0.056 to 0.084 ppm). This threshold may be due to lower power in detecting effects at low concentrations. In addition, the studies indicate some increased risks observed at below threshold concentrations. Regardless, if a zero slope (implying a threshold) is applied to the lower portion of the data, the concentration-response function for the remaining portion of the data must be larger than the slope for the entire data set. Below we describe how to adjust the slope of the CR function to investigate the implications of imposing a threshold on the CR function.

School Absences

In addition to hospital admissions and ER visits, there is considerable scientific research that has reported significant relationships between elevated ozone levels and other morbidity effects. Controlled human studies have established relationships between ozone and symptoms such as cough, pain on deep inhalation, shortness of breath, and wheeze. In addition, epidemiological research has found relationships between ozone exposure and acute infectious diseases (e.g., bronchitis, and sinusitis) and a variety of "symptom-day" categories. Some "symptom-day" studies examine excess incidences of days with identified symptoms such as wheeze, cough, or other specific upper or lower respiratory symptoms. Other studies estimate relationships with a more general description of days with adverse health impacts, such as "respiratory restricted activity days" or work loss days. We selected a few endpoints that reflect some minor morbidity effects and carefully adjusted estimates to avoid double counting (e.g., adjusted minor restricted activity days by number of asthma-related emergency room visits).

One of these studies demonstrated that 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). For illness-related absences, verified through telephone contact, further questions assessed whether the illness was respiratory or gastrointestinal, with respiratory including runny nose/sneeze, sore throat, cough, earache, wheezing, or asthma attack. Associations were observed between 8-hour average ozone and school absenteeism due to several different respiratory-related illnesses. Specifically, the authors report a 62.9% (95% CI = 18.4 -124.1%) change in new episodes of absences from all illnesses associated with a 20 ppb change in 8-hour average ozone. This provides the basis for our quantitative estimate, which was applied to all schoolchildren aged 5-17. On the 1-hour scale, 62.9% change per 20 ppb change of 8-hour ozone is about 21.2% per 10

ppb change in 1-hour daily maximum ozone using the assumed ratio between 1-hour and 8-hour ozone of 1.33 and between 1-hour and 24-hour of 2.5 (Schwartz 1997). Thus, we estimated:

$$\frac{62.9\%}{20\,ppb8hr} x \frac{1\,ppb8hr}{1.33\,ppb1hr} = \frac{21.2\%}{10\,ppb8hr}$$

In calculating the change in episodes of school loss, we assumed children did not attend school during weekends and holidays, that about 20% of students attended year-round schools, and adjusted the attendance rate for each month of the year. The baseline absence rate reported by Hall et al. (2003), based on a telephone survey of school districts, was applied. To convert episodes of school loss into days, we estimated 1.265 days as the average duration of an illness-related school absence, the result of dividing the average daily school loss rate from BenMAP by the episodic absence rate from Gilliland et al. (2001).

Minor Restricted Activity Days

Ostro and Rothschild (1989) estimated the impact of PM2.5 on the incidence of minor restricted activity days (MRADs) and respiratory-related restricted activity days (RRADs) in a national sample of the adult working population, ages 18 to 65, living in metropolitan areas. The annual national survey results used in this analysis were conducted in 1976-1981. Controlling for PM2.5, two-week average ozone concentration has a highly variable but statistically significant association with MRADs but not with RRADs. MRADs are days where people reduced their activity, but did not miss work, and can therefore be viewed as relatively minor and transient symptom days.

For our MRAD estimate, we initially reanalyzed on an individual year basis each of the six years of data from Ostro and Rothschild (1989) using their multi-pollutant model that included PM2.5. We then used an inverse variance-weighted meta-analysis to combine the six individual year results. This resulted in an estimate of a 0.112% change (95%CI 0.046 – 0.178%) per $\mu g/m^3$ of 1-hour maximum ozone. Conversion to ppb yielded an effect estimate of 2.24% change (95%CI = 0.92 – 3.56%) per 10 ppb change in 1-hour maximum ozone concentration. This estimate was applied to all adults above age 18.

Sensitivity Analyses

We also performed several additional analyses to evaluate the sensitivity of the results to our assumptions. In our first analysis, we considered two alternative ways to characterize ozone exposure and population. First, we estimated ozone concentration at the census-tract level. Specifically, we used population data from the year 2000 census and determined the population centroid for every census tract in the state. The assigned ozone concentration at each centroid was determined using the inverse square distance weighted interpolation of the ozone concentrations observed at the monitors within a 50-kilometer radius of the centroid. This value was then assigned to each resident in the census tract. Second, we averaged the observed concentrations at

the monitors within each county and assigned the county average concentration to the entire county population.

As a second sensitivity analysis, we imposed a threshold on all of the CR functions and accompanied this assumption with a re-estimated, higher CR function for the remaining data. Most of existing studies assume a non-threshold model, either linear or logistic, over the entire range of ozone concentrations. If one were to impose a threshold or no-effects level over the lower range of the data, the remaining slope estimate would have to increase to fit the remaining observations. Unfortunately, there is only limited data to suggest the magnitude of the increase in the slope. Specifically, several of the studies of emergency room visits for asthma estimated a slope for both the full range and for an upper portion of the data. Therefore, as a sensitivity analysis, we attempted to draw inference about how the slope would increase, drawing on both the direct and indirect evidence, described below.

Stieb et al. (1996) examined the effects of ozone on emergency department (ED) visits for asthma in Saint John, Canada. In the basic analysis, they report a beta coefficient for the full population of 0.0035 for a change of 1 ppb in 1-hr maximum ozone, using a lag of 0 and 1 day. When a dichotomous model was developed to examine the effect of concentrations above versus below 75 ppb, the beta increased to 0.45. Based on graphical and descriptive data presented in the paper, the mean concentrations above and below 75 ppb were assumed to be 95 and 35 ppb, a difference of 60. This results in a beta of 0.0076 and a ratio of the slope using the highest quartile, where effects are observed, versus the slope for the full range of data of approximately 2.16.Tolbert et al. (2000) examined the effects of ozone on pediatric ED visits in Atlanta. In the basic analysis, a relative risk (RR) of 1.042 was reported for a 20 ppb change in the 8-hour maximum daily ozone. This relates to a beta of 0.00206 ($\beta = \ln(1.042)/20$)) or converting to a 1-hr maximum using a ratio of 1.33, a beta of 0.0015. The authors also report an RR of 1.23 for concentrations above 100 ppb range versus low concentrations (< 50 ppb) of ozone. Assuming the mean for concentrations above 100 ppb was 105 ppb and the mean concentrations for values below 50 ppb was 40 ppb, the resulting beta coefficient is 0.00318 ($\beta = \ln(1.23/(105-40))$) for an 8-hour change in ozone or 0.0024 for a 1-hour change which is 1.6 times the slope using all of the ozone data. Finally, Romieu et al. (1995) studied ozone and pediatric ED visits in Mexico City. The authors report an RR of 1.43 for a 50 ppb change in 1-hour maximum ozone, using a one-day lag. This relates to a beta of 0.00715 for a 1-hour change in ozone. However, when they examined multiple days with high peaks greater than 110 ppb, the RR increased to 1.68 for a cumulative lag of 0 and 1 and to a RR of 2.33 for a cumulative lag of 1 and 2 days. Based on personal communication with the authors, the mean concentration for days below 110 ppb was 67 ppb versus a mean for days above 110 ppb of 127 ppb. Thus, the resultant betas become 0.0086 ($\beta = \ln(1.68)/60$)) and 0.0141 ($\beta = \ln(2.33)/60$)), respectively. This suggests a ratio of the slope based on data above a threshold relative to the slope for the full data of between 1.21 and 1.97. Overall, the empirical evidence confirms the logical expectation that the slope for only the upper end of the distribution of concentrations will be much larger than that for the entire distribution. The existing evidence, however, involves different cutpoints for the higher end and different

averaging times, which clearly will affect the ultimate slope. However, given these results, it appears that for a sensitivity analysis, an increase of 40% in the slope above a threshold of 60 ppb (8-hour average) is a reasonable approximation. We also examined a presumed threshold of 50 ppb (8-hour average) using a slope increase of 100%. As additional sensitivity analysis, we determined, assuming a 40% increase in the slope in the upper segment of the data, what the threshold concentration would have to be to generate effects similar to those from a non-threshold model. Finally, we determined what the increase in the slope would have to be in the upper segment, given a threshold of 70 ppb 8-hour average, to generate effects similar to a non-threshold model.

Note, however, that these presumed threshold values are well within the range of concentrations observed in most, if not all, of the original epidemiologic studies. In fact, these values are often in the upper end of the range of values, rendering this assumption somewhat unlikely. Nevertheless, it is of interest to examine the effects of such an assumption.

Health Effects Results

Table B-3 presents the estimated statewide annual health impacts of current (2001-2003) levels of ozone, compared to attainment of the Federal 8-hour standard of 0.08 ppm. For most of the endpoints, the 95% confidence intervals around each central estimate reflects the uncertainty associated with the beta coefficient derived from the epidemiological studies used in the calculation. For mortality, the uncertainty was based on the range of estimates generated from several meta-analyses. For example, the results indicate that the impact of not attaining the federal 8-hour standard of 0.08 ppm statewide is 360 cases of premature mortality (probable range = 180 – 550), 2,400 hospital admissions (95% CI = 1,400 – 3,500), 380 emergency room visits for asthma (95% CI = 230-530), 2.5 million days of illness-related school loss (95% CI = 690,000 – 4,200,000), and 1.8 million (95% CI = 730,000 – 2,800,000) minor restricted activity days per year. Since the results for premature mortality due to short-term exposures were derived from evidence from several papers, rather than combining the results into a confidence interval, we use the terminology of “probable range,” rather than 95% confidence interval.

Table B-4 presents the estimated annual statewide health impacts of current (2001-2003) levels of ozone, compared to attainment of the State 1-hour standard of 0.09 ppm. Again, for most of the endpoints, the 95% confidence intervals around each central estimate reflect the uncertainty associated with the beta coefficient derived from the epidemiological studies used in the calculation. As discussed above, for mortality, the uncertainty was based on the range of estimates generated from several meta-analyses. The results indicate that the current impact of ozone compared to attainment of the proposed 1-hour standard of 0.09 ppm statewide is 540 cases of premature mortality (probable range = 270 – 810), 3,600 hospital admissions (95% CI = 2,000 – 5,000), 560 emergency room visits for asthma (95% CI = 340-790), 3.8 million days of illness-related school loss (95% CI = 1,040,000 – 6,900,000), and 2.6 million (95% CI = 1,100,000 – 4,200,000) minor restricted activity days per year.

Similar to Table B-4, Table B-5 presents the impact of current ozone levels compared to attainment of the proposed State 8-hour standard of 0.070 ppm. Generally speaking, the health impacts of not attaining the 8-hour standard are greater than those associated with not attaining the 1-hour standard. For example, the results indicate that the impact of current ozone levels, compared to full attainment of the proposed 8-hour standard statewide is 630 cases of premature mortality (probable range = 310 – 950), 4,200 hospital admissions (95% CI = 2,400 – 5,800), 660 emergency room visits for asthma (95% CI = 400-920), 4.7 million days of illness-related school loss (95% CI = 1,200,000 – 8,600,000), and 3.1 million (95% CI = 1,300,000 – 5,000,000) minor restricted activity days per year.

Tables B-6, B-7 and B-8 present estimates of the annual health impacts of not attaining the federal 8-hour, and the proposed State 1-hour and 8-hour standards, respectively, by air basin.

Incremental Impacts Discussion

The differences between the results in Tables B-3 (federal 8-hour standard), B-4 (State 1-hour standard) and B-5 (State 8-hour standard) are the "incremental" impacts of not attaining the State 1-hour and 8-hour standards, compared to the federal 8-hour ozone standard, respectively. However, it is more reasonable to consider attainment of the two state standards together, compared to current ozone levels, since it is unlikely that control strategies will be geared to first attain one standard and then the other. Therefore, the impacts are not separable and should not be treated as such. Nonetheless, the following discussion can be helpful in understanding the incremental impacts.

Comparing Tables B-3 and B-5, the current impact of not attaining the federal 8-hour standard is about 360 premature deaths, with an additional 270 deaths associated with not attaining the proposed State 8-hour standard, making the total estimated impact of not attaining both standards 630 premature deaths. Similarly, statewide about 2,400 hospital admissions annually are associated with nonattainment of the federal 8-hour standard, and an additional 1,800 hospitalizations are due to nonattainment of the proposed State 8-hour standard, making the total estimated impact of not attaining both the federal 8-hour and State 8-hour standards 4,200 hospital admissions. For ER visits, we estimate that 380 cases are associated with nonattainment of the federal 8-hour standard, and an additional 280 cases with nonattainment of the proposed State 8-hour standard, for a total of 660 cases associated annually with nonattainment of the proposed 8-hour standard. For school loss, 2.5 million days are estimated to be associated with the nonattainment of the federal 8-hour standard, and an additional 2.2 million days, a total of 4.7 million days, associated with nonattainment of the proposed State 8-hour standard. Lastly, we estimated that the impact of current ozone levels is about 1.8 million minor restricted activity days due to nonattainment of the federal 8-hour standard, and an additional 1.3 million, for a total of 3.1 million days, associated with nonattainment of the proposed State 8-hour standard.

A similar examination of Tables B-4 and B-5 reveals the incremental impacts of not attaining the proposed State 8-hour standard compared to the State 1-hour standard. The current impact of not attaining the State 1-hour standard is about 540 premature deaths, with an additional 90 deaths associated with not attaining the proposed 8-hour standard, making the total estimated impact of not attaining both the State 1-hour and 8-hour standards 630 premature deaths. Similarly, statewide about 3,600 hospital admissions annually are associated with nonattainment of the State 1-hour standard, and an additional 600 hospitalizations are due to nonattainment of the proposed 8-hour standard, making the total impact of not attaining both the State 1-hour and 8-hour standards 4,200 hospital admissions. For ER visits, we estimate that 560 cases are associated with nonattainment of the 1-hour standard, and an additional 100 cases with nonattainment of the proposed 8-hour standard, for a total of 660 cases associated annually with nonattainment of the proposed 8-hour standard. For school loss, 3.8 million days are estimated to be associated with the nonattainment of the State 1-hour standard, and an additional 900,000 days, a total of 4.7 million days, associated with nonattainment of the proposed 8-hour standard. Lastly, we estimated that the impact of current ozone levels is about 2.6 million minor restricted activity days due to nonattainment of the 1-hour standard, and an additional 500,000, for a total of 3.1 million days, associated with nonattainment of the proposed 8-hour standard.

Figure B-2: Incremental Impacts of Ozone Exposures Compared to Attainment of Ozone Standards for Premature Deaths and School Absences (Annual statewide cases avoided with attainment of ozone standards. Details are given in the text.)

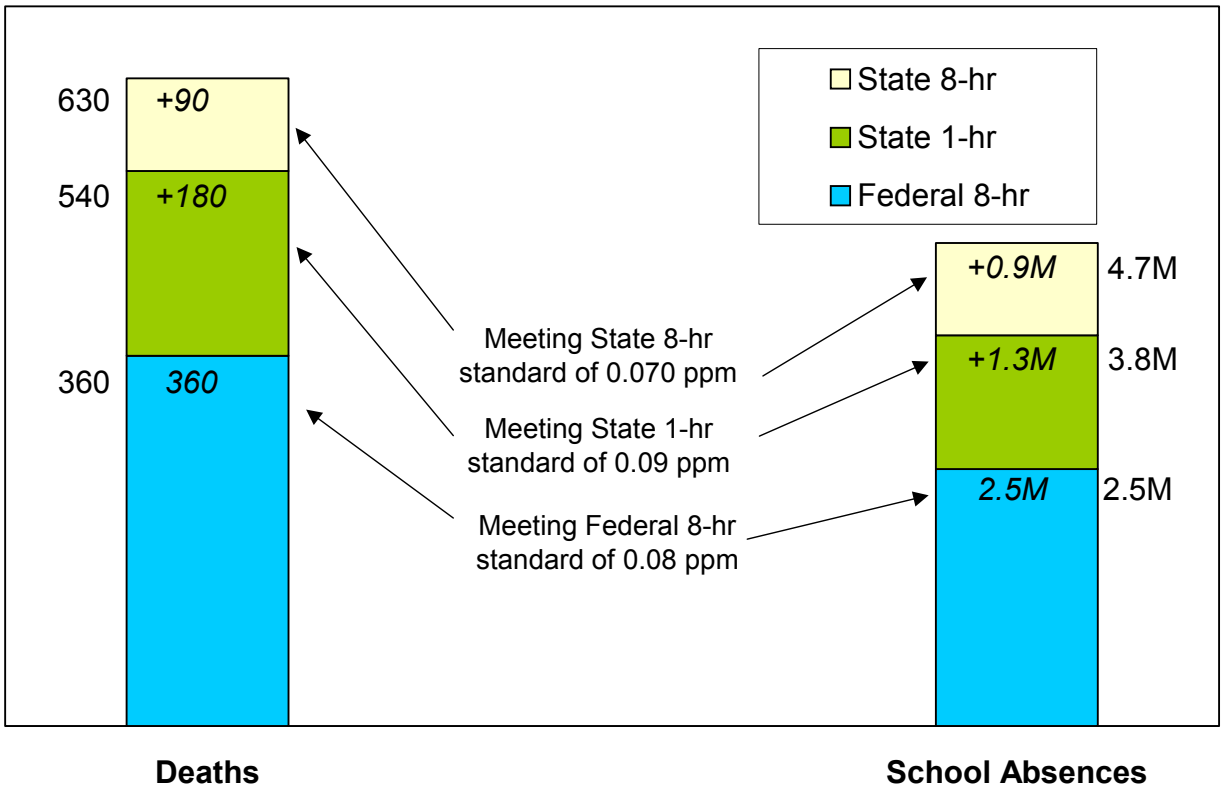


Figure B-2 summarizes the results of the above incremental impacts discussion for two endpoints: premature death and school absences. The numbers within each stacked bar represent the incremental impacts analysis. For premature death, attainment of the federal 8-hour standard would avoid 360 deaths annually in California. An additional 180 deaths, as indicated inside the stacked bar, would be avoided with attainment of the State 1-hour standard, and another 90 deaths would be avoided with attainment of the proposed State 8-hour standard, for a total of 630 deaths avoided annually at full attainment, as indicated on the side next to the bar. For illness-related school absences, the incremental analysis estimates annual avoidance of about 2.5 million school absences with attainment of the federal 8-hour standard, an additional 1.3 million avoided school absences with attainment of the current State 1-hour standard, and another 900,000 (indicated by +0.9M inside the stacked bar) with attainment of the proposed 8-hour standard, for a total of 4.7 million fewer school absences.

Sensitivity Analysis Results

We performed several sensitivity analyses to investigate two key assumptions in our analysis. Here, we discuss results on both the State 8-hour standard and the 1-hour

standard. Our first sensitivity analysis examined the implications of alternative exposure assessments. In the first reassessment, we interpolated concentrations for each census tract using nearby monitoring data. The exposure of the population within each census tract was determined using the inverse square distance weighted interpolation of the ozone concentrations observed at the monitors within a 50-kilometer radius of the centroid. The results for mortality are similar to those obtained using the base-case approach. Not attaining the proposed California 8-hour ozone standard using census-tract interpolations led to an estimated current impact of 610 deaths compared to 630 deaths using our base-case approach. Not attaining the State 1-hour ozone standard using census-tract interpolations lead to an estimated current impact of 530 deaths compared to 540 deaths using our base-case approach. In the second reassessment of exposure, we assigned residents to their county-wide average ozone concentrations. Similar impacts of about 680 deaths was obtained for the proposed 8-hour standard and 580 deaths was obtained for the State 1-hour standard.

In our second sensitivity analysis, we examined the implications of assuming alternative threshold models. If we assumed a threshold of 60 ppb and a 40% increase of the slope of the remaining higher concentrations, it resulted in about 10% to 14% decrease in estimated health impacts. For example, the estimated mortality impact would decrease from 630 to 540 comparing current ozone levels with attainment of the proposed 8-hour standard, and from 540 to 490 comparing current ozone levels with attainment of the 1-hour standard. The breakeven point associated with a 40% increase in the slope would be about 55 ppb for both the 1-hour and 8-hour standards. In other words, if the slope at the higher end of exposure was 40% greater than the slope for the full range of exposures, we would obtain the same impact estimate as in the base case, if the higher slope estimate was applied to concentrations greater than 55 ppb. For an assumed threshold of 70 ppb, the slope would have to increase by about 150% for the 8-hour standard or about 130% for the 1-hour standard to obtain about the same impact as in the base case, non-threshold model. If we assumed a threshold at 50 ppb with a 100% increase in the remaining slope, the estimated impact would increase by about 70% for the 8-hour standard and about 75% for the 1-hour standard.

Uncertainties and Limitations

There are a number of uncertainties involved in quantitatively estimating the impacts on health associated with outdoor ozone air pollution. Over time, some of these will be reduced as new research is conducted. However, some uncertainty will remain in any estimate. Below, we briefly discuss some of the major uncertainties and limitations of these estimated health impacts. These issues are discussed in more detail in Chapter 10 (also see Levy et al., 2001; Thurston and Ito, 1999).

Developing concentration-response functions

A primary uncertainty is the choice of the specific studies and concentration-response functions used in the quantification. Several challenges and unresolved issues present themselves with respect to designing and interpreting time-series studies of ozone-related health effects. The principal challenge facing the analyst is to remove

bias due to confounding by short-term temporal factors operating over time scales from days to seasons. The correlation of ozone with these confounding terms tends to be higher than that for PM or other gaseous pollutants. Thus, model specifications that may be appropriate for PM, the primary focus of much of the available literature, may not necessarily be adequate for ozone. Few studies to date have thoroughly investigated these potential effects with reference to ozone, introducing an element of uncertainty into the analysis.

Of particular importance is the strong seasonal cycle for ozone, high in summer and low in winter, opposite to the usual cycle in daily mortality and morbidity, which is typically high in winter and low in summer. Inadequate control for seasonal patterns in time series analyses leads to biased effect estimates. In the case of ozone, inadequate seasonal pattern control generally yields statistically significant inverse associations between ozone and health outcomes. In contrast, for winter-peaking pollutants such as CO and NO_2, the bias is toward overly positive effect estimates. Also, temporal cycles in daily hospital admissions or emergency room visits are often considerably more episodic and variable than is usually the case for daily mortality. As a result, smoothing functions that have been developed and tuned for analyses of daily mortality data may not work as well at removing cyclic patterns from morbidity analyses.

Potential confounding by daily variations in co-pollutants and weather is another analytical issue to be considered. With respect to co-pollutants, daily variations in ozone tends not to correlate highly with most other criteria pollutants (e.g., CO, NO_2, SO_2, PM10), but may be more correlated with secondary fine particulate matter (e.g., PM2.5) measured during the summer months. Assessing the independent health effects of two pollutants that are somewhat correlated over time is problematic. However, much can be learned from the classic approach of first estimating the effects of each pollutant individually, and then estimating their effects in a two-pollutant model. For this reason, we have emphasized use of studies that have also controlled for PM.

The choice of the studies and concentration-response functions used for health impact assessment can affect the impact estimates. Because of differences, likely related to study location, subject population, study size and duration, and analytical methods, effect estimates differ somewhat between studies. We have addressed this issue by emphasizing meta-analyses and multi-city studies, and also by presenting estimates derived from several studies.

To a substantial degree, the growing literature on acute ozone effects is an artifact of interest in studying acute PM effects. For example, of the 84 time-series mortality studies published between 1995 and mid-2004, 35 studies examined PM but not ozone; 47 studies examined both PM and ozone; and only 2 studies examined ozone but not PM. In many of the multi-pollutant studies, ozone is treated primarily as a potential confounder of the PM effects under study. As a result, many of these studies lack specific hypotheses regarding mortality effects of ozone, and fail to provide the range and depth of analyses, including sensitivity analyses, that would be most useful in judging whether ozone is an independent risk factor for acute mortality. This is in

contrast to morbidity studies where hypotheses regarding ozone effects on respiratory symptoms, lung function, hospitalization and ER visits, etc. have been studied with ozone treated as a key pollutant. Fortunately, studies of short-term exposure and mortality have been replicated in many cities throughout the world, under a wide range of exposure conditions, climates and covarying pollutants. As a result, the evidence of an effect of ozone on premature mortality is compelling. Nevertheless, uncertainty remains about the actual magnitude of the effect and the appropriate confidence interval.

Thresholds

A second major uncertainty relates to the general shape of the concentration-response function and the existence of a threshold. This is discussed in detail earlier, with the conclusion that there is little evidence for a threshold. An important consideration in determining if a safe level of ozone can be identified is whether the CR relationship is linear across the full concentration range or instead shows evidence of a threshold. Among the ozone epidemiology literature, only a few studies of hospital admissions and emergency room visits have examined the shape of the CR function. These studies also provide the only epidemiologic investigations into whether or not there is an ozone effect threshold. Since only a few studies have investigated whether there is an effect threshold, and the few studies available do not cover all endpoints, the epidemiologic literature does not provide a basis for concluding whether or not there is a population level effect threshold. However, many of the available studies were conducted at fairly low concentrations of ambient ozone, so we are never extrapolating beyond the range of the studies. Therefore, for this analysis, we have assumed that there is no threshold for ozone effects and we estimated impacts down to an assumed background concentration of 0.04 ppm. To the extent that there may not be health effects below the proposed ozone standard, the analysis may overestimate the impacts of ambient ozone. However, we also conducted a sensitivity analysis assuming several different possible thresholds. In doing so, we also adjusted the slope of the upper segment of the ozone concentrations to conform with the implications of a threshold model. If we had assumed zero impacts accrue below the proposed standards and provided no adjustment to the concentration-response functions, our estimates would be reduced by about 80%.

A related issue is that limited data suggest that ozone effects may be seasonal. While analysis of year round data suggests positive associations between a number of endpoints and ozone exposure, some data sets that have been analyzed seasonally report positive RR estimates for summer and negative RR estimates for winter. The cause of this phenomenon has not been adequately investigated, but may be related to thresholds, differences in personal exposure between seasons, or to co-pollutant exposures. In light of this uncertainty, this analysis used year-round effect estimates, although the relatively long, warm season in California may make the summer estimates more relevant than those of the winter season.

Assumptions about rollback

A further uncertainty concerns the process used to design and implement strategies for controlling ozone-producing compounds. Such control strategies have been designed with the objective of reducing ozone episodes during worst-case meteorological conditions. In addition, basin-wide strategies have focused on the ozone concentrations at the highest (design) site in each basin. How these strategies would affect other sites during dissimilar episodes cannot be answered with certainty. Site-by-site analyses almost always have found that trends for multiple sites within a basin are very similar to each other. Similarly, monthly trends within a basin have usually proved to be similar, while the prevalence of different episode types may be markedly different for different months during the overall ozone season. (See trend analysis in the Supplement).

Unquantified adverse effects

An additional limitation in this analysis is the inability to quantify all possible impacts that are associated with current ozone concentrations, since estimates are provided for only a subset of possible adverse outcomes. For example, estimates of the effects of ozone on asthma exacerbation, asthma induction, respiratory symptoms, airway inflammation, and acute and long-term changes in lung function are not presented. Although there is some evidence for such effects, the available data were either too inconsistent or sparse to justify quantification of possible impacts of not achieving the proposed ozone standards, or the evidence comes from controlled exposure studies that can not be used to make population level effects estimates. To the extent that certain important health outcomes were excluded, we may have underestimated the health impacts of the proposed standards.

Baseline rates of mortality and morbidity

There is also uncertainty in the baseline rates for the investigated health outcomes in the studied population. Often, one must assume a baseline incidence level for the city or country of interest. In addition, incidence can change over time as health habits, income and other factors change.

Exposure assessment

There are likely uncertainties in the statewide exposure assessment, and in whether the existing monitoring network provides representative estimates of exposure for the general population. We have attempted to reproduce the same relationship between monitor readings and exposure as in the original epidemiological studies. Most of these studies use population-oriented, background, fixed site monitors, often aggregated to the county level. The available epidemiological studies have used multiple pollutant averaging times, and we have proposed conversion ratios for 1-hour to 8-hour and 24-hour ozone concentrations based on national estimates. A preliminary examination of the California monitoring data indicates that the ratios are similar to those found in the highly populated areas of the State. However, uncertainty is added to the estimated

impacts of not attaining the proposed standards to the extent the converted concentration bases differ from monitored concentrations.

Summary

The purpose of this appendix is to provide quantitative estimates of some of the health impacts of current levels of ozone, compared to those that would occur under a hypothetical control strategy that brings the State into attainment with the proposed ozone standards. This assessment should not be regarded as exhaustive, since we have provided estimates only for a subset of health effects endpoints. However, the results presented support the conclusion that significant public health impacts are associated with current ozone concentrations in California that would not occur with attainment of the proposed ozone standards. It is estimated that attainment of the proposed ozone standards throughout California would significantly reduce the number of ozone-related adverse health effects each year. Specifically, we estimate that the current impact of exposures above the proposed standard is:

- 630 (310 – 950, probable range) premature deaths for all ages.
- 4,200 (2,400 – 5,800, 95% confidence interval (CI)) hospitalizations due to respiratory diseases for all ages.
- 660 (400 – 920, 95% CI) emergency room visits for asthma for children under 18 years of age.
- 4.7 million (1,200,000 – 8,600,000, 95% CI) illness-related school absences for children 5 to 17 years of age.
- 3.1 million (1.3 million – 5.0 million, 95% CI) minor restricted activity days for adults above 18 years of age.

These estimates are based on attainment of the proposed State 8-hour standard, as it is the more stringent of the two (1-hour and 8-hour) proposed standards. The reader is cautioned that since 1-hour and 8-hour concentrations are highly correlated, it is <u>not</u> appropriate to add the estimated ~~m Tables B-2 and B-3 together.~~ impacts from Tables B-3, B-4 and B-5 together. Instead, the estimates above represent the total impacts estimated to accrue from attaining both the proposed 8-hour and 1-hour standards. For a discussion of the incremental impacts of current ozone levels compared to attainment of the proposed 8-hour standard over the State 1-hour standard and the federal 8-hour standard, see page B-19.

As noted above, there are several important assumptions and uncertainties in this analysis. Some concern the study design, the statistical modeling methodologies used, and the selection of studies from which the CR functions are derived. Few studies have investigated the shape of the CR function, or whether there is a population response threshold for health endpoints other than emergency room visits for asthma. Further, but likely small, uncertainty is added by assumptions in the statewide exposure assessment. Nonetheless, when new evidence on mortality from short-term exposures to ozone is published from the recent meta-analyses sponsored by the US EPA, we will

update our estimates and use the census-tract interpolation to characterize ambient ozone exposure. It should also be noted that since several health effects related to acute and chronic ozone exposures are not included in the estimates, the health impacts associated with lowering ozone exposure are likely underestimated.

Table B-2: Summary of Meta-Analyses Linking Daily Ozone to Mortality (for 10 ppb change in 24-hour average ozone)

Study Number	Author	# of studies	% Change in Mortality (95% CI)	comment
1	Anderson (2004)	15	1.13 (0.38 - 1.51)	European studies only
2	Anderson (2004)	20	0.75 (0.19 – 1.32)	European studies corrected for possible publication bias
3	Thurston+Ito (2001)	7	1.37 (0.78 – 1.96)	Earlier studies using non-linear specification for temperature
4	Thurston+Ito (2001)	19	0.89 (0.56 – 1.22)	All earlier studies
5	Stieb et al. (2003)	109	1.12 (0.32 – 1.92)	Meta-analysis including single and mult-pollutant models
6	Bell et. al. (2004)	95	0.25 (0.12 – 0.39)	NMMAPS, using lag(01)
7	Bell et. al. (2004)	95	0.52 (0.27 – 0.77)	NMMAPS,lag(06)
8	Levy et al. (2001)	4	0.98 (0.59 – 1.38)	Using relatively stringent inclusion criteria
9	Levy et al. (2001)	15	0.80 (0.60 – 1.00)	Using less stringent inclusion criteria
10	Gryparis et al. (2004)	23	0.5 (-0.38 – 1.30)	APEAH2 studies in Europe, all year
11	Gryparis et al. (2004)	23	1.65 (0.85 – 2.60)	APEAH2 studies in Europe, summer only

Table B-3: California Annual Health Impacts of Current Ozone Concentrations Compared to the Federal 8-hour Ozone Standard of 0.08 ppm*

Health Endpoint	Population	Estimated Beta** (% per 10 ppb 1-hour ozone) (95% Confidence Interval)	Incidence (cases/year) (95% Confidence Interval)
Premature Mortality due to Short-term Exposures	All ages	0.0040 (0.0020 - 0.0060)	360 (180 – 550) ***
Hospital Admissions for Respiratory Diseases	All ages	0.0164 (0.0095 - 0.0228)	2,400 (1,400 – 3,500)
Emergency Room Visits for Asthma	Age < 18	0.0237 (0.01446 – 0.0329)	380 (230 – 530)
School Loss Days	Age 5-17	0.2123 (0.06672 – 0.3295)	2,500,000 (690,000 – 4,200,000)
Minor Restricted Activity Days	Age > 18	0.0222 (0.0092 - 0.0350)	1,800,000 (730,000 – 2,800,000)

*Base period 2001-2003. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-3, B-4 and B-5 together. Due to rounding conventions in the process of determining attainment of the federal 8-hour standard, the concentration of 0.084 ppm was used since this is the highest value considered in attainment.

**As discussed in detail in the text, the evaluation of impacts of not attaining the 8-hour standard was based on the equivalent 1-hour concentration. Therefore, the beta coefficients here are in 1-hour scale.

***Results for premature mortality represent a probable range of likely values rather than a 95% confidence interval since the coefficients were derived from examining the evidence from several studies separately rather than combining their results in a formal meta-analysis.

Table B-4: California Annual Health Impacts of Current Ozone Concentrations Compared to the State 1-hour Ozone Standard of 0.09 ppm*

Health Endpoint	Population	Estimated Beta (% per 10 ppb 1-hour ozone) (95% Confidence Interval)	Incidence (cases/year) (95% Confidence Interval)
Premature Mortality due to Short-term Exposures	All ages	0.0040 (0.0020 - 0.0060	540 (270 – 810) **
Hospital Admissions for Respiratory Diseases	All ages	0.0164 (0.0095 - 0.0228)	3,600 (2,000 – 5,000)
Emergency Room Visits for Asthma	Age < 18	0.0237 (0.01446 – 0.0329)	560 (340 – 790)
School Loss Days	Age 5-17	0.2123 (0.06672 – 0.3295)	3,800,000 (1,040,000 – 6,900,000)
Minor Restricted Activity Days	Age > 18	0.0222 (0.0092 - 0.0350)	2,600,000 (1,100,000 – 4,200,000)

*Base period 2001-2003. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-3, B-4 and B-5 together. Due to rounding conventions in the process of determining attainment of the 1-hour standard, the concentration of 0.094 ppm was used since this is the highest value considered in attainment.

**Results for premature mortality represent a probable range of likely values rather than a 95% confidence interval since the coefficients were derived from examining the evidence from several studies separately rather than combining their results in a formal meta-analysis.

Table B-5: California Annual Health Impacts of Current Ozone Concentrations Compared to the State 8-hour Ozone Standard of 0.070 ppm*

Health Endpoint	Population	Estimated Beta** (% per 10 ppb 1-hour ozone) (95% Confidence Interval)	Incidence (cases/year) (95% Confidence Interval)
Premature Mortality due to Short-term Exposures	All ages	0.0040 (0.0020 - 0.0060)	630 (310 – 950) ***
Hospital Admissions for Respiratory Diseases	All ages	0.0164 (0.0095 - 0.0228)	4,200 (2,400 –5,800)
Emergency Room Visits for Asthma	Age < 18	0.0237 (0.01446 – 0.0329)	660 (400 – 920)
School Loss Days	Age 5-17	0.2123 (0.06672 – 0.3295)	4,700,000 (1,200,000 – 8,600,000)
Minor Restricted Activity Days	Age > 18	0.0222 (0.0092 - 0.0350)	3,100,000 (1,300,000 – 5,000,000)

*Base period 2001-2003. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-3, B-4 and B-5 together.

**As discussed in detail in the text, the evaluation of impacts of not attaining the 8-hour standard was based on the equivalent 1-hour concentration. Therefore, the beta coefficients here are in 1-hour scale.

***Results for premature mortality represent a probable range of likely values rather than a 95% confidence interval since the coefficients were derived from examining the evidence from several studies separately rather than combining their results in a formal meta analysis.

~~Table B-4: Annual Health Benefits from Attaining 1-hour Ozone Standard of Air Basin of 0.09 ppm~~ Table B-6: Annual Health Impacts of Current Ozone Concentrations Compared to the Federal 8-hour Ozone Standard of 0.08 ppm by Air Basin (cases/year).

Air Basin	Mortality	Hospital Admissions	Emergency Room Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	<1	<1	280	250
Lake County	0	0	0	0	0
Lake Tahoe	<1	<1	<1	450	360
Mountain Counties	7	36	5	33,000	29,000
Mojave Desert	26	180	29	190,000	130,000
North Coast	0	0	0	0	0
North Central Coast	0	0	0	0	0
Northeast Plateau	0	0	0	0	0
South Coast	220	1,500	230	1,400,000	1,100,000
South Central Coast	7	48	7	50,000	36,000
San Diego	11	71	10	67,000	55,000
San Francisco Bay	1	5	1	3,700	3,900
San Joaquin Valley	62	400	70	480,000	280,000
Salton Sea	15	86	14	105,000	62,000
Sacramento Valley	19	103	15	94,000	78,000
Statewide	360	2,400	380	2,500,000	1,800,000

Note: Some columns may not add up to the statewide totals due to rounding. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-6, B-7 and B-8 together. Table B-8 should be used to estimate the maximum health impact per air basin.

The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

Table B-7: Annual Health Impacts of Current Ozone Concentrations Compared to the State 1-hour Ozone Standard of 0.09 ppm by Air Basin (cases/year).

Air Basin	Mortality	Hospital Admissions	Emergency Room Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	<1	<1	300	260
Lake County	0	0	0	0	0
Lake Tahoe	<1	2	<1	1,900	1,400
Mountain Counties	9	48	6	45,000	38,000
Mojave Desert	40	270	45	310,000	200,000
North Coast	<1	<1	<1	0	0
North Central Coast	1	7	1	7,600	5,300
Northeast Plateau	0	0	0	0	0
South Coast	290	2,000	310	2,000,000	1,500,000
South Central Coast	14	97	14	106,000	73,000
San Diego	21	140	19	130,000	100,000
San Francisco Bay	20	1 40	18	110,000	110,000
San Joaquin Valley	89	570	100	740,000	410,000
Salton Sea	19	110	19	140,000	81,000
Sacramento Valley	39	200	29	200,000	150,000
Statewide	540	3,600	560	3,800,000	2,600,000

Note: Some columns may not add up to the statewide totals due to rounding. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-6, B-7 and B-8 together. Table B-8 should be used to estimate the maximum health impacts per air basin. The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

Table B-8: Annual Health Impacts of Current Ozone Concentrations Compared to the State 8-hour Ozone Standard of 0.070 ppm by Air Basin (cases/year).

Air Basin	Mortality	Hospital Admissions	Emergency Room Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	<1	<1	1,100	870
Lake County	0	0	0	49	48
Lake Tahoe	<1	8	<1	8,600	6,400
Mountain Counties	10	61	8	61,000	49,000
Mojave Desert	50	340	58	420,000	250,000
North Coast	<1	1	<1	1,400	990
North Central Coast	2	17	3	19,000	12,700
Northeast Plateau	0	0	0	270	270
South Coast	320	2,200	350	2,300,000	1,600,000
South Central Coast	19	130	19	150,000	97,000
San Diego	33	220	30	220,000	170,000
San Francisco Bay	21	140	19	120,000	110,000
San Joaquin Valley	100	650	110	880,000	460,000
Salton Sea	24	150	24	200,000	110,000
Sacramento Valley	45	250	37	250,000	190,000
Statewide	630	4,200	660	4,700,000	3,100,000

Note: Some columns may not add up to the statewide totals due to rounding. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated impacts from Tables B-6, B-7 and B-8 together. Table B-8 should be used to estimate the maximum health impacts per air basin. The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

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Supplement to Appendix B

Rollback Formulae

For the technical reader, the mathematical formulae for our rollback procedure follow. Denote:

OzCurrent = current daily ozone observed value,
BasinMax = design value based on three years of measured data,
BG = background ozone of 0.04 ppm,
Std = 0.094 ppm for 1-hour, or basin-specific equivalent 1-hour design value for 8-hour standard of 0.070 ppm, or basin-specific equivalent 1-hour design value for federal 8-hour standard of 0.084 ppm, and
OzAttain = rolled-back ozone value in the "attainment" scenario.

First, the reduction percentage (or reduction factor RF) was calculated for each basin as follows:

If BasinMax > Std, then RF = (BasinMax - Std) / (BasinMax – BG).
If BasinMax <= Std, then RF = 0.

The rollback factor, 1-RF, is applied as follows. For all sites within the basin, the portion of the site's current ozone levels above background was adjusted:

If OzCurrent > BG, then OzAttain = BG + (1 - RF) $\times$ (OzCurrent – BG).
If OzCurrent <= BG, then OzAttain = OzCurrent.

The change in ozone concentrations is OzCurrent – OzAttain, calculated at the daily level for each site, which is the difference between the observed value and the rolled-back value for each site on each day of the year.

Note that we used the actual levels of the standards, 0.09 and 0.070 ppm, in the rollback rather than the maximal values that round to the standards as is done with air quality modeling. Such modeling usually assumes worst-case meteorology, unlike our methodology of using the three-year high value.

Rollback Method Development

The assumption of a constant rollback factor applied to an entire air basin was justified through an empirical analysis of the trends in the percentiles at South Coast Air Basin monitoring sites. This air basin was selected for the analysis since the air quality trends were clear, there is a range of coastal and inland environments, and a majority of benefits are projected to occur in that air basin. Figures B-3 through B-12 and Tables B-9 through B-18 provide examples of the results from that analysis, and the materials are representative of the results used for development of the rollback factor applied in the benefits analysis. In the graphs, the dotted line indicates the ozone standard, and the dashed line represents the assumed background level. Due to space limitations, the

legend for every percentile line was not provided. However, the reader is advised to examine the solid lines in each graph, from top to bottom, to represent the maximum, 90th percentile, 80th percentile, 70th percentile, 60th percentile, 50th percentile, and 40th percentile of the annual distribution of ozone measurements.

Briefly, the analysis showed that since 1980, the trend in the monitored values associated with the distribution of percentiles was consistently downward, and that the relationships were relatively parallel and linear. Consequently, we assumed a constant rollback factor based on a basin's three-year high value, and applied it to all daily high values at all sites within the basin. In other words, when a control strategy is geared towards reducing the highest ozone levels in an air basin, its impact on days with low and moderate ozone levels is comparable to those days with high ozone levels.

Estimation of Exposed Population

To estimate the number of people exposed to the ozone changes observed at each monitoring site, the county population was divided by the number of monitoring sites in a given county. For example, suppose a county has N monitoring stations and population POP according to year 2000 census. Then we would estimate that (POP/N) persons were exposed to ozone levels at each of the N monitors within this county. The health incidences were then calculated based on the concentration-response functions relating changes in ozone concentrations and exposed population for each day at each monitor. The sensitivity of this methodology is discussed in detail on page B-16.

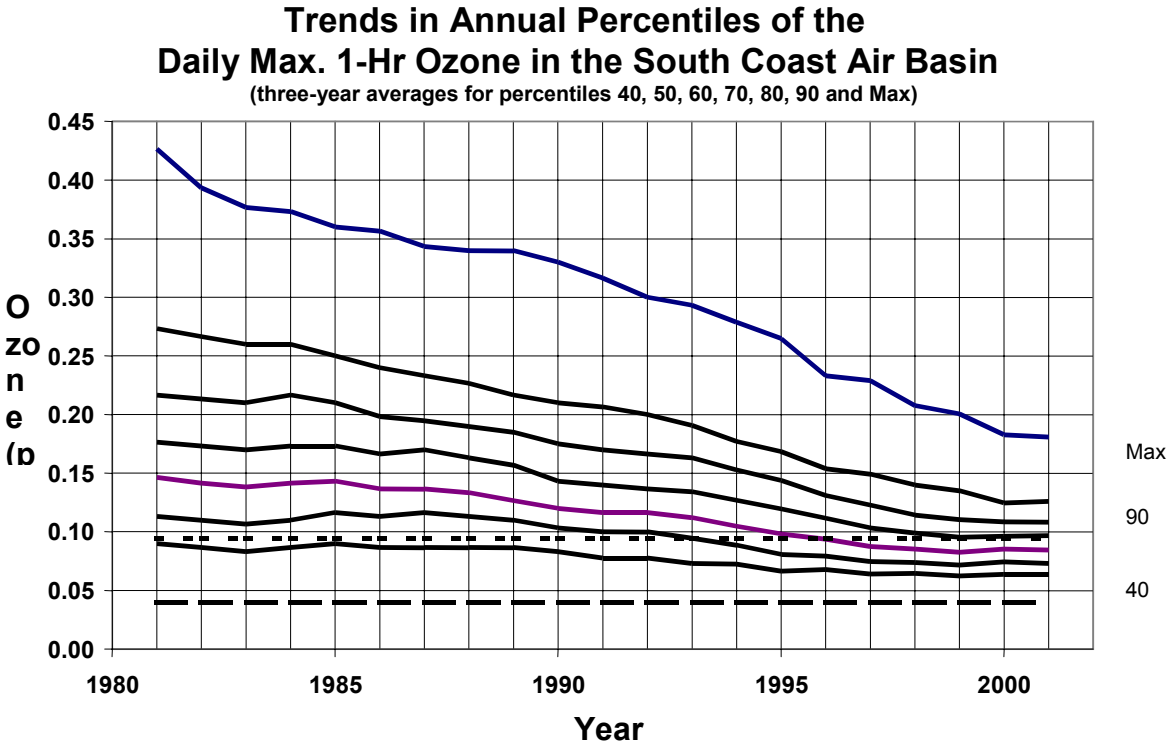

Figure B-3: Trends in Annual Percentiles of Daily Max 1-hour Ozone in the South Coast Air Basin

Table B-9: Summary of Trends in Annual Percentiles of the Daily Max. 1-Hr Ozone in the South Coast Air Basin

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.427**	**0.317**	**0.183**
Δ% above background		**28%**	**63%**
90th Percentile	**0.273**	**0.207**	**0.125**
Δ% above background		**29%**	**64%**
80th Percentile	**0.217**	**0.170**	**0.109**
Δ% above background		**26%**	**61%**
70th Percentile	**0.177**	**0.140**	**0.096**
Δ% above background		**27%**	**59%**
60th Percentile	**0.147**	**0.117**	**0.086**
Δ% above background		**28%**	**57%**
50th Percentile	**0.113**	**0.100**	**0.075**
Δ% above background		**18%**	**53%**
40th Percentile	**0.090**	**0.078**	**0.064**
Δ% above background		**24%**	**52%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

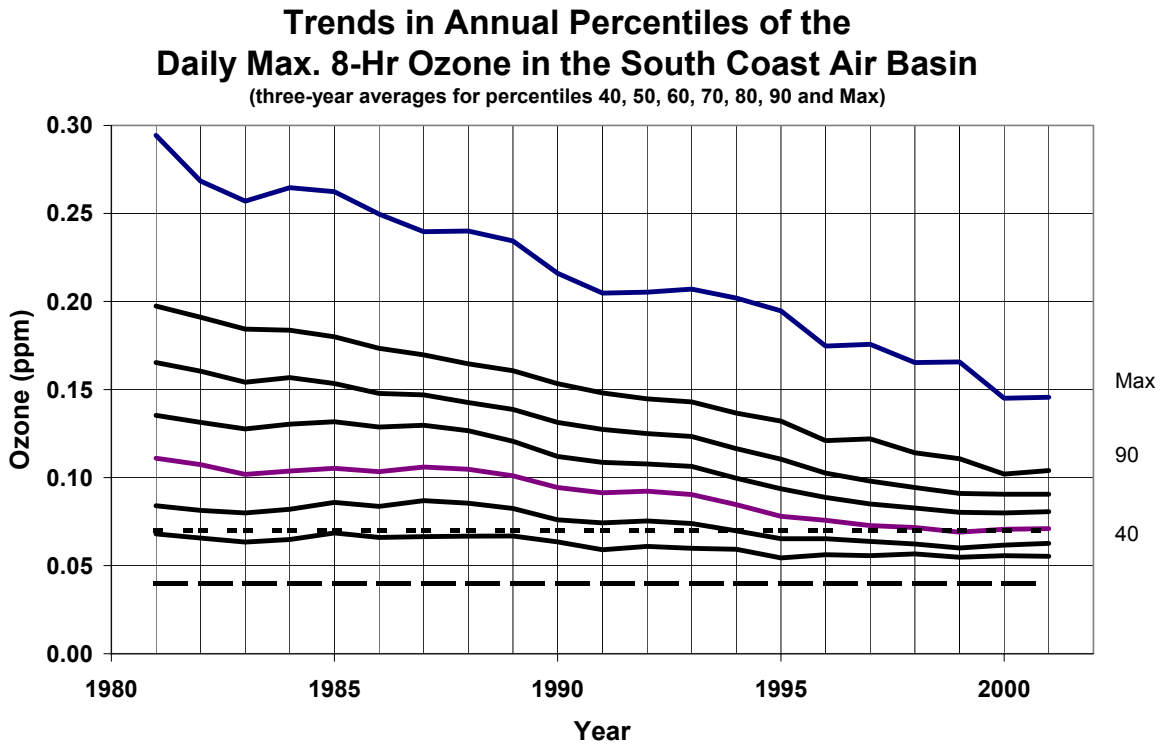

Figure B-4: Trends in Annual Percentiles of Daily Max 8-hour Ozone in the South Coast Air Basin

Table B-10: Summary of Trends in Annual Percentiles of the Daily Max. 8-hr Ozone in the South Coast Air Basin

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.294**	**0.205**	**0.145**
Δ% above background		**35%**	**59%**
90th Percentile	**0.197**	**0.148**	**0.102**
Δ% above background		**31%**	**61%**
80th Percentile	**0.165**	**0.127**	**0.091**
Δ% above background		**30%**	**60%**
70th Percentile	**0.135**	**0.109**	**0.080**
Δ% above background		**28%**	**58%**
60th Percentile	**0.111**	**0.091**	**0.071**
Δ% above background		**28%**	**57%**
50th Percentile	**0.084**	**0.074**	**0.062**
Δ% above background		**22%**	**51%**
40th Percentile	**0.068**	**0.059**	**0.056**
Δ% above background		**32%**	**44%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

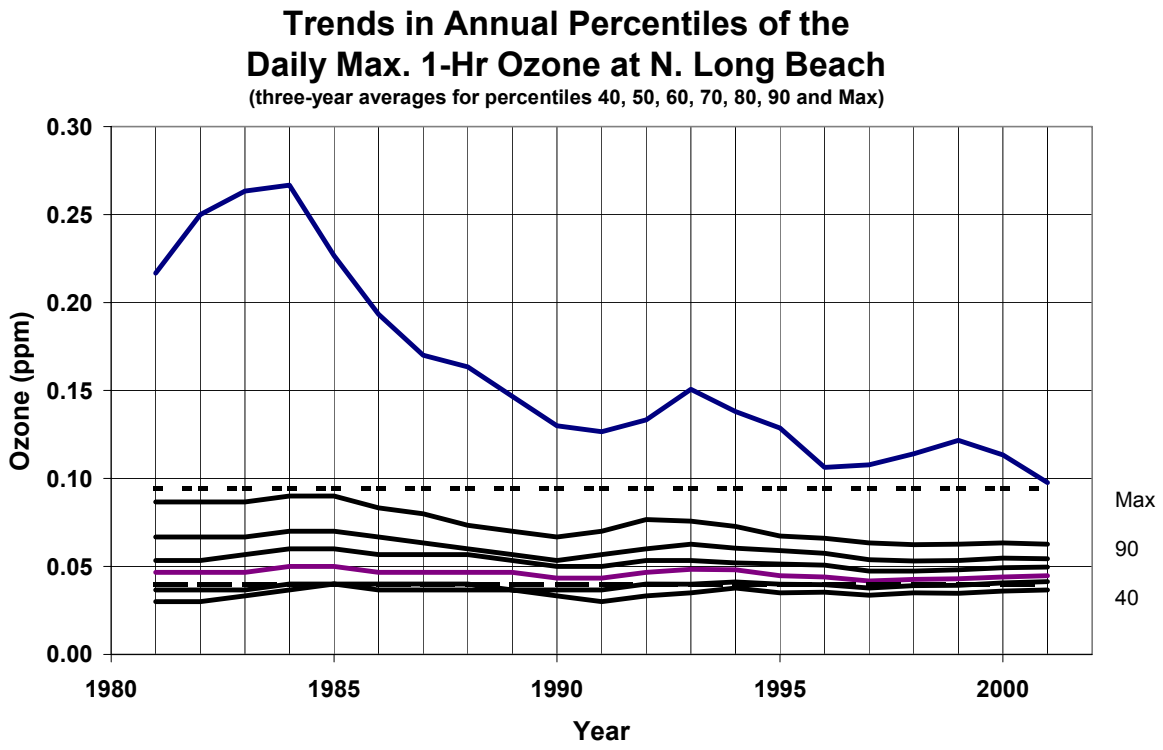

Figure B-5: Trends in Annual Percentiles of Daily Max 1-hour Ozone at N. Long Beach

Table B-11: Summary of Trends in Annual Percentiles of the Daily Max 1-hour Ozone at N. Long Beach

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.217**	**0.127**	**0.113**
Δ% above background		**51%**	**58%**
90th Percentile	**0.087**	**0.070**	**0.063**
Δ% above background		**36%**	**50%**
80th Percentile	**0.067**	**0.057**	**0.055**
Δ% above background		**38%**	**45%**
70th Percentile	**0.053**	**0.050**	**0.049**
Δ% above background		**25%**	**30%**
60th Percentile	**0.047**	**0.043**	**0.044**
Δ% above background		**50%**	**40%**
50th Percentile	**0.037**	**0.037**	**0.041**
Δ% above background		**Percentiles are below background.**	
40th Percentile	**0.030**	**0.030**	**0.036**
Δ% above background		**Percentiles are below background.**	

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

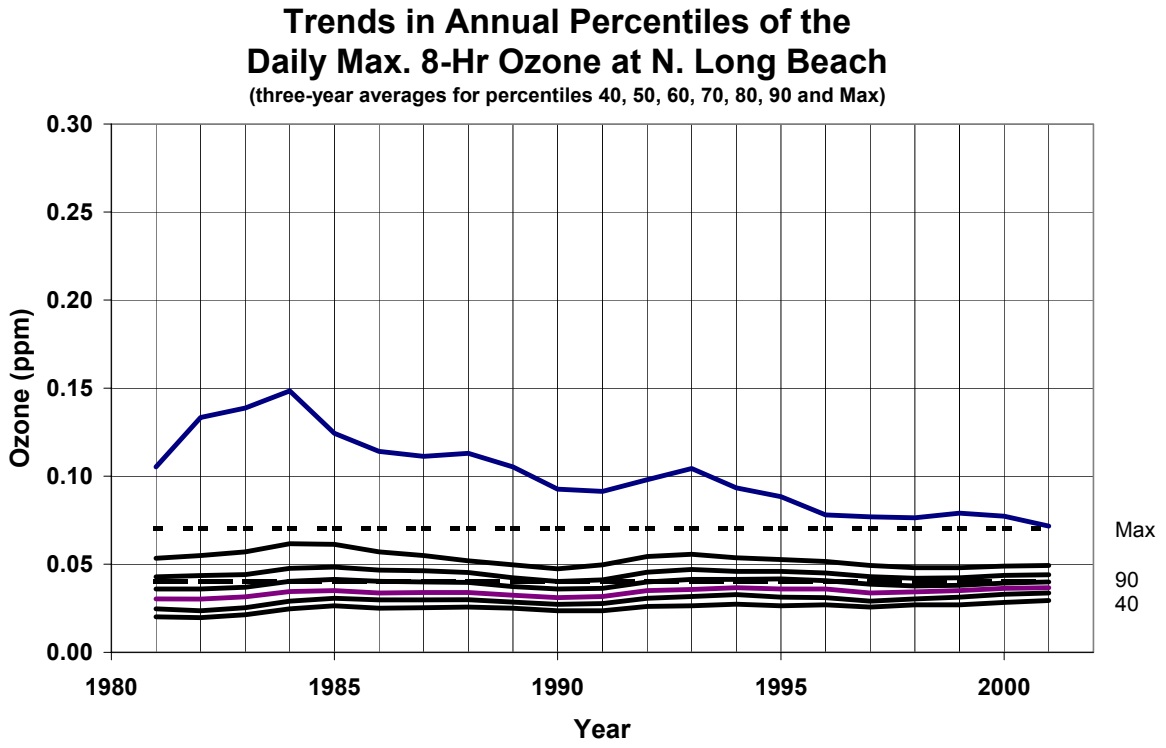

Figure B-6: Trends in annual percentiles of daily max 8-hour ozone at N. Long Beach

Table B-12: Summary of Trends in Annual Percentiles of the Daily Max 8-hour Ozone at N. Long Beach

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.105**	**0.091**	**0.077**
Δ% above background		**21%**	**43%**
90th Percentile	**0.053**	**0.050**	**0.049**
Δ% above background		**28%**	**33%**
80th Percentile	**0.043**	**0.041**	**0.044**
Δ% above background		**59%**	**-29%**
70th Percentile	**0.036**	**0.036**	**0.039**
Δ% above background		**Percentiles are below background.**	
60th Percentile	**0.030**	**0.032**	**0.036**
Δ% above background		**Percentiles are below background.**	
50th Percentile	**0.025**	**0.028**	**0.033**
Δ% above background		**Percentiles are below background.**	
40th Percentile	**0.020**	**0.024**	**0.028**
Δ% above background		**Percentiles are below background.**	

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

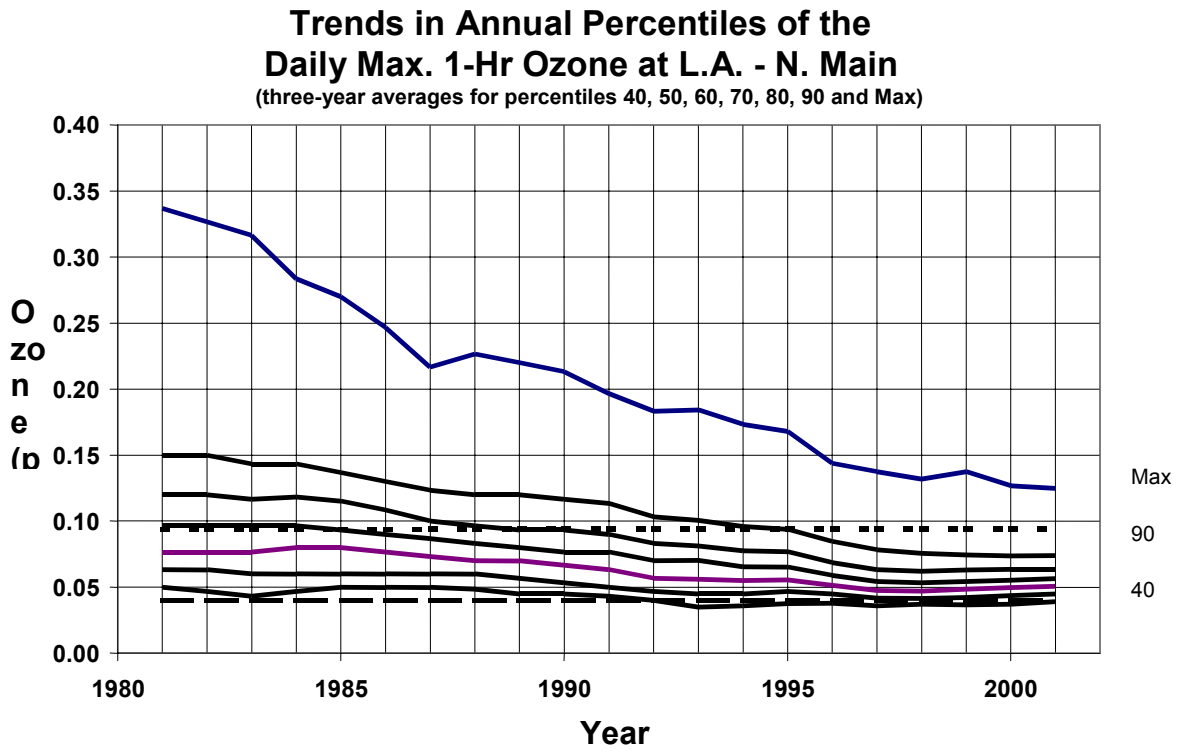

Figure B-7: Trends in annual percentiles of daily max 1-hour ozone L.A. – N. Main

Table B-13: Summary of Trends in Annual Percentiles of the Daily Max 1-hour Ozone at L.A. - N. Main

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.337**	**0.197**	**0.127**
Δ% above background		**47%**	**71%**
90th Percentile	**0.150**	**0.113**	**0.074**
Δ% above background		**33%**	**69%**
80th Percentile	**0.120**	**0.090**	**0.064**
Δ% above background		**38%**	**70%**
70th Percentile	**0.097**	**0.077**	**0.055**
Δ% above background		**35%**	**73%**
60th Percentile	**0.077**	**0.063**	**0.050**
Δ% above background		**36%**	**74%**
50th Percentile	**0.063**	**0.050**	**0.044**
Δ% above background		**57%**	**84%**
40th Percentile	**0.050**	**0.043**	**0.037**
Δ% above background		**67%**	**100%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

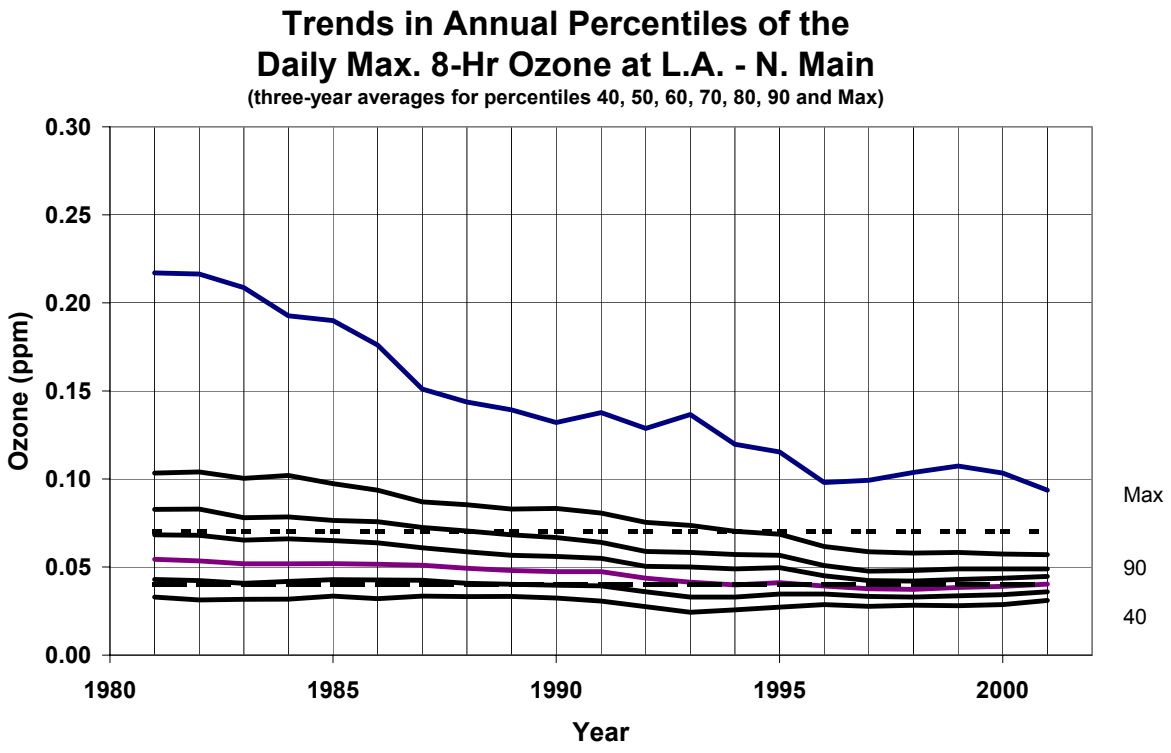

Figure B-8: Trends in annual percentiles of daily max 8-hour ozone at L.A.-N. Main

Table B-14: Summary of Trends in Annual Percentiles of the Daily Max 8-hour Ozone at L.A. - N. Main

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.217**	**0.138**	**0.103**
Δ% above background		**45%**	**64%**
90th Percentile	**0.103**	**0.081**	**0.057**
Δ% above background		**36%**	**73%**
80th Percentile	**0.083**	**0.064**	**0.049**
Δ% above background		**44%**	**79%**
70th Percentile	**0.068**	**0.055**	**0.044**
Δ% above background		**47%**	**87%**
60th Percentile	**0.054**	**0.047**	**0.039**
Δ% above background		**49%**	**100%**
50th Percentile	**0.043**	**0.039**	**0.034**
Δ% above background		**100%**	**100%**
40th Percentile	**0.033**	**0.031**	**0.029**
Δ% above background		**Percentiles are below background.**	

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

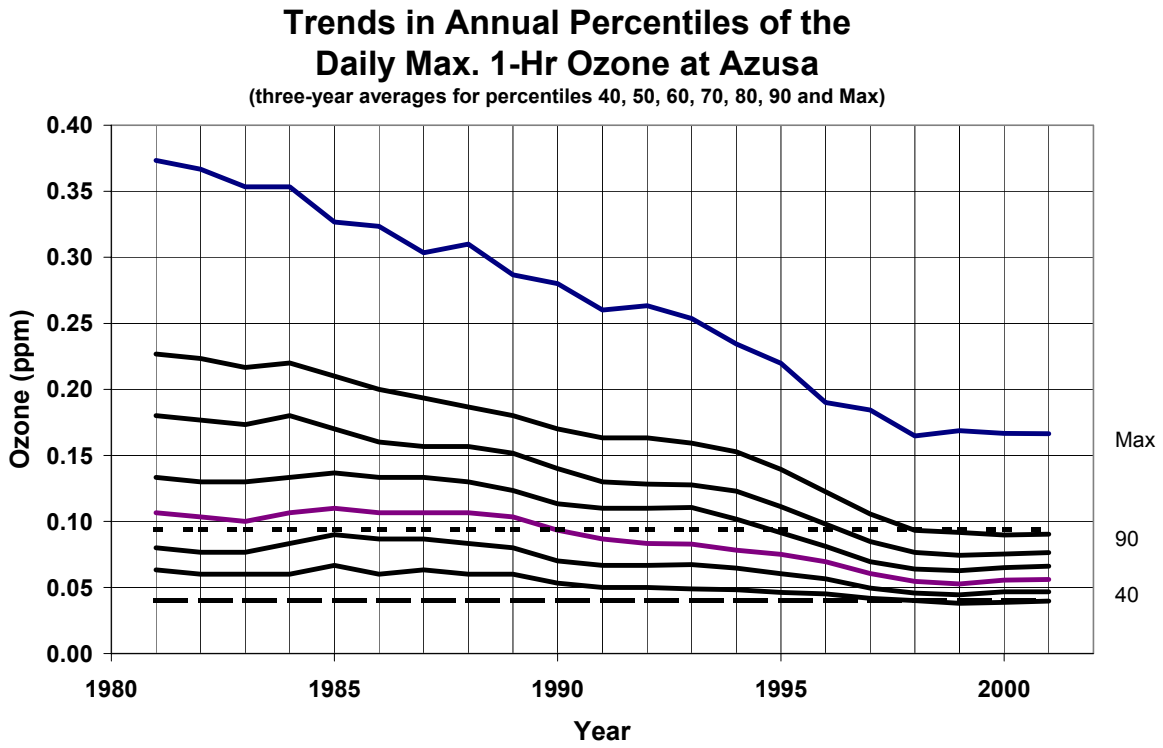

Figure B-9: Trends in annual percentiles of daily max 1-hour ozone at Azusa

Table B-15: Summary of Trends in Annual Percentiles of the Daily Max 1-hour Ozone at Azusa

Summary of Trends in Annual Percentiles of the Daily Max. 1-Hr Ozone at Azusa			
	Average Value During Period		
Indicator	1980-1982	1990-1992	2000-2002
Maximum	0.373	0.260	0.167
Δ% above background		34%	62%
90th Percentile	0.227	0.163	0.090
Δ% above background		34%	73%
80th Percentile	0.180	0.130	0.075
Δ% above background		36%	75%
70th Percentile	0.133	0.110	0.065
Δ% above background		25%	73%
60th Percentile	0.107	0.087	0.056
Δ% above background		30%	77%
50th Percentile	0.080	0.067	0.047
Δ% above background		33%	83%
40th Percentile	0.063	0.050	0.039
Δ% above background		57%	100%

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

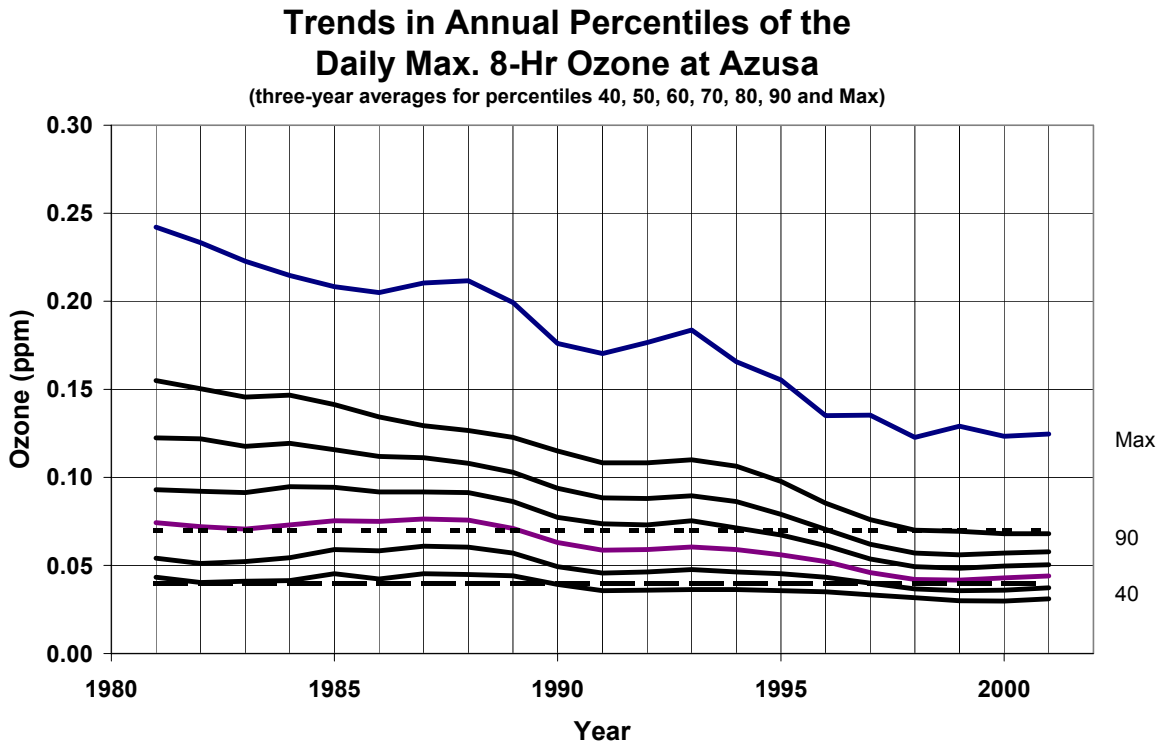

Figure B-10: Trends in annual percentiles of daily max 8-hour ozone at Azusa

Table B-16: Summary of Trends in Annual Percentiles of the Daily Max 8-hour Ozone at Azusa

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.242**	**0.170**	**0.123**
Δ% above background		**35%**	**59%**
90th Percentile	**0.155**	**0.108**	**0.068**
Δ% above background		**41%**	**76%**
80th Percentile	**0.123**	**0.088**	**0.057**
Δ% above background		**41%**	**79%**
70th Percentile	**0.093**	**0.074**	**0.050**
Δ% above background		**36%**	**82%**
60th Percentile	**0.074**	**0.059**	**0.043**
Δ% above background		**46%**	**100%**
50th Percentile	**0.054**	**0.046**	**0.036**
Δ% above background		**60%**	**100%**
40th Percentile	**0.043**	**0.036**	**0.030**
Δ% above background		**100%**	**100%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

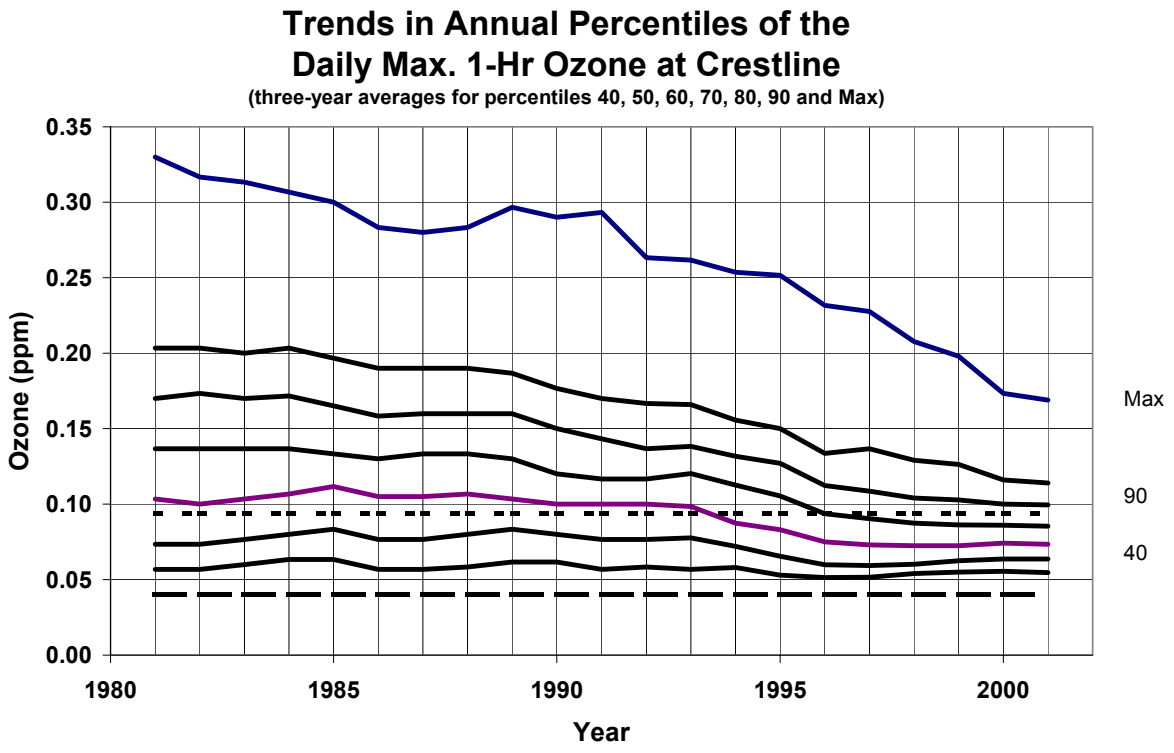

Figure B-11: Trends in annual percentiles of daily max 1-hour ozone at Crestline

Table B-17: Summary of Trends in Annual Percentiles of the Daily Max 1-hour Ozone at Crestline

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.330**	**0.293**	**0.173**
Δ% above background		**13%**	**54%**
90th Percentile	**0.203**	**0.170**	**0.116**
Δ% above background		**20%**	**53%**
80th Percentile	**0.170**	**0.143**	**0.100**
Δ% above background		**21%**	**54%**
70th Percentile	**0.137**	**0.117**	**0.086**
Δ% above background		**21%**	**52%**
60th Percentile	**0.103**	**0.100**	**0.074**
Δ% above background		**5%**	**46%**
50th Percentile	**0.073**	**0.077**	**0.064**
Δ% above background		**-10%**	**29%**
40th Percentile	**0.057**	**0.057**	**0.056**
Δ% above background		**0%**	**7%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

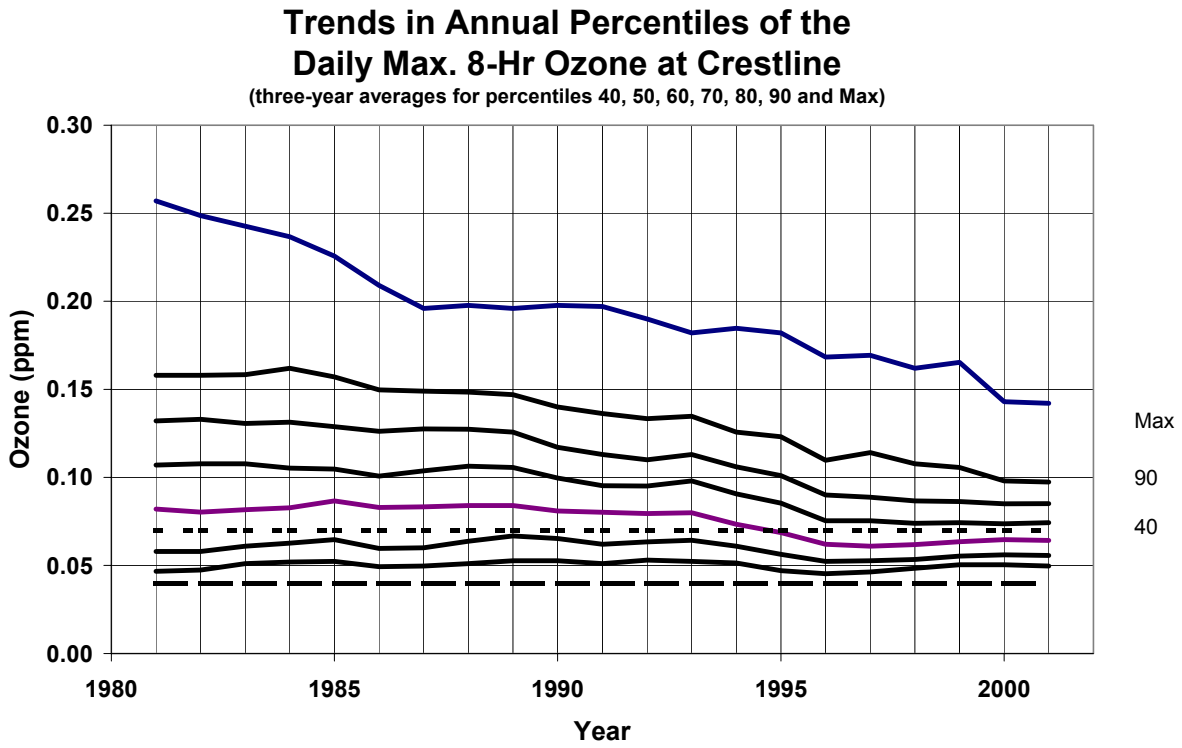

Figure B-12: Trends in annual percentiles of daily max 8-hour ozone at Crestline

Table B-18: Summary of Trends in Annual Percentiles of the Daily Max 8-hour Ozone at Crestline

	Average Value During Period		
Indicator	**1980-1982**	**1990-1992**	**2000-2002**
Maximum	**0.257**	**0.197**	**0.143**
Δ% above background		**28%**	**53%**
90th Percentile	**0.158**	**0.136**	**0.098**
Δ% above background		**18%**	**51%**
80th Percentile	**0.132**	**0.113**	**0.085**
Δ% above background		**21%**	**51%**
70th Percentile	**0.107**	**0.095**	**0.074**
Δ% above background		**17%**	**50%**
60th Percentile	**0.082**	**0.080**	**0.065**
Δ% above background		**4%**	**41%**
50th Percentile	**0.058**	**0.062**	**0.056**
Δ% above background		**-22%**	**11%**
40th Percentile	**0.047**	**0.051**	**0.050**
Δ% above background		**-65%**	**-55%**

note: Delta % above background is the change in the portion of measured ozone since 1980-82 above "background", where background is defined as 0.04 ppm.

Table B-19: Baseline Incidence Rates (Incidence/1000 Persons/Year)

County Name	Mortality (Short-Term Exposures) Non-Accidental, All Ages	Hospital Admissions, All Respiratory, All Ages	ER Visits for Asthma, Age Under 18	School Loss Days, All Illness, Age 5-17	MRAD Age>18
Alameda County	6.60	10.13	3.81	5990.10	7805.39
Alpine County	7.40	10.13	3.81	5990.10	7805.39
Amador County	9.99	10.13	3.81	5990.10	7805.39
Butte County	10.40	10.13	3.81	5990.10	7805.39
Calaveras County	8.90	10.13	3.81	5990.10	7805.39
Colusa County	7.10	10.13	3.81	5990.10	7805.39
Contra Costa County	6.78	10.13	3.81	5990.10	7805.39
Del Norte County	8.41	10.13	3.81	5990.10	7805.39
El Dorado County	6.29	10.13	3.81	5990.10	7805.39
Fresno County	6.41	10.13	3.81	5990.10	7805.39
Glenn County	7.71	10.13	3.81	5990.10	7805.39
Humboldt County	8.51	10.13	3.81	5990.10	7805.39
Imperial County	5.44	10.13	3.81	5990.10	7805.39
Inyo County	11.81	10.13	3.81	5990.10	7805.39
Kern County	6.60	10.13	3.81	5990.10	7805.39
Kings County	5.66	10.13	3.81	5990.10	7805.39
Lake County	13.13	10.13	3.81	5990.10	7805.39
Lassen County	5.75	10.13	3.81	5990.10	7805.39
Los Angeles County	6.08	10.13	3.81	5990.10	7805.39
Madera County	6.35	10.13	3.81	5990.10	7805.39
Marin County	7.47	10.13	3.81	5990.10	7805.39
Mariposa County	9.48	10.13	3.81	5990.10	7805.39
Mendocino County	8.89	10.13	3.81	5990.10	7805.39
Merced County	6.29	10.13	3.81	5990.10	7805.39
Modoc County	11.62	10.13	3.81	5990.10	7805.39
Mono County	3.87	10.13	3.81	5990.10	7805.39
Monterey County	5.88	10.13	3.81	5990.10	7805.39
Napa County	10.45	10.13	3.81	5990.10	7805.39
Nevada County	8.56	10.13	3.81	5990.10	7805.39
Orange County	5.68	10.13	3.81	5990.10	7805.39

Placer County	7.00	10.13	3.81	5990.10	7805.39
Plumas County	10.08	10.13	3.81	5990.10	7805.39
Riverside County	7.37	10.13	3.81	5990.10	7805.39
Sacramento County	7.14	10.13	3.81	5990.10	7805.39
San Benito County	5.06	10.13	3.81	5990.10	7805.39
San Bernardino County	6.10	10.13	3.81	5990.10	7805.39
San Diego County	6.41	10.13	3.81	5990.10	7805.39
San Francisco County	8.78	10.13	3.81	5990.10	7805.39
San Joaquin County	6.98	10.13	3.81	5990.10	7805.39
San Luis Obispo County	7.87	10.13	3.81	5990.10	7805.39
San Mateo County	6.77	10.13	3.81	5990.10	7805.39
Santa Barbara County	6.80	10.13	3.81	5990.10	7805.39
Santa Clara County	5.19	10.13	3.81	5990.10	7805.39
Santa Cruz County	6.56	10.13	3.81	5990.10	7805.39
Shasta County	9.50	10.13	3.81	5990.10	7805.39
Sierra County	9.26	10.13	3.81	5990.10	7805.39
Siskiyou County	10.42	10.13	3.81	5990.10	7805.39
Solano County	5.90	10.13	3.81	5990.10	7805.39
Sonoma County	8.17	10.13	3.81	5990.10	7805.39
Stanislaus County	7.22	10.13	3.81	5990.10	7805.39
Sutter County	7.43	10.13	3.81	5990.10	7805.39
Tehama County	9.90	10.13	3.81	5990.10	7805.39
Trinity County	10.73	10.13	3.81	5990.10	7805.39
Tulare County	6.71	10.13	3.81	5990.10	7805.39
Tuolumne County	9.50	10.13	3.81	5990.10	7805.39
Ventura County	5.76	10.13	3.81	5990.10	7805.39
Yolo County	6.37	10.13	3.81	5990.10	7805.39
Yuba County	7.26	10.13	3.81	5990.10	7805.39

Appendix C

Findings of the Air Quality Advisory Committee and Responses

UNIVERSITY OF CALIFORNIA, IRVINE
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February 24, 2005

Dr. Richard Bode
Research Division
California Air Resources Board
1001 I Street
Sacramento, CA 95812

Sacramento, CA

Dear Dr. Bode:

The Air Quality Advisory Committee met on January 11and 12, 2005 to evaluate the draft document "Review of the California Ambient Air Quality Standard For Ozone." The examination of the current air quality standards and the recommendations for modification of those standards derived from the Children's Environmental Health Protection Act (Senate Bill 25) and a resulting document "Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act" which was published as a staff report in 2000. SB 25 prompted an analysis of the scientific basis of the California air quality standards for particulate matter, sulfates, ozone, carbon monoxide, nitrogen dioxide, lead, and sulfur dioxide.

In response to SB 25, an up to date examination of the scientific information relevant to each of these standards that was published in peer reviewed documents was commissioned to determine if the current California standards were adequately protective of children's health. The staff of the Office of Environmental Health Hazard Assessment (OEHHA) made an analysis of the findings and recommended a list of standards that required re-review. The OEHHA analysis was deliberated by AQAC in a public meeting and the list of standards to be reviewed was prioritized. The standard for ozone was among those of highest priority for review.

In most respects, the committee was pleased with the document "Review of the California Ambient Air Quality Standard for Ozone." The committee went on record to complement the staffs of the ARB and OEHHA for performing a very comprehensive and careful compilation and analysis of the peer reviewed literature on sources, monitoring and health effects of ambient ozone.

The draft document made the following recommendations.

1. Retain ozone as the indicator for oxidant air pollution.

2. Retain the 1-hour-average standard for ozone at 0.09 ppm.

3. Establish an 8-hour-average standard for ozone at 0.070 ppm.

4. For both the 1-hour and 8-hour ozone standards, the concentrations for the standards noted above are to be established as "not to be exceeded".

5. Retain the current monitoring method for ozone which uses the ultraviolet (UV) absorption method for determining compliance with the state Ambient Air Quality Standard for ozone. Incorporate all federally approved UV methods (listed at http://www.epa.gov/ttn/amtic/criteria.html) as California Approved Samplers for ozone.

The AQAC discussed whether the UV-absorption method adequately measured other oxidant gases. The AQAC submitted comments to the Chair and a list of findings and suggested modifications was prepared. The committee suggests the deletion of recommendation # 1 and unanimously endorses recommendations 2 through 5.

The specific comments of the AQAC on the draft document are appended to this letter.

The AQAC is extremely appreciative of the responsiveness and expertise of the the staffs of OEHHA and the ARB. We commend them on the excellent job they did in reviewing and summarizing the scientific literature in the complex area of ozone and its effects on human health, and in establishing a set of ambient air quality standards that will protect the health of California's citizens and especially their children.

Finally, the AQAC strongly recommends that additional research is needed on the possible effects of ozone on fetal and neonatal development, and that the ozone standard should be reviewed in 5 years or less if significant new research results become available.

Sincerely,

Michael T. Kleinman, Chair
Air Quality Advisory Committee

Cc: Bart Croes, Research Division

Summary Comments of the Air Quality Advisory Committee

The staffs of OEHHA and the ARB provided an excellent review of the current literature relevant to the sources, transport and health effects of ambient ozone (O_3). The review provided a firm basis for establishing the needs for health-based O_3 air quality standards and the committee was unanimous in its appreciation of the effort and diligence involved in producing the report.

The staffs' recommendations for retaining the 1-hour O_3 standard and adding a new 8-hour standard at 70 ppb are well supported by the scientific evidence summarized in the document. Given the charge to set standards protective of human health, the key factor is the lowest exposure at which health effects have been demonstrated. This is inevitably a matter of interpretation, but there are convincing clinical studies showing lung function impairment at exposures as low as 80 ppb (6.6-hour average) and in some cases lower. Epidemiology and toxicology studies, although not as useful for pinpointing a lowest effect level, provide ample evidence of serious health effects of O_3, including hospitalizations for respiratory illness and asthma exacerbation. Recent evidence also suggests that that long-term exposure may be associated with permanent lung injury and that higher daily O_3 concentrations are associated with higher mortality rates.

The Air Quality Advisory Committee (AQAC) provided comments on a chapter by chapter basis and also addressed specific overarching questions that were submitted to them during their review of the report.

Children's protection, with an adequate margin of safety, is of paramount importance to public health. While the measurable injury and morbidity may be small, there is a developing body of knowledge that suggests that O_3 exposures early in life may contribute to lung compromise later in life (i.e. effects may be cumulative). As the committee indicates this is an area that has not been adequately researched and more work is needed. In addition, children with chronic lung diseases such as bronchopulmonary dysplasia, asthma and cystic fibrosis could be at special risk but, with the possible exception of asthma, there has been little research effort in these areas. Since asthma affects nearly 10% of the child population, the effects of O_3 on this group is of special importance. Although commented on in the draft document, it is important to recognize that children have higher minute ventilation rates per unit lung volume than do adults, hence their lungs receive greater doses of inhaled pollutants than do adults for comparable exposures. It is important to recognize that children are not "miniature adults" and this should be stressed in discussions of dose-response relationships.

Although Chapters 11 and 12 and Appendix A summarize the literature regarding the effects that ozone has on subjects (epidemiological and experimental) with chronic respiratory diseases, most specifically asthma, this information is not mentioned in Chapter 11 (Staff Recommendations). Individuals with chronic respiratory diseases are more likely to have acute adverse effects than healthy individuals.

Since there is little experimental data regarding the long-term effects of ozone on infants and children, the evidence has been interpreted cautiously. This should be highlighted as an area for research.

The Committee's primary responsibility is to assess the adequacy of the scientific basis for the proposed standards to protect public health. For this reason, our specific comments are more detailed when dealing with health-related chapters than for other chapters of the Draft Report. Our comments on the other chapters are primarily focused on factors that might influence the interpretation of ambient air quality vis-à-vis public health implications.

The document is in general extremely comprehensive and the committee appreciates the extensive effort undertaken in its preparation. Below are suggestions and comments of a more specific nature on a chapter-to-chapter basis. The committee supports the suggested standards and the suggested form of the standards being expressed as not to be exceeded, but suggests that even though this document does not specifically deal with the efforts to meet the proposed standards, greater precision in the discussion of how O_3 is measured, what constitutes an exceedance and how limitations in the monitoring capabilities may affect the exact level that "will not be exceeded".

The committee does have some concerns. The previous standard was assessed with respect to whether it adequately protected the health of children with some margin of safety. The proposed 8 hr standard provides some margin of safety by limiting the incidences of peak exposures that could be important in children's exposures. The decreased FEV1 reported in Kunzeli et al [Environ Res 72:8-23, 1997] in college students and Gauderman et al [N Engl J Med 351:1057-67, 2004] suggest that O_3 exposure during lung development may permanently impact lung function. One can ask whether these effects start during fetal life similar to the impact of pre-natal ETS exposure on the fetus (Hanrahan et al Amer J Respir Crit Care Med 145:1129-35, 1992 - higher airways resistance and smaller lungs; Tepper et al Am J Respir Crit Care Med 171:78-82, 2005 - lower airway function [FEF50, FEF25-75%, and 30% reduction in FEF75%] but not increased airway reactivity [to methacholine] in infants with pre-natal ETS exposure). The parallel to ETS exposure is should provoke interest in other studies on newborns and early infancy to determine whether there are other similar untoward effects of O_3. Pre-natal ETS exposure is well documented to impair development of respiratory control and increase the incidence of infant apnea and SIDS. While there have been reports of a similar effect of O_3 to ETS on birth weight [Parker et al, Pediatrics 115:121-8, 2004] and body growth during adolescents [Jedrychowski et al Environ Res 90:12-20, 2002], potential impaired CNS development with pre-natal O_3 exposure has not been studied. The Committee feels that additional research efforts on maternal, in utero and exposures during lung development are needed. If this preliminary evidence is supported in future research results it may be necessary to reconsider the form of the standard and include a longer terms exposure limit.

The Committee also feels strongly that an ozone-related research agenda should be supported over the next 5 years and that it is of very high priority that the ozone air quality standards be revisited in at most 5 years from now.

Important research issues to be addressed prior to the next cycle of review for ozone?

Acute toxicity mechanisms in sensitive populations (i.e. individuals with chronic respiratory and heart diseases)

Long-term effects of early exposure to ozone on cardiorespiratory system, nervous system and the developing organism.

Effects of O_3 exposure below 0.08 ppm using current more sensitive methods related to mechanisms of O_3 effects on the cardiopulmonary system.

Interactions of ozone with organic vapors to form secondary organic aerosols (the toxicity of these compounds is nearly unknown).

Several other suggestions are interspersed in the specific comments.

SPECIFIC COMMENTS

Chapter 1

Executive Summary – some modifications will be needed to include suggested changes in specific chapters below. The standards are adequately supported. The document is very comprehensive and it might be useful to insert into the Summary of Staff recommendations, a list (not a paragraph) of known adverse effects for ozone exposure to make it easier to put the rationale for the standards into context.

Chapter 2

Introduction and Overview – This chapter was very well written and provides the context for the process of setting the O_3 standard in a well balanced manner.

Chapter 3

Physics and Chemistry of O_3 - To avoid any chance of confusion it should be specified that ozone concentration is measured by volume, usually indicated with '(v)' following the unit. It would be less confusing if a single way of expressing concentration were chosen and used throughout the document. Another issue is 'significant figures.' This could impact the interpretation of the standard. The attribution at 0.070 ppm suggests a precision with 3 significant figures. Some discussion of how this is taken into account in the establishment of guidelines for ozone monitoring and reporting should be inserted to Chapter 6.

Chapter 4

Background O_3 in California - For research issues in the next cycle: background vs. elevation, season and region might be further addressed – although 40 ppb(v) is a reasonable estimate of the background for the discussion of the standard. The issue of unusual incursions of O_3 are important in the context of defining what constitutes an

exceedance for regulatory purposes. This should be specified in this chapter as well as in the monitoring chapter.

Chapter 5

O_3 Precursor Emissions – This chapter does not mention natural emissions of precursors. The information in Chapter 4 could be reintroduced to put the anthropogenic precursors in perspective. This is especially important since unusual circumstances (e.g. wildfires) will be considered in the evaluation of whether an area exceeds the standard. If there are not enough data to include in the pie charts, perhaps a qualitative summary statement could be included.

Chapter 6

The precision of ozone measurements is an issue that should be discussed. If a monitoring method has a standard deviation of x, than any given reading would really have a true value (t) of t ± 2x.(i.e. there is a limit on what would constitute an exceedance). It would be useful to spell out what we mean by exceedance in Chapter 8.

Chapter 7

Exposure to O_3 – The Committee did not request any additions.

Chapter 8

As mentioned for Chapter 6, there is some ambiguity with respect to precision of measurements as to what constitutes a measurable difference above the standard. If it is specified that the data will be in ppm with one significant figure rounding would allow 0.0749ppm to be truncated down to 0.07ppm—dropping to meet the standard as a result. Rounding specification have been used in the past by USEPA. (For example, EPA guidelines for data handling sometimes specify such round-off: see EPA-454/R-98-017, which allows 0.084ppm to be “less than, or equal to, 0.08ppm”.)

On the other hand using ppb(v), with 70ppb(v) as the standard (to be reported to the nearest 1ppb(v)), any concentration above 70.5ppb(v) is correctly seen as an exceedance, rather than allowing 74 to comply.

It might make sense to specify something like …ozone will be measured by volume fraction, and recorded in ppb(v) to the nearest 1ppb(v).” The standards could be stipulated as 90ppb(v) and 70ppb(v), respectively.

Chapter 9

The Committee did not address the Welfare Benefits, since its priority was human health effects. It might be worthwhile, however, to mention that the benefits analysis does not include the value of reducing ozone damage to cash crops, degradation of property (i.e. premature wearing of painted surfaces).

Chapter 10 Health Benefits Analysis (now listed as Appendix B)

The health benefits assessment is not being used to set the health standards, and it is not being used in a cost-benefit analysis, so an explanation about its purpose would be helpful. Many comments from the public concerned the differences between the studies used as the basis of the standard selection versus the studies used in the health benefits assessment. It is appropriate that the two are different because the purposes of

the two analyses are different, as the staff have pointed out in the response to public comments. The introduction would help clarify and respond to some of these comments if it included: (1) an explanation about the purpose of the health benefit assessment in the context of the health standard review process, (2) an explanation of the reasons why clinical studies are useful for standard setting but are not as useful for health benefit assessment, and (3) an explanation of why monetary values for health effects are not included.

Given the significance of the threshold assumptions for the results of the assessment, and the limited information from the literature, it is appropriate to calculate benefits under two alternative assumptions: (1) no threshold for any health effect category and (2) the same threshold (based on asthma emergency room visits studies if that is the best source) for all health effect categories, with adjustments to the estimated slope of the concentration response above the threshold.

It is appropriate to change to a census tract level extrapolation from ambient monitor concentrations for the health benefits assessment, rather than a county level aggregation. A more detailed exposure assessment than this is not needed for the health benefits assessment based on epidemiology studies because these are also based on ambient concentrations.

Bell et al. (2004) recently published an analysis of the NMMAPS data focused on ozone and their mean results are slightly higher than the previous NMMAPS results reported: 0.52% per 10 ppb 24-hour average ozone, which translates to about 0.21% per 10 ppb daily 1-hour high. This is still lower than the WHO central estimate, and the analysis still includes the use of multiple temperature and season variables. However, it covers 95 US cities, including 12 in California. The authors suggest that publication bias could be one reason why their results are lower than Anderson et al., Levy et al., and Stieb et al. report because the latter are based mostly on published studies for individual cities. It also may be appropriate to include a sensitivity analysis based on the "nearly significant" results for summer ozone based on recent ACS publication to show what the implications are of these results relative to the daily mortality estimates. There were also public comments given regarding forthcoming publications in Epidemiology reporting new analyses of the potential relationship between ozone and mortality. Given the significance of this health effect, the staff should consider incorporating this new evidence if possible.
There are inevitably important uncertainties in a quantitative benefits analysis, not so much about the nature of the health benefits but about their specific quantitative level. The uncertainties have been described in Section 10.6, but it is a difficult section to read. We suggest that the discussion of uncertainties in section 10.6 be edited to clarify the main points and incorporate the results of the revised threshold sensitivity analysis.

Chapter 11

1. Controlled Exposures:

The committee find that the review of human exposure studies was complete, current and accurate, with a few small exceptions. Some areas could be strengthened. For example, with respect to effective dose, the paragraph on p 11-212 could be improved by repeating some of the details given on p 11-4, citing Adams' (2003) comparison of FEV1 responses to 6.6 hr exposure to 0.08 ppm vs. 2 hr exposure to 0.30 ppm O_3.

In several places, reference is made to O_3 inhalation effects on respiratory symptoms or respiratory irritation when symptoms of breathing discomfort would be more accurate.

The examination of gender differences appears to be based on the corresponding section of the USEPA Criteria O_3 Document. It is the Committee's understanding that this section of the USEPA Criteria Document has been revised and there might be some updated material that could be incorporated into the revised report.

The section on heat and humidity effects on O_3-induced pulmonary function and symptoms responses does not mention that Gibbons and Adams (1984) noted that the ability to complete a given O_3 exposure was shortened when subjects were exercised under higher temperature conditions than when studies were performed under normal room temperature conditions. This could have some implications for summer exposures in California when O_3 exposures might be highest.

The summary statement on Adaptation (p 11-174) ["First, research suggests that ventilatory responses and reduced exercise performance do not show response attenuation with repeated exposures to O_3 concentrations that lead to diminution of pulmonary function responses"] is not accurate. Foxcraft and Adams (1986) performed a repeated O_3 exposure study. They did find reduced symptoms and improved exercise performance after 4 consecutive days of 0.35 ppm O_3 exposure, while they also reported diminution of the Day 1 pulmonary function reduction by Day 4 of exposure.

The summary statement on p 11-17 ["exercise performance can be reduced under conditions where O_3 inhalation has induced pulmonary function decrements and/or symptoms of respiratory discomfort. Significant reductions in exercise performance have been reported at O_3 concentrations as low as 0.06 ppm."] should be qualified. The Linder (1988) observations have not been observed by others using similar protocols at 0.06 ppm and higher (0.12 ppm) concentrations (Gong et al. 1986; Schelegle and Adams, 1986). Also exercise tolerance and PF changes are not always seen in concert (Gong et al., 1986; Foxcraft and Adams, 1986; Schlegle et al., 1987).

2. Toxicological Studies

Although there is to be discussion regarding ozone toxicity in infants and children, some of the literature is missing in this document (Chapters 11 and Appendix A). Also, the information regarding pre/postnatal exposure to ozone could be highlighted in separate sections in Chapter 11 and Appendix A. Doing so could make it easier to tease out the important information regarding age susceptibility/toxicity.

A few additional articles could be considered:

Carl, J., Bruce, H., and Jacob, F. (2004). Differential proinflammatory cytokine responses of the lung to ozone and lipopolysaccharide exposure during postnatal development. *Exp Lung Res* 30, 599-614.

Elsayed, N. M., Mustafa, M. G., and Postlethwait, E. M. (1982) Age-dependent pulmonary response of rats to ozone exposure. J Toxicol Environ Health 9:835-48.

Finkelstein, J. N., and Johnston, C. J. (2004). Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. Pediatrics 113, 1092-1096.

Mariassy, A. T., Sielczak, M. W., McCray, M. N., Abraham, W. M., and Wanner, A. (1989) Effects of ozone on lamb tracheal mucosa. Quantitative glycoconjugate histochemistry. Am J Pathol 135:871-9.

Myers, B. A., Dubick, M. A., Gerriets, J. E., Reiser, K. M., Last, J. A., and Rucker, R. B. (1986). Lung collagen and elastin after ozone exposure in vitamin B-6-deficient rats. Toxicol Lett 30, 55-61.

Phalen, R. F., Crocker, T. T., McClure, T. R., and Tyler, N. K. (1986). Effect of ozone on mean linear intercept in the lung of young beagles. J Toxicol Environ Health 17, 285-296.

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

Romero-Velazquez, R. M., Alfaro-Rodriguez, A., Gonzalez-Pina, R., and Gonzalez-Maciel, A. (2002). Effect of ozone prenatal exposure on postnatal development of cerebellum. Proc West Pharmacol Soc 45, 65-67.
Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., and Clewell, H. J., 3rd (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15, 987-1016.

Sorace, A., de Acetis, L., Alleva, E., and Santucci, D. (2001). Prolonged exposure to low doses of ozone: short- and long-term changes in behavioral performance in mice. Environ Res 85, 122-134.

Stephens, R. J., Sloan, M. F., Groth, D. G., Negi, D. S., and Lunan, K. D. (1978) Cytologic responses of postnatal rat lungs to O3 or NO2 exposure. Am J Pathol 93:183-200.

Tyson, C. A., Lunan, K. D., and Stephens, R. J. (1982) Age-related differences in GSH-shuttle enzymes in NO2- or O3-exposed rat lungs. Arch Environ Health 37:167-76.

3. Have potential differential exposure and dose patterns among infants and children been examined sufficiently in the document?

Sections 8.4 (Consideration of Infants and Children) and 8.7.4 (Consideration of Infants and Children in Recommending the Ozone Standards) present general statements to the effect that children receive a larger exposure of ozone. There is some literature on this topic that could be cited. A table similar to that in Kleinman (1991) could be used.

Kleinman, M.T. Effects of ozone on pulmonary function: The relationship of response to effective dose. J. Exposure Analysis and Environmental Epidemiology, 1:309-325, 1991.

Chapter 12. Epidemiologic Studies.

General comments:

Overall, this chapter provides a very thoughtful and comprehensive review of the epidemiologic literature that fairly points to methodological weaknesses that in general, have likely underestimated the impact of ozone on human health. This critique adds some additional interpretation of these weaknesses. The choice of an ozone standard based on susceptible populations is well supported by the evidence presented. Below are some additional data to support the protection of populations at risk.

Although studies conducted in other parts of the country and internationally are clearly relevant, studies in California are particularly relevant to this review. It is important for this review to further interpret results of studies in California with respect to the misclassification of O_3 exposure based on region. The details of this were covered in the exposure section but results of epidemiologic studies need to be interpreted with this in mind. The use of air conditioning, air exchange rates and time indoors will all dramatically influence personal O_3 exposure. This was described in 12.2 under time series studies in the last paragraph on page 12-22, but it also applies to the other study designs. Studies conducted in inland areas of California where outdoor O_3 is highest may have subjects who are less exposed to O_3 than areas closer to the coast. The California studies most influenced by this phenomenon are the studies by Delfino et al. cited in section 12.1 and above, the 7th Day Adventist Cohort, and the Children's Health Study (CHS) (Gilliland et al., 2001, and 12.3.5 CHS references). The CHS included schoolchildren living in hotter inland areas of southern California. This phenomenon may have partly explained the isolated results in the CHS for the increased risk of asthma onset only among children playing three or more sports in the six out of 12 communities with higher O_3 (McConnell et al., 2002). More outdoor exposure and

increased O_3 dose may have been a function of the physical activities. The text reviewing McConnell et al, 2002 on p 12-54 only refers to "effect modification by physical activity."

The impact of weather on behavior, air conditioning use, and therefore indoor exposure to O_3, may have also led to null results for lung function growth and O_3 in the prospective analysis of 4th graders in the CHS (12.3.5, p. 12-52, Gauderman et al, 2000). This contrasts significant results for particles, which have considerably greater penetration and persistence in indoor environments. Note that the Gauderman et al. (2000) study was notably updated recently with an 8-year follow-up of fourth graders (Gauderman et al, 2004) in contrast to the 4-year follow-up in the 2000 publication. The new study also found acid vapor and elemental carbon were associated with lung function declines along with $PM_{2.5}$ and NO_2.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. The effect of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351(11):1057-67.

Also of great importance in interpreting epidemiologic results is the issue of excessive control for presumed confounding by outdoor temperature on effects of outdoor (ambient) O_3, particularly where there is lack of evidence for a direct heath effect of local temperature ranges. Often results are not presented for O_3 models without temperature. This issue was described in section 12.2 under time series studies in the second paragraph on page 12-22 and later in reference to the studies reviewed, but it also needs to be referenced to the other study designs. An example of why ambient temperature can have little direct relevance to health is shown in a personal PM exposure assessment study of 19 asthmatic children living in inland San Diego County. The magnitude of correlation between personal temperature and ambient O_3 was far less than for central site temperature over a 14-day monitoring period (r = 0.50 for 8-hr O_3 and 1-hr maximum outdoor temperature, vs. r = 0.10 for 8-hr O_3 and 1-hr maximum personal temperature) (Delfino et al., 2003). There was no association between personal temperature and lung function in that study, but there were strong inverse associations between personal PM and lung function. Ambient O_3 was not associated with lung function but the study was designed to assess personal PM effects and had limited power to assess effects of central site exposures.

Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV_1 in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. Environ Health Perspect 2004; 112:932-41.

Specific comments:

12.1. Some relevant acute field studies were not discussed in this section, including studies conducted in California. These include:

Delfino RJ, Zeiger RS, Seltzer JM, Street DH. Symptoms in pediatric asthmatics and air pollution: Differences in effects by symptom severity, anti-inflammatory medication use, and particulate averaging time. Environ Health Perspect, 1998; 106: 751-61.

This study of schoolchildren with asthma in inland San Diego County showed significant associations between asthma symptoms (bothersome or interfered with daily activities) and O_3, with similar associations for minimum to 90th percentile 1-hr (58 ppb) and 8-hr O_3 maximums (46 ppb). Associations for O_3 and PM_{10} were largely independent in models incorporating both pollutants, and O_3 associations were not confounded by outdoor fungal spores. The study also showed significantly stronger associations between asthma symptoms and O_3 in a subset of asthmatics not taking anti-inflammatory medications. Threshold analyses suggested effects below 80 ppb 1-hr O_3 maximum in this subset, but not among other subjects. 80 ppb 8-hr maximum O_3 was exceeded 25 times during the three-month study.

Mortimer KM, Tager IB, Dockery DW, Neas LM, Redline S. The Effect of Ozone on Inner-City Children with Asthma. Identification of Susceptible Subgroups. Am J Respir Crit Care Med 162:1838-1845 (2000).

Mortimer, et al. (2000) reported results of a series of 2-week asthma panels in 846 inner city children with asthma living in low income neighborhoods. They found that O_3 was inversely associated with PEF and positively associated with symptoms with the strongest associations among children born of low birth weight or premature.

Delfino RJ, Gong H Jr, Linn WS, Hu Y, Pellizzari ED. Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 2003; 111:647-656.

This study of Hispanic schoolchildren with asthma in LA showed significant associations between asthma symptoms (bothersome or interfered with daily activities) and ambient VOCs, PM_{10} elemental and organic carbon, but not O_3. However, O_3, along with formaldehyde and acetone were similarly associated with more severe symptoms interfering with daily activities among a subset of children, particularly those on maintenance medication. Odds ratios (OR) for interquartile increases in 1-hr O_3 (14 ppb) were identical to 8-hr O_3 (11 ppb) (both ORs around 2.0), even though 1-hr O_3 never exceeded 52 ppb. See Table 4 in that paper for details.

12.1.3. Page 12-5:
The study by Gent and colleagues (2003) is large panel study with key findings. The review should put the findings of effect modification from maintenance medication into proper perspective. First, the biological mechanism of O_3 is in large part related to airway inflammation as discussed in the Toxicology section. Therefore, medication that controls airway inflammation such as inhaled corticosteroids would be expected to dampen the effects of O_3. However, finding the opposite in a panel study such as Gent

et al. (2003) is not unexpected if use of such medication is largely restricted to more severe asthmatics, who are expected top be more susceptible to O_3. The results contrast findings of Delfino et al. (1998) showing significantly stronger association between asthma symptom severity and O_3 in asthmatic children not taking anti-inflammatory medications, largely inhaled corticosteroids (ICS). Mortimer, et al. (2000, discussed above) compared effects on asthma outcomes by outdoor O_3 levels across medication groups based on baseline data for prescribed medication. Associations between incidence of symptoms and an increase of 15 ppb in O_3 was largest among those prescribed cromolyn but not ICS (OR 1.46, 95% CI 1.06, 2.01) followed by nonsignificant ORs for those prescribed ß-agonists or xanthines only (1.18), ICS (1.08), and no medication (1.04). The percentage change in PEF was also greatest among those prescribed cromolyn but not ICS (-1.27, 95% CI -2.47, -0.06) followed by nonsignificant PEF changes of around -0.5 for the other groups.

Section 12.2.

The review made the important point of describing residual confounding of ozone effects by the co-adjustment approach in time series models, and the lack of stratified analyses by season. This issue has not received adequate attention in the literature and may explain many null findings. These potential analytic weaknesses and control for temperature (see above) is particularly troubling for the null results in Los Angeles (Linn et al, 2000; Mann et al., 2002 and Nauenberg and Basu, 1999) suggesting that new studies and reanalysis of these studies are needed.

The committee concur with Dr. Bates that the Atlanta study by Friedman et al. (2001) is particularly important in suggesting that lowering ozone will have major benefits in reducing hospital admissions and ED visits. It is also important to point out that the effects detected in Atlanta were related to a reduction in traffic, which includes a wide range of toxic air pollutants including particle-bound in addition to ozone. Strong correlation between ozone and PM in Atlanta has made it impossible to separate effects of the two on asthma ED visits as reported by Tolbert (2000) reviewed in section 12-35.

typo in title of Table 12-2 Hospital was misspelled.

The statement on p 12-36, third paragraph, lines 11-12 is unclear. What is meant by "self-selected" and "not quantitatively useful." All of these studies are subject to exposure misclassification and air pollutant components (most unmeasured) could differ by season, year and geographic location. These factors will lead to inconsistencies. For instance, for the Delfino 1997a study, concentrations of PM_{10}, $PM_{2.5}$, SO_4, and H^+ were significantly higher during 1992 than 1993 due to sulfate transport episodes, and O_3 lower. Therefore, finding significant results in 1993 alone are not unexpected.

12.4.2: Similar to section 12.2.1, the presentation of important issues to understand in time series analysis is excellent and provides thoughtful direction to further research. The criticisms of smoothing functions that include midrange temperatures of questionable clinical relevance are particularly informative and suggest that studies using this method may have underestimated the effects of air pollutants including ozone.

Appendix D

Responses to Comments From the Air Quality Advisory Committee

Responses to Comments of the Air Quality Advisory Committee

Note: Staff responses are given in bold italics following each point raised by AQAC.

The staffs of OEHHA and the ARB provided an excellent review of the current literature relevant to the sources, transport and health effects of ambient ozone (O_3). The review provided a firm basis for establishing the needs for O_3 air quality standards and the committee was unanimous in its appreciation of the effort and diligence involved in producing the report.

The Air Quality Advisory Committee (AQAC) provided comments on a chapter by chapter basis and also addressed specific overarching questions that were submitted to them during their review of the report.

Children's protection, with an adequate margin of safety, is of paramount importance to public health. While the measurable injury and morbidity may be small, there is a developing body of knowledge that suggests that O_3 exposures early in life may contribute to lung compromise later in life (i.e. effects may be cumulative). As the committee indicates this is an area that has not been adequately researched and more work is needed. In addition, children with chronic lung diseases such as bronchopulmonry displasia, asthma and cyctic fibrosis could be at special risk but, with the possible exception of asthma, there has been little research effort in these areas. Since asthma affects nearly 10% of the child population, the effects of O_3 on this group is of special importance. Although commented on in the draft document, it is important to recognize that children have higher minute ventilation rates per unit lung volume than do adults, hence their lungs receive greater doses of inhaled particles than do adults for comparable exposures. It is important to recognize that children are not "miniature adults" and this should be stressed in discussions of dose-response relationships.

A discussion of dosimetry in children has been added to the controlled studies chapter. The review of animal toxicology studies that investigated effects in pre- and post-natal animals in the controlled studies chapter has been expanded.

Although Chapters 11 and Appendix A summarize the literature regarding the effects that ozone has on subjects (epidemiological and experimental) with chronic respiratory diseases, most specifically asthma, this information is not mentioned in Chapter 8, Staff Recommendations. Individuals with chronic respiratory diseases are more likely to have acute adverse effects than healthy individuals.

A section on effects of ozone on people with chronic disease has been added to the recommendation chapter (Chapter 11).

Since there is little experimental data regarding the long-term effects of ozone on infants and children, the evidence has been interpreted cautiously. This should be highlighted as an area for research.

This topic has been added to the recommendations for future research.

The Committee's primary responsibility is to assess the adequacy of the health basis for the proposed standards. For this reason, our specific comments are more detailed when dealing with health-related chapters than for other chapters of the Draft Report. Our comments on the other chapters are primarily focused on factors that might influence the interpretation of ambient air quality vis-à-vis public health implications.

The document is in general extremely comprehensive and the committee appreciates the extensive effort undertaken in its preparation. Below are suggestions and comments of a more specific nature on a chapter-to-chapter basis. The committee supports the suggested standards and the suggested form of the standards being expressed as not to be exceeded, but suggests that even though this document does not specifically deal with the efforts to meet the proposed standards, greater precision in the discussion of how O_3 is measured, what constitutes an exceedance and how limitations in the monitoring capabilities may affect the exact level that "will not be exceeded".

Discussion of monitoring capabilities has been added to Chapter 6, which discusses monitoring methods.

The committee does have some concerns. The previous standard was assessed with respect to whether it adequately protected the health of children with some margin of safety. The proposed 8 hr standard provides some margin of safety by limiting the incidences of peak exposures that could be important in children's exposures. We have been provided information on the effects of ozone exposure on the developing lung, albeit in non-human primates. The effects of ozone on the developing lung is one of several areas that the Committee feels needs additional research efforts.

Discussion of this topic in the controlled studies chapter has been expanded, and the topic added to the list of recommendations for future research.

The Committee also feels strongly that an ozone-related research agenda should be supported over the next 5 years and that it is of very high priority that the ozone air quality standards be revisited in at most 5 years from now.

Staff thanks the Committee for their comment.

Important research issues to be addressed prior to the next cycle of review for ozone?

Acute toxicity mechanisms in sensitive populations (i.e. individuals with chronic respiratory and heart diseases)

Long-term effects of early exposure to ozone on cardiorespiratory system, nervous system and the developing organism.

Effects of O_3 exposure below 0.08 ppm using current more sensitive methods related to mechanisms of O_3 effects on the cardiopulmonary system.

Interactions of ozone with organic vapors to form secondary organic aerosols (the toxicity of these compounds is nearly unknown).

Several other suggestions are interspersed in the specific comments.

The Committee's suggestions have been added to the research recomendations section of the Staff recommendation.

SPECIFIC COMMENTS

Chapter 1

Executive Summary – some modifications will be needed to include suggested changes in specific chapters below. The standards are adequately supported. The document is very comprehensive and it might be useful to insert into the Summary of Staff recommendations, a list (not a paragraph) of known adverse effects for ozone exposure to make it easier to put the rationale for the standards into context.

The necessary modifications have been made, and the requested list added.

Chapter 2

Introduction and Overview – This chapter was very well written and provides the context for the process of setting the O_3 standard in a well balanced manner.

Chapter 3

Physics and Chemistry of O_3 - To avoid any chance of confusion it should be specified that ozone concentration is measured by volume, usually indicated with '(v)' following the unit. It would be less confusing if a single way of expressing concentration were chosen and used throughout the document. Another issue is 'significant figures.' This could impact the interpretation of the standard. The attribution at 0.070 ppm suggests a precision with 3 significant figures. Some discussion of how this is taken into account in the establishment of guidelines for ozone monitoring and reporting should be inserted to Chapter 6.

Agency policy is to use ppm, and we have done so throughout the report. We added an indication in Chapter 3 that ozone is measured by volume. Discussion on the issue of the number of decimal places for the recomended standards (Chapter 11), rounding conventions (Chapter 7), and precision (Chapter 6) have been added.

Chapter 4

Background O_3 in California - For research issues in the next cycle: background vs. elevation, season and region might be further addressed – although 40 ppb(v) is a reasonable estimate of the background for the discussion of the standard. The issue of unusual incursions of O_3 are important in the context of defining what constitutes an exceedance for regulatory purposes. This should be specified in this chapter as well as in the monitoring chapter.

Discussion on exceptional events and their identification has been added to Chapters 4 and 7.

Chapter 5

O_3 Precursor Emissions – This chapter does not mention natural emissions of precursors. The information in Chapter 4 could be reintroduced to put the anthropogenic precursors in perspective. This is especially important since unusual circumstances (e.g. wildfires) will be considered in the evaluation of whether an area exceeds the standard. If there are not enough data to include in the pie charts, perhaps a qualitative summary statement could be included.

A paragraph on natural emissions has been added.

Chapter 6

The precision of ozone measurements is an issue that should be discussed. If a monitoring method has a standard deviation of x, than any given reading would really have a true value (t) of t ± 2x.(i.e. there is a limit on what would constitute an exceedance). It would be useful to spell out what we mean by exceedance in Chapter 8.

During oral discussion at the AQAC meeting AQAC also requested that Staff clarify what the proposed monitoring method measures, the extent to which other oxidants were also measured, the sampler calibration methods, and the limitations of the samplers and agency plans for operating improvement.

Discussion on the precision of ozone measurements has been added to Chapter 6.

The proposed method measures ultra-violet light absorbtion at a wavelength of 254 nm. Since light at 254 nm is strongly absorbed by ozone, in proportion to the amount present, ozone concentration is determined. With regard to interference, UV Photometer manufacturers must demonstrate that their analyzers successfully reject interference from common oxidants, specifically sulfur dioxide and nitrogen dioxide, in order to receive equivalent method designation.

Specifically, 40CFR Part 53 - Ambient Air Monitoring Reference and Equivalent Methods; Subpart B - Procedures for Testing Performance Characteristics of Automated Methods SO2, CO, O3 and NO2, addresses performance requirements for interference tests. 40CFR53 part 53.23 (d) (2) states "The test analyzer shall be tested for all substances likely to cause a detectable response. The test analyzer shall be challenged, in turn, with each (potentially) interfering agent specified in table B-3." Table B-3 requires testing of NO2 and SO2, each at 0.500 ppm-v, for gas-phase photometric ozone methods.

Ozone instruments are calibrated by comparing the responses of an ambient ozone analyzer to a certified ozone transfer standard. The response to ozone gas is compared at 4 levels and regressed using the "least squares" method. The four levels are approximately 0.400 ppm-v, 0.300 ppm-v, 0.200 ppm-v, and 0.090 ppm-v. Calibration gas at each of these levels is introduced into the ozone analyzer until a steady and unchanging analyzer response is achieved. Typically, a steady reading of 10 minutes is taken as the calibration data point. The regression results are not used to correct data; they are used to determine the instrument's linearity and deviation from the true based on the regression slope. An instrument is not adjusted to match the transfer standard unless it is beyond 2% from true (slope of 0.98 to 1.02).

We would not expect any difference in accuracy for the average 8-hour measured concentration. Accuracy, comprised of systematic bias and random precision, is neither gained nor lost by arithmetic operations.

The U.S.EPA is planning to revise federal air quality monitoring regulations in 2005. Included in the U.S.EPA National Monitoring Strategy, which provides rationale for the regulatory revisions, is a proposal to tighten ozone data quality objectives to 7% precision and 7% bias. If U.S.EPA does adopt these criteria, the Air Resources Board and local air pollution districts will be obliged to adhere to them. However, it is not certain whether tighter criteria will actually improve the observed precision or bias of the network.

Chapter 7

Exposure to O_3 – ***The Committee did not request any changes.***

Chapter 8

As mentioned for Chapter 6, there is some ambiguity with respect to precision of measurements as to what constitutes a measurable difference above the standard. If it is specified that the data will be in ppm with one significant figure rounding would allow 0.0749ppm to be truncated down to 0.07ppm—dropping to meet the standard as a result. Rounding specification have been used in the past by USEPA. (For example,

EPA guidelines for data handling sometimes specify such round-off: see EPA-454/R-98-017, which allows 0.084ppm to be “less than, or equal to, 0.08ppm”.)

On the other hand using ppb(v), with 70ppb(v) as the standard (to be reported to the nearest 1ppb(v)), any concentration above 70.5ppb(v) is correctly seen as an exceedance, rather than allowing 74 to comply.

It might make sense to specify something like …ozone will be measured by volume fraction, and recorded in ppb(v) to the nearest 1ppb(v).” The standards could be stipulated as 90ppb(v) and 70ppb(v), respectively.

Staff has clarified the discussion of the reason for three decimal places in the recommended 8-hour average standard of 0.070 ppm, compared to the two decimal places recommended for the 1-hour average stndard of 0.09 ppm.

Chapter 9

The Committee did not address the Welfare Benefits, since its priority was human health effects. It might be worthwhile, however, to mention that the benefits analysis does not include the value of reducing ozone damage to cash crops, degradation of property (i.e. premature wearing of painted surfaces).

A sentence has been added to the introduction of the chapter indicating that the benefits analysis does not include calculation of welfare benefits.

Chapter 10 Health Benefits Analysis (now listed as Appendix B)

The health benefits assessment is not being used to set the health standards, and it is not being used in a cost-benefit analysis, so an explanation about its purpose would be helpful. Many comments from the public concerned the differences between the studies used as the basis of the standard selection versus the studies used in the health benefits assessment. It is appropriate that the two are different because the purposes of the two analyses are different, as the staff has pointed out this out in the response to public comments. The introduction would help clarify and respond to some of these comments if it included: (1) an explanation about the purpose of the health benefit assessment in the context of the health standard review process, (2) an explanation of the reasons why clinical studies are useful for standard setting but are not as useful for health benefit assessment, and (3) an explanation of why monetary values for health effects are not included.

The text has been modified to address the issues raised.

Given the significance of the threshold assumptions for the results of the assessment, and the limited information from the literature, it is appropriate to calculate benefits under two alternative assumptions: (1) no threshold for any health effect category and (2) the same threshold (based on asthma emergency room visits studies if that is the best source) for all health effect categories, with adjustments to the estimated slope of the concentration response above the threshold.

We have performed, and present the results of, sensitivity analyses to address this issue.

It is appropriate to change to a census tract level extrapolation from ambient monitor concentrations for the health benefits assessment, rather than a county level aggregation. A more detailed exposure assessment than this is not needed for the health benefits assessment based on epidemiology studies because these are also based on ambient concentrations.

Bell et al. (2004) recently published an analysis of the NMMAPS data focused on ozone and their mean results are slightly higher than the previous NMMAPS results reported: 0.52% per 10 ppb 24-hour average ozone, which translates to about 0.21% per 10 ppb daily 1-hour high. This is still lower than the WHO central estimate, and the analysis still includes the use of multiple temperature and season variables. However, it covers 95 US cities, including 12 in California. The authors suggest that publication bias could be one reason why their results are lower than Anderson et al., Levy et al., and Stieb et al. report because the latter are based mostly on published studies for individual cities. It also may be appropriate to include a sensitivity analysis based on the "nearly significant" results for summer ozone based on recent ACS publication to show what the implications are of these results relative to the daily mortality estimates. There were also public comments given regarding forthcoming publications in Epidemiology reporting new analyses of the potential relationship between ozone and mortality. Given the significance of this health effect, the staff should consider incorporating this new evidence if possible.

The Bell et al. paper has been included in the analyses presented in the revised chapter.

There are inevitably important uncertainties in a quantitative benefits analysis, not so much about the nature of the health benefits but about their specific quantitative level. The uncertainties have been described in Section 10.6, but it is a difficult section to read. We suggest that the discussion of uncertainties in section 10.6 be edited to clarify the main points and incorporate the results of the revised threshold sensitivity analysis.

The section has been edited to improve clarity and readability.

Chapter 11

Controlled Exposures:

The committee find that the review of human exposure studies was complete current and accurate, with a few small exceptions. Some areas could be strengthened. For example, with respect to effective dose, the paragraph on p 11-212 could be improved by repeating some of the details given on p 11-4, citing Adams' (2003) comparison of FEV1 responses to 6.6 hr exposure to 0.08 ppm vs. 2 hr exposure to 0.30 ppm O_3.

This section was edited as suggested.

In several places, reference is made to O_3 inhalation effects on respiratory symptoms or respiratory irritation when symptoms of breathing discomfort wold be more accurate.

The text was reviewed, and revised as appropriate.

The examination of gender differences appears to be based on the corresponding section of the USEPA Criteria O_3 Document. It is the Committee's understanding that this section has been revised and there might be some updated material that could be incorporated into the revised report.

The section was revised, and some new material indluded.

The section on heat and humidity effects on O_3-induced pulmonary function and symptoms responses does not mention that Gibbons and Adams (1984) noted that the ability to complete a given O_3 exposure was shortened when subjects were exercised under higher temperature conditions than when studies were performed under normal room temperature conditions. This could have some implications for summer exposures in California when O_3 exposures might be highest.

The cited paper was added to the section on heat and humidity effects, and the text revised appropriately.

The summary statement on Adaptation (p 11-174) ["First, research suggests that ventilatory responses and reduced exercise performance do not show response attenuation with repeated exposures to O3 concentrations that lead to diminution of pulmonary function responses"] is not accurate. Foxcraft and Adams (1986) performed a repeated O_3 exposure study. They did find reduced symptoms and improved exercise

performance after 4 consecutive days of 0.35 ppm O_3 exposure, while they also reported diminution of the Day 1 pulmonary function reduction by Day 4 of exposure.

The section has been revised to incorporate the cited paper.

The summary statement on p 11-17 ["exercise performance can be reduced under conditions where O_3 inhalation has induced pulmonary function decrements and/or symptoms of respiratory discomfort. Significant reductions in exercise performance have been reported at O_3 concentrations as low as 0.06 ppm."] should be qualified. The Linder (1988) observations have not been observed by others using similar protocols at 0.06 ppm and higher (0.12 ppm) concentrations (Gong et al. 1986; Schelegle and Adams, 1986). Also exercise tolerance and PF changes are not always seen in concert (Gong et al., 1986; Foxcraft and Adams, 1986; Schlegle et al., 1987).

The section was revised to address the comment.

4. Toxicological Studies

Although there is to be discussion regarding ozone toxicity in infants and children, some of the literature is missing in this document (Chapters 11 and Appendix A). Also, the information regarding pre/postnatal exposure to ozone could be highlighted in separate sections in Chapter 11 and Appendix A. Doing so could make it easier to tease out the important information regarding age susceptibility/toxicity.

A few additional articles could be considered:

Carl, J., Bruce, H., and Jacob, F. (2004). Differential proinflammatory cytokine responses of the lung to ozone and lipopolysaccharide exposure during postnatal development. *Exp Lung Res* 30, 599-614.

Elsayed, N. M., Mustafa, M. G., and Postlethwait, E. M. (1982) Age-dependent pulmonary response of rats to ozone exposure. J Toxicol Environ Health 9:835-48.

Finkelstein, J. N., and Johnston, C. J. (2004). Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. Pediatrics 113, 1092-1096.

Mariassy, A. T., Sielczak, M. W., McCray, M. N., Abraham, W. M., and Wanner, A. (1989) Effects of ozone on lamb tracheal mucosa. Quantitative glycoconjugate histochemistry. Am J Pathol 135:871-9.

Myers, B. A., Dubick, M. A., Gerriets, J. E., Reiser, K. M., Last, J. A., and Rucker, R. B. (1986). Lung collagen and elastin after ozone exposure in vitamin B-6-deficient rats. Toxicol Lett 30, 55-61.

Phalen, R. F., Crocker, T. T., McClure, T. R., and Tyler, N. K. (1986). Effect of ozone on mean linear intercept in the lung of young beagles. J Toxicol Environ Health 17, 285-296.

Raub, J. A., Mercer, R. R., and Kavlock, R. J. (1983) Effects of Prenatal Nitrogen Exposure on Postnatal Lung Function in the Rat. Toxicology and Applied Pharmacology 94:119-134.

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

Romero-Velazquez, R. M., Alfaro-Rodriguez, A., Gonzalez-Pina, R., and Gonzalez-Maciel, A. (2002). Effect of ozone prenatal exposure on postnatal development of cerebellum. Proc West Pharmacol Soc 45, 65-67.

Sarangapani, R., Gentry, P. R., Covington, T. R., Teeguarden, J. G., and Clewell, H. J., 3rd (2003). Evaluation of the potential impact of age- and gender-specific lung morphology and ventilation rate on the dosimetry of vapors. Inhal Toxicol 15, 987-1016.

Sorace, A., de Acetis, L., Alleva, E., and Santucci, D. (2001). Prolonged exposure to low doses of ozone: short- and long-term changes in behavioral performance in mice. Environ Res 85, 122-134.

Stephens, R. J., Sloan, M. F., Groth, D. G., Negi, D. S., and Lunan, K. D. (1978) Cytologic responses of postnatal rat lungs to O3 or NO2 exposure. Am J Pathol 93:183-200.

Tyson, C. A., Lunan, K. D., and Stephens, R. J. (1982) Age-related differences in GSH-shuttle enzymes in NO2- or O3-exposed rat lungs. Arch Environ Health 37:167-76.

A new section on toxicological effects in pre- and post-natal animals has been added, which includes consideration of the papers suggested by the Committee.

5. Have potential differential exposure and dose patterns among infants and children been examined sufficiently in the document?

Sections 8.4 (Consideration of Infants and Children) and 8.7.4 (Consideration of Infants and Children in Recommending the Ozone Standards) present general statements to the effect that children receive a larger exposure of ozone. There is some literature on this topic that could be cited. A table similar to that in Kleinman (1991) could be used.

Kleinman, M.T. Effects of ozone on pulmonary function: The relationship of response to effective dose. J. Exposure Analysis and Environmental Epidemiology, 1:309-325, 1991.

This paper has been added to the discussion of dosimetry in Chapter 9.

Chapter 12. Epidemiologic Studies.

General comments:

Overall, a very thoughtful and comprehensive review of the epidemiologic literature that fairly points to methodological weaknesses that in general, have likely underestimated the impact of ozone on human health. My critique adds some additional interpretation of these weaknesses. The choice of an ozone standard based on susceptible populations is well supported by the evidence presented. I have added some additional data to support the protection of populations at risk.

Although studies conducted in other parts of the country and internationally are clearly relevant, studies in California are particularly relevant to this review. It is important for this review to further interpret results of studies in California with respect to the misclassification of O_3 exposure based on region. The details of this were covered in the exposure section but results of epidemiologic studies need to be interpreted with this in mind. The use of air conditioning, air exchange rates and time indoors will all dramatically influence personal O_3 exposure. This was described in 12.2 under time series studies in the last paragraph on page 12-22, but it also applies to the other study designs. Studies conducted in inland areas of California where outdoor O_3 is highest may have subjects who are less exposed to O_3 than areas closer to the coast. The California studies most influenced by this phenomenon are the studies by Delfino et al. cited in section 12.1 and above, the 7th Day Adventist Cohort, and the Children's Health Study (CHS) (Gilliland et al., 2001, and 12.3.5 CHS references). The CHS included schoolchildren living in hotter inland areas of southern California. This phenomenon may have partly explained the isolated results in the CHS for the increased risk of asthma onset only among children playing three or more sports in the six out of 12 communities with higher O_3 (McConnell et al., 2002). More outdoor exposure and increased O_3 dose may have been a function of the physical activities. The text reviewing McConnell et al, 2002 on p 12-54 only refers to "effect modification by physical activity."

The text has been revised to incorporate these recommendations.

The impact of weather on behavior, air conditioning use, and therefore indoor exposure to O_3, may have also led to null results for lung function growth and O_3 in the prospective analysis of 4th graders in the CHS (12.3.5, p. 12-52, Gauderman et al, 2000). This contrasts significant results for particles, which have considerably greater penetration and persistence in indoor environments. Note that the Gauderman et al. (2000) study was notably updated recently with an 8-year follow-up of fourth graders (Gauderman et al, 2004) in contrast to the 4-year follow-up in the 2000 publication. The new study also found acid vapor and elemental carbon were associated with lung function declines along with $PM_{2.5}$ and NO_2.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. The effect

of air pollution on lung development from 10 to 18 years of age. N Engl J Med. 2004;351(11):1057-67.

The text has been revised to incorporate these recommendations.

Also of great importance in interpreting epidemiologic results is the issue of excessive control for presumed confounding by outdoor temperature on effects of outdoor (ambient) O_3, particularly where there is lack of evidence for a direct heath effect of local temperature ranges. Often results are not presented for O_3 models without temperature. This issue was described in section 12.2 under time series studies in the second paragraph on page 12-22 and later in reference to the studies reviewed, but it also needs to be referenced to the other study designs. An example of why ambient temperature can have little direct relevance to health is shown in a personal PM exposure assessment study of 19 asthmatic children living in inland San Diego County. The magnitude of correlation between personal temperature and ambient O_3 was far less than for central site temperature over a 14-day monitoring period (r = 0.50 for 8-hr O_3 and 1-hr maximum outdoor temperature, vs. r = 0.10 for 8-hr O_3 and 1-hr maximum personal temperature) (Delfino et al., 2003). There was no association between personal temperature and lung function in that study, but there were strong inverse associations between personal PM and lung function. Ambient O_3 was not associated with lung function but the study was designed to assess personal PM effects and had limited power to assess effects of central site exposures.

Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV_1 in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect* 2004; 112:932-41.

The text has been revised to incorporate these recommendations.

Specific comments:

12.1. Some relevant acute field studies were not discussed in this section, including studies conducted in California. These include:

Delfino RJ, Zeiger RS, Seltzer JM, Street DH. Symptoms in pediatric asthmatics and air pollution: Differences in effects by symptom severity, anti-inflammatory medication use, and particulate averaging time. *Environ Health Perspect*, 1998; 106: 751-61.

This study of schoolchildren with asthma in inland San Diego County showed significant associations between asthma symptoms (bothersome or interfered with daily activities) and O_3, with similar associations for minimum to 90th percentile 1-hr (58 ppb) and 8-hr O_3 maximums (46 ppb). Associations for O_3 and PM_{10} were largely independent in models incorporating both pollutants, and O_3 associations were not confounded by

outdoor fungal spores. The study also showed significantly stronger associations between asthma symptoms and O_3 in a subset of asthmatics not taking anti-inflammatory medications. Threshold analyses suggested effects below 80 ppb 1-hr O_3 maximum in this subset, but not among other subjects. 80 ppb 8-hr maximum O_3 was exceeded 25 times during the three-month study.

Mortimer KM**,** Tager IB, Dockery DW, Neas LM, Redline S. The Effect of Ozone on Inner-City Children with Asthma. Identification of Susceptible Subgroups. Am J Respir Crit Care Med 162:1838-1845 (2000).

Mortimer, et al. (2000) reported results of a series of 2-week asthma panels in 846 inner city children with asthma living in low income neighborhoods. They found that O_3 was inversely associated with PEF and positively associated with symptoms with the strongest associations among children born of low birth weight or premature.

Delfino RJ, Gong H Jr, Linn WS, Hu Y, Pellizzari ED. Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. *Environ Health Perspect* 2003; 111:647-656.

This study of Hispanic schoolchildren with asthma in LA showed significant associations between asthma symptoms (bothersome or interfered with daily activities) and ambient VOCs, PM_{10} elemental and organic carbon, but not O_3. However, O_3, along with formaldehyde and acetone were similarly associated with more severe symptoms interfering with daily activities among a subset of children, particularly those on maintenance medication. Odds ratios (OR) for interquartile increases in 1-hr O_3 (14 ppb) were identical to 8-hr O_3 (11 ppb) (both ORs around 2.0), even though 1-hr O_3 never exceeded 52 ppb. See Table 4 in that paper for details.

Discussion of these four papers has been added to the chapter.

12.1.3. Page 12-5:

The study by Gent and colleagues (2003) is large panel study with key findings. The review should put the findings of effect modification from maintenance medication into proper perspective. First, the biological mechanism of O_3 is in large part related to airway inflammation as discussed in the Toxicology section. Therefore, medication that controls airway inflammation such as inhaled corticosteroids would be expected to dampen the effects of O_3. However, finding the opposite in a panel study such as Gent et al. (2003) is not unexpected if use of such medication is largely restricted to more severe asthmatics, who are expected top be more susceptible to O_3. The results contrast findings of Delfino et al. (1998) showing significantly stronger association between asthma symptom severity and O_3 in asthmatic children <u>not</u> taking anti-inflammatory medications, largely inhaled corticosteroids (ICS). Mortimer, et al. (2000, discussed above) compared effects on asthma outcomes by outdoor O_3 levels across medication groups based on baseline data for prescribed medication. Associations between incidence of symptoms and an increase of 15 ppb in O_3 was largest among

those prescribed cromolyn but not ICS (OR 1.46, 95% CI 1.06, 2.01) followed by nonsignificant ORs for those prescribed ß-agonists or xanthines only (1.18), ICS (1.08), and no medication (1.04). The percentage change in PEF was also greatest among those prescribed cromolyn but not ICS (-1.27, 95% CI -2.47, -0.06) followed by nonsignificant PEF changes of around -0.5 for the other groups.

Discussion of these papers has been added to the chapter.

Section 12.2.

The review made the important point of describing residual confounding of ozone effects by the co-adjustment approach in time series models, and the lack of stratified analyses by season. This issue has not received adequate attention in the literature and may explain many null findings. These potential analytic weaknesses and control for temperature (see above) is particularly troubling for the null results in Los Angeles (Linn et al, 2000; Mann et al., 2002 and Nauenberg and Basu, 1999) suggesting that new studies and reanalysis of these studies are needed.

The committee concur with Dr. Bates that the Atlanta study by Friedman et al. (2001) is particularly important in suggesting that lowering ozone will have major benefits in reducing hospital admissions and ED visits. It is also important to point out that the effects detected in Atlanta were related to a reduction in traffic, which includes a wide range of toxic air pollutants including particle-bound in addition to ozone. Strong correlation between ozone and PM in Atlanta has made it impossible to separate effects of the two on asthma ED visits as reported by Tolbert (2000) reviewed in section 12-35.

Discussion of the Friedman et al. paper has been added to the chapter.

typo in title of Table 12-2 Hospital was misspelled.

This has been corrected.

The statement on p 12-36, third paragraph, lines 11-12 is unclear. What is meant by "self-selected" and "not quantitatively useful." All of these studies are subject to exposure misclassification, and air pollutant components (most unmeasured) could differ by season, year and geographic location. These factors will lead to inconsistencies. For instance, for the Delfino 1997a study, concentrations of PM_{10}, $PM_{2.5}$, SO_4, and H^+ were significantly higher during 1992 than 1993 due to sulfate transport episodes, and O_3 lower. Therefore, finding significant results in 1993 alone are not unexpected.

These points have been clarified.

12.4.2: Similar to section 12.2.1, the presentation of important issues to understand in time series analysis is excellent and provides thoughtful direction to further research. The criticisms of smoothing functions that include midrange temperatures of questionable clinical relevance are particularly informative and suggest that studies using this method may have underestimated the effects of air pollutants including ozone.

Thank you for the comment.

Appendix E

Summaries of Public Comments and Responses

Ozone Standard Review Staff Report
Summary of Comments (by Commenter)

Note: Comments are in regular type, and responses are italicized.

David Bates

Several suggestions/differences in emphasis related to specific health studies, but no disagreement with conclusions/findings.

1. Page 11-12: The point might be made that there is concordance between the dosimetric calculations of the target area for the highest concentration of ozone (the terminal bronchiole), and the observed morphological effects, which is the centriacinar region. The dosimetric calculations also indicate the higher delivered dose of ozone as the tidal volume increases, and this is consistent with the increased effects on exercise.

This point is addressed in the report in the section on the effective dose concept.

2. Page 11-15: The complex problem of the variation of effect with different time courses of ozone delivery is well described. The genetic basis for differences in sensitivity to ozone demonstrated in breeding experiments deserves more analysis.

We are unclear what breeding experiments the commenter is referring to. Although it is generally agreed that there is a genetic contribution to between-subject differences in sensitivity to ozone, research on this topic is just beginning. Investigations into possible contributions from several genes are underway, but data are not yet available.

3. Page 11-45: If the length of time between exposures is important, how can this be related to the time course of exposure that would usually occur to an exposed child? This is mentioned on Page 11-46: "The episodic nature of ambient exposure conditions in humans suggests that reliable assessments of risk must include a clear understanding of the impact of cyclic exposure ". There is no follow-up as to how this might be done.

The statement on p 11-46 was not meant to imply that we could quantitatively evaluate the effects of cyclic exposure in adults or in children, but to point out the responses observed in animals from cyclic exposure. Animal studies give some indications as to exposure intervals of concern, but unfortunately the data are not readily extrapolated to humans at this point.

4. Page11-48: First paragraph: the FEV1 has the smallest coefficient of variation, but the FEF25-75 is much more sensitive than the FEV1 to changes in terminal bronchioles. More emphasis on the work of Weinman on the small airway effects of ozone is needed. This is important to offset the early FVC change which is due to

stimulation by ozone of the C-fiber system – the changes in small airways are slower to resolve and very likely more important in terms of long term effects.

The issue of small airway effects has been alluded to with reference to several papers reviewed in the Staff Report, although it has not been discussed as a separate topic. We will add a section discussing small airway effects in the revised report. It should be noted that there is little literature on the effects of ozone on the small airways, and more research on this topic would be useful.

5. Page 11-51: Is it fair to assume that human variability in response to ozone is genetic in origin? What is the role of anti-oxidants such as superoxide dismutase? What about the protective effect of Vitamin C?

It is likely that the largest part of the variability is genetically based. Differences between individuals in superoxide dismutase or other anti-oxidant enzymes are largely genetically determined. Anti-oxidant vitamins have been shown to influence responsiveness to ozone, but do not necessarily fully mitigate responses to ozone exposure. Antioxidant vitamin supplements are likely a modifying factor, rather than a determinant factor in responsiveness.

6. Page 11-52: The reader should be told that although a single subject may have a meaningful threshold value for the effects of ozone, no such threshold is derivable for a group if a statistically significant shift in the mean is taken as the criterion of some effect.

This is true. A group mean value does not represent a population threshold. In fact, due to the variability between individuals, determining a population threshold implies finding the threshold level for the most responsive people, which is likely to be a very low concentration. We will clarify this point in the revised report.

7. Page11-87: Insufficient attention is given to the work of Frank, R. et al (Repetitive ozone exposure of young adults: evidence of persistent small airway dysfunction: Am J Respir Crit Care Med 164: 1253-1260; 2001). The reference is quoted on page 11-226. In evaluating acute exposure data, it is important to separate the early FVC effect due to stimulation of the C-fiber system, and the later and more persistent small airway effects as shown by these authors. Their work also suggests that the reduced effect of ozone on subsequent days after an initial effect is to be explained by the protective mucus layer induced by the inflammatory response to the exposure on the first day, which has the effect of diminishing the response on subsequent days. These observations are relevant to standard setting.

We will discuss this paper more fully in the section to be added on small airway function. However, it should be pointed out that the primary measure of small airway function used in this paper is a unique measurement that was developed by the investigators. It has not been validated or used in any other study. In addition, although the investigators speculate that increased mucus production may explain their findings,

there were no measurements made in the experiment that could support or refute the suggested mechanism.

8. Page11-92: The complex data on asthmatics is well described here.

Thank you for the comment.

9. Page 11-110: The emphasis on the joint ozone/allergen exposures is important, even though, as noted on the top of page 11-111, "they do not directly contribute to the evaluation of the level of the standard". It should be noted here that sequential exposures to ozone and allergens must be very common in real life situations.

We covered this information because it addresses a common exposure pattern, and one that explores a possible explanation for observations that asthmatics have higher risk of being admitted to the emergency room or hospital on high ozone days.

10. Page 11-112: Summary: the Southern California Children's study found that lung development, as judged by lung function tests, was being adversely affected by exposures to vehicle exhausts, but higher exposures to ozone were without effect.

These studies are discussed on pg 12-52, in the review of epidemiology studies. Although ozone effects on lung function were weak, associations between ozone exposure and other effects were found.

11. Page 11-114; second paragraph: the point might be made that exacerbations of asthma are now thought to be primarily inflammatory in nature and hence aggravation by ozone, which causes inflammation at very low doses, is to be expected.

We agree that it is quite likely that for many asthmatics, inflammation related to ozone exposure may represent an additive effect and be of particular concern. We will alter our text to reflect this point.

12. Page 11-127: Penultimate paragraph: might be better expressed as follows: "Chronic obstructive pulmonary disease, as well as chronic asthma, lead to nonuniform distribution of inhaled air in the lungs. This will have the effect of increasing the delivered dose of an inhaled pollutant to the regions of the lung which are relatively over-ventilated".

Thank you for the suggestion. We will consider the wording of the paragraph.

13. Page 11-149: The interaction between heat stress and the effects of ozone is important, and as noted below, there have been recent attempts to separate the higher mortality in heat waves into the deaths attributable to heat and the deaths attributable to the concomitant elevated ozone levels. Increased temperature leads

to increased ventilation, which in turn will increase the delivered dose of ozone to the lungs.

This point is well taken, and in fact heat may contribute to increased delivered dose. However, activity levels, especially outdoors, tend to be lower on very hot days. Also, the chamber studies that have investigated this topic did not find that concurrent heat exposure altered responses compared to those observed with completion of the same protocol at room temperature.

14. Page11-172: Second paragraph: note the work of Frank et al which suggests that the mucus secretion initiated by the first ozone exposure plays a part in lessening the effect (on FVC) of subsequent exposures. It should be noted that it is not clear whether successive exposures result in a reduced effect at the level of the small airways, although the work of Christian et al noted on Page 11-173 suggests that the effects on distal airways may also be attenuated. As noted on Page 11-174, whether this applies to lung tissue is unclear. These distinctions should be made clear in the Summary on page 11-174. My opinion is that the reduced FVC response on successive exposures cannot be assumed to indicate a reduction of effect in other parameters within the lung.

See comments above regarding the Frank et al. paper page 11-87. Christian et al. do not report small airway function data, although the bronchoalveolar lavage fluid analysis suggests that with four consecutive days of exposure to ozone some, but not all, inflammatory measures had shifted toward the normal range. However, the measured values suggest that after four days of exposure inflammation was still evident in the lower airways. We agree with the commenter's opinion that FVC is not necessarily representative of all responses. We will edit the text for clarity.

15. Page11-177: In the Summary, a reference should be given to the reduction in exercise performance noted at ozone levels of 0.06 ppm.

Thank you for the suggestion. We will add this to the document.

16. Page 11-198: Tokyo-Yokohama asthma was almost certainly due to high particulate and SO_2 levels and had nothing to do with ozone. It is not really relevant to this review.

Your point is well taken. We had it in the document because it was an early recognition that air pollution might affect asthma. We will clarify this in the document.

17. Page 11-200: Peden's observation about an increased eosinophilic response should be put earlier when the interaction of ozone and allergens was being reviewed.

Thank you for this suggestion. We will consider this.

18. Page 11-207: First paragraph: more emphasis should be given to this work in the interaction between combined O_3 and allergen exposures.

Although interesting, this material is not part of the basis for the standards recommendations; it serves as important supporting material. In addition, it is difficult to extrapolate between monkeys and humans so that the material could be used quantitatively.

19. Page 11-211: Pollutant mixtures: More discussion is needed on the factors affecting simultaneous exposure to ozone on the one hand, and to vehicle exhaust on the other. Perhaps a few paragraphs specifically on patterns of exposure would be helpful. This is because PM2.5 in the urban environment is associated with a variety of adverse health effects.

Little is known about combined exposure to particulate matter and ozone in human or animal subjects. The small amount of available literature suggests that ozone is more significant than particulate matter in inducing acute respiratory effects.

CHAPTER 12:

20. An important point should be mentioned at the outset, which is that it is now known that a peak in asthma attendances and admissions occurs in the third week of September. This was first documented in Vancouver (see Environ Research 51: 51-70; 1990 quoted in another context in the reference list here) but has since been shown by the group at McMaster (see ATS Abstracts) to occur across Canada. It is independent of air pollution, but may interfere with ongoing panel studies by obscuring an association with air pollution during other periods of the year. See Gent et al 2003 quoted here for a September asthma peak not detected by the authors, which might have affected their ongoing panel study. See annotation of the Gent study also in the second paragraph on Page 12-5.

This is not addressed in the epidemiological literature we reviewed. We will investigate this point, and revise the section appropriately. The fall peak in asthma would on average add noise to epidemiological studies but could also bias results of an individual study if by chance it correlated with either an episode (unlikely given the season) or a trough in ozone concentrations. We will note this in the document. For the studies that are of longer-term duration such as those examining hospital admissions, this should not have a major impact on the findings.

21. Page 12-3: In relation to data on PM2.5 and ozone in Mexico City, see comment on pg. 11-211.

See response to comment on pg. 11-211 above.

22. Page 12-4: A comment should be added to the note on Brauer's study that the ozone exposures were measured by personal badges as well as by an ozone monitor very close to the workers.

We will add this to the document.

23. Page 12-7: The recent study by Hall et al of the economic costs of school absences, based on the Gilliland study, might be noted here.

We can note the study. However, the Hall study examines the quantitative implication of the Gilliland study for the L.A. basin. We have conducted our own quantification using this study and others, using more recent and complete data.

24. Page12-23: I was surprised that no mention was made of the Atlanta study: FRIEDMAN, M.S., POWELL, K.E., HUTWAGNER, L., GRAHAM, L.M., & TEAGUE, W.G. Impact of changes in transportation and Commuting behaviors during the 1996 Summer Olympic Games in Atlanta on Air Quality and Childhood Asthma. JAMA 2001: 285; 897-905.

For many people, the documentation of a reduced adverse health effect synchronous with a reduced ambient ozone level constitutes very convincing evidence that the data being derived from epidemiological associations is real. My own opinion is that this study deserves special emphasis, not least when the effect of a possible "standard" is being discussed.

Thank you for pointing this out. Omission of this study was an oversight. We intend to add it to the next draft of the report.

25.Page12-25:This comment on the Petroeschevsky study in Brisbane fails to make two important points, first that it involved over 13,000 hospital admissions for asthma, and second that aerosol sulfates were not present so the effect was due to ambient ozone alone.

Thank you for this suggestion. We will add these two good points to the text.

26. Page 12-39: Last paragraph: "On this issue, the evidence is fairly supportive of independent effects for ozone". This is too weak a statement in my opinion. It should read: "On this issue, the evidence is conclusive that ozone is responsible for exerting direct effects" – see data from Mexico City and from Brisbane and Atlanta already discussed.

We will remove the word "fairly" and just say supportive.

Joint submission endorsed by: American Chemistry Council, California Business Properties Association, California Cement Manufacturers Environmental Coalition, California Chamber of Commerce, California Citrus Mutual, California Cotton Ginners Association, California Cotton Growers Association, California Farm Bureau Federation, California Independent Oil Marketers Association, California Independent Petroleum Association, California League of Food Processors, California Manufacturers and Technology Association, California Natural Gas Producers Association, Construction Materials Association of California, Council of Shopping Centers, Industrial Environmental Association, National Association of Industrial and Office Properties-California Chapters, Nisei Farmers League, Retail Industry Leaders Association.

1. There is not sufficient scientific support for the proposed 8-hr standard.

The commenter may misunderstand the CA definition of ambient air quality standards. In California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects (H&S Code 39014). The standards represent the greatest outdoor exposure that is acceptable. The number of people who experience these exposures is immaterial.

The averaging times have been selected to represent common exposure patterns. The 1-hr average standard relates to peak exposure concentrations, and also represents a frequent duration of outdoor activity for many people, for example, children playing after school, adults exercising, people doing yard work or home maintenance for a relatively short time period. In this case, the standard means that for a 1-hr exposure, the maximum average ozone concentration estimated to be without adverse consequences is 0.09 ppm. Likewise, the 8-hr average standard relates to both the ozone concentration profile frequently observed in down wind areas and the activity pattern of outdoor workers, and adults and children who spend multi-hour periods in outdoor activity, including work, play and recreation. In this case, the standard means that for an 8-hr exposure, the highest average concentration estimated to be without adverse effects is 0.070 ppm.

The concept of margin of safety includes the idea that a standard must be set at a level below the lowest concentration at which adverse effects have been documented to provide protection for potentially sensitive subjects who were not included in the study groups. Since State law requires that ambient air quality standards protect the most sensitive people in the population, we have looked not only at group mean responses, the basis of U.S. EPA developed ambient air quality standards, but have also evaluated individual responses. The scientific literature clearly shows that there is a very wide range of responses among individuals. This is not adequately factored into U.S. EPA ambient air quality standards. In the case of our 1-hr recommendation, multi-hour exposure studies did not find statistically significant responses with exposure to 0.10 ppm during the first one to two hours of a 6.6 to 8 hr exposure, while there are group mean and individual changes of concern with 2-hr exposure to 0.12 ppm ozone. This

suggests a threshold in exercising people somewhere below 0.12 ppm and above 0.10 ppm for one to two hour exposures. We have included a margin of safety, and recommended a 1-hr standard of 0.09 ppm.

The body of findings from studies of 6.6 hr exposures to 0.08 ppm ozone indicates that about 26% of people who undergo similar exposures will experience symptoms and pulmonary function decrements of 10% or larger, with some experiencing decrements in excess of 30%. Since responses are related to the inhaled dose, larger decrements, and a larger fraction of people experiencing effects would be expected if the exposure period had been extended from 6.6 to 8 hours. This led to the conclusion that an 8-hr average concentration of 0.08 ppm was not adequately protective of public health. The few data available suggested that multi-hour exposure to 0.04 or 0.06 ppm ozone was unlikely to result in adverse responses.

In the case of the 8-hr average recommendation, there is less guidance for determining an adequate margin of safety, since only one chamber study at 0.04 ppm, and one at 0.06 ppm have examined responses to ozone concentrations below 0.08 ppm. Both studies found no significant pulmonary function or symptoms effects at the group level, although there were a few individual responders at 0.06 ppm. The margin of safety is supported by several epidemiologic studies, which report associations between ozone and a wide range of severe health outcomes. While we agree that this margin of safety is a more uncertain estimate than available for the 1-hr average standard, it incorporates consideration of all available data.

The primary health endpoints from the chamber studies used to develop these recommendations are acute responses (decrements in pulmonary function, respiratory symptoms, airway hyperreactivity and airways inflammation). Reduced lung function is not a benign effect because it is due to a neural reflex, as asserted by some commentators. Activation of the neural reflex represents an attempt by the body to limit inhalation of a toxic substance, in this case ozone, to protect the airway lining tissues from oxidant damage, and resulting airway inflammation. Furthermore, reduced lung function and symptoms can reduce ability to work, as well as to participate in healthful exercise and recreation. These seemingly minor effects, temporarily reduced lung function and symptoms, can impact on ability to earn a living, and to maintain a healthy lifestyle, and clearly qualify as adverse by ATS standards, both physiologically and as aspects of quality of life. Asthmatics already have underlying chronic airway inflammation and reduced lung function. The additional ozone insult to the airway can result in exacerbation of asthma. Children are disproportionately impacted by asthma as they have higher prevalence rates, and the highest hospitalization rates are for 0-4 year olds. This is likely due at least partially to physics – the airway resistance is inversely proportional to the 4th power of the radius. Thus in a small child a little airway constriction can result in serious breathing difficulty. This will be clarified in the revised report

In addition, repeated episodes of airway inflammation lead to morphological changes in the lungs, and may contribute to long-term respiratory health impacts. Animal studies

clearly support this line of reasoning. There is also convincing evidence that children who grow up in high ozone communities have lower lung function values at maturity than children who grow up in low ozone communities (Kunzli et al., 1997; Galizia and Kinney, 1999). This is a significant finding, in that low lung function is a known risk factor for chronic lung disease and premature death. The epidemiologic studies include such endpoints as premature mortality, hospitalization for respiratory and cardiovascular disease, emergency room visits for asthma, and respiratory symptoms.

Although no directly stated, this comment may include concern that ARB/OEHHA and U.S. EPA have recommended different ozone standards. There are several differences in the California standard review process that may clarify for the commenter why California and the U.S. EPA review the same literature and arrive at different recommended standards. California law requires that the standards protect the most sensitive subgroup of the population. This requires that we consider the range of individual responses to different exposure protocols to understand the range of variability in the population as a whole, and then to base our recommendations on the sensitive sub-group. In contrast, U.S. EPA primarily looks at group mean responses, with little consideration of the variability among individuals.
Second, California standards are based solely on health considerations, not on risk analysis. As noted above, our model, set by State law, is for selection of a concentration and averaging time combination that is unlikely to induce adverse effects in anyone who happens to undergo that exposure pattern. The exposure patterns used are based on a combination of patterns identified by ambient air quality monitoring, and on likely outdoor activity patterns. California standard setting does not consider the likelihood of exposure. As noted above, in California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects. Furthermore, the proposed standards are based on responses of subject groups most likely to have significant exposure – people who are active outdoors.

Third, when EPA last considered the ozone standard in 1996/1997, there were far fewer epidemiologic studies showing severe outcomes associated with ozone exposure.

2. The proposed 8-hr average is not the appropriate form for such a standard.

The California Code of Regulations (Title 17 section 70200) establishes the form of the ambient air quality standard for ozone as "not to be exceeded". The Expected Peak Day Concentration methodology used for area attainment designations is defined in Title 17 of the California Code of Regulations section 70306 Appendix 2. This section is unrelated to the section of the Health & Safety Code that has been opened in the present regulatory action. The EPDC method for attainment designation can be changed, but a completely separate regulatory action would be required from that for standard review. We have not opened the attainment designation procedure for review, and have no plans to do so.

3. The proposed standard would be lower than relevant background concentrations and as such is not attainable.

Our analysis determined that 0.04 ppm is a reasonable average background ozone concentration. This value is in agreement with the conclusions of the 1996 U.S. EPA ozone criteria document, and also with the World Health Organization's 2000 document outlining Air Quality Criteria for Europe. See page 50 for an in-depth discussion of the comments received on background ozone (Chapter 4).

American Petroleum Institute & Western States Petroleum Association & Paul Switzer, Stanford University

1. The epidemiological studies of ozone and mortality use inadequate models. The report does not adequately address the statistical concerns with these models.

See #3 below.

2. It is not appropriate to use PM epidemiological studies to assess acute ozone mortality effects.

We agree that there are methodological issues with the ozone epidemiology literature, and these are discussed and acknowledged in the Staff Report. However, the recommended standards are not based primarily on epidemiology. They are based on controlled human exposure studies. Epidemiologic studies do figure into the margin of safety considerations since they strongly suggest the possibility of severe health outcomes. Thus, even if there are uncertainties about the actual effect level, measurement error, and treatment of weather and time trend, these studies are too numerous and the effects too severe to be ignored.

3. Epidemiological studies about the effects of ozone exposure on mortality and other serious health endpoints need further analysis.

API provided a commentary that points out several issues relative to time-series studies, and seeks to discredit the findings, primarily of ozone mortality studies. While the issues the commenter raises are not new, it is interesting that they focus on epidemiology and mortality, in that neither epidemiology nor mortality formed the primary basis for the standard recommendations. In the past 8 to 10 years, the focus of air pollution epidemiology has been PM. The issues the commenter raised have been investigated at length with reference to PM. Unfortunately, few studies have been designed with ozone-related hypotheses, and consequently few of the issues raised have been adequately investigated with reference to ozone. This is acknowledged in the Staff Report, which includes considerable discussion of statistical modeling issues associated with the epidemiologic literature on ozone. The Staff Report also addresses other modeling issues not mentioned by the commenter as they relate to the different types of epidemiology studies discussed in the Staff Report.

The commenter uses the NMMAPS study to support the view that ozone effects are highly variable between cities, and consequently uncertain. However, greater variability may be expected among cities simply because the effect estimate is so low and small variation in co-factors may exert more influence. This is why meta-analytic results from a large set of cities are used in preference to results from single cities. Variability is not a good enough reason to discount all of the studies. While NMMAPS (which is primarily a PM study that reports a few results for ozone) is an important study, the observation that it found a number of negative associations for ozone suggests that some of the modeling methods used may not fully control for seasonality and time trend.

It is likely that statistical modeling designed to remove weather confounding in PM studies is not the same as what would be used to control for weather confounding relative to ozone, and that the modeling requirements for removing this confounding vary by weather pattern between geographical areas. Most ozone results, particularly for mortality, come from studies that have been modeled for PM effects, and were part of the analysis of possible confounding factors, not primary analyses. Evaluation of the controlled exposure literature clearly shows that there is no biological plausibility for the reported negative effect, implicating inadequate statistical modeling and perhaps measurement error of exposure.

The commenter also asserts that heat and humidity effects may totally confound the effects of ozone. However, it is unlikely that weather would totally explain away these effects. The existing time series studies suggest that the temperature effect is very immediate; mortality usually occurs on the day of or day after high temperature. Humidity doesn't appear to play an independent role (Schwartz et al., 2004). Most existing studies carefully control for these effects and still report an independent effect of ozone. Also, summer-specific studies also report effects of ozone. Regardless, temperature, of course, peaks in the summer while mortality peaks in the winter so the correlation between the two is usually very low or negative; therefore, failure to control for temperature is unlikely to generate a positive association between ozone and mortality. In fact, as reported in our recommendation document, a study by Thurston and Ito showed that when weather was modeled most carefully using non-linear functions, the effect estimate for ozone increased. Thus, it does not appear that temperature is responsible for reported associations between ozone and mortality. In addition, ozone is often elevated in a given city for several days, and not all ozone excursions are accompanied by temperatures that are high enough to cause mortality. Regarding interactive effects, human studies on this subject indicate that concurrent heat exposure does not impact responses to ozone. NMMAPS used basically the same weather and time trend modeling methodology in all cities, regardless of the local weather patterns, which may not adequately address differences in pattern among cities. It is likely that If the weather and time parameters had been modeled correctly and differently in each city, there may have been fewer negative, biologically implausible results for some cities. There are many possible explanations for the heterogeneity in the effect estimates and the fact that Samet et al. could not identify any effect modifiers is not evidence that they don't exist. Only six general socioeconomic status (SES) variables were tested. Factors such as monitor placement, spatial

variability, SES, background health status, use of air conditioners, and housing characteristics all could contribute to heterogeneity in response. Finally, while it is possible that no benefits would result from ozone reductions, the existing meta-analysis of studies suggest that, on average, health benefits would occur. A few null findings cannot lead us to ignore all of the positive findings and the meta-analysis results.

The commenter raises the issue of heterogeneity of ozone exposure within a study area. Since ambient ozone is a regional pollution, most studies that have examined this issue report fairly similar concentrations and a high intra-city correlation among monitors on a daily basis. Regardless, random exposure measurement error would tend to reduce the likelihood of finding an effect, and would be unlikely to result in a positive and significant association.

The commenter raises the issue of a possible non-linear effect of ozone and mortality. Previous studies have suggested that the functions look fairly linear in response. However, it is true that if there is significant measurement error in exposure, it will be more difficult to find a threshold if one exists. However, as discussed above, an absolute threshold at the population level is unlikely. In addition, we are not able to reestimate the functions that have been reported in many of these studies. Finally, if the models are, in fact, non-linear, the resulting positive slope estimate would have to be larger than that produced by the linear function. This increase may fully offset the application of a threshold.

The commenter raises the issue of lag selection and ozone averaging time as impacting interpretation of ozone health effects. Based on human and animal studies, ozone effects would be expected within a day or two of exposure. There are also some studies, which suggest greater effects from cumulative exposures over 3 to 5 days. This has been considered in our interpretation of the literature. Further, since all ozone averaging times (i.e., 1, 8, 24 hr) are highly correlated, it is difficult to use epidemiology results to determine the specific averaging time of interest. As noted above, epidemiology literature is not the primary basis for either the concentrations or averaging times recommended. Epidemiology was used in a qualitative manner, as support for the controlled exposure studies.

The commenter points out that staff has not addressed mortality displacement in the Staff Report, and that this is necessary if epidemiologic studies are to be used as the basis for ambient air quality standards. It is true that we have not discussed this topic in the report. However, issues of displacement are more appropriate when one is attempting to determine the amount of life years lost and for economic valuation issues. It is not necessarily relevant for standard setting purposes.

4. A more precise and quantitative definition of adverse effects is needed.

Adverse effects were evaluated in accordance with the American Thoracic Society guidelines outlined in the Staff Report. An effect was considered significant if it was large enough to reduce or limit work or exercise capacity, or was sufficient to impact

quality of life. Obviously, some of the categories suggested in the guidelines do not pertain to effects observed with ozone exposure; however, we believe that we have appropriately applied the recommended criteria.

5. Further justification for the Staff Report's recommendations is needed.

In California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects (H&S Code 39014). The standards represent the greatest outdoor exposure that is acceptable. The number of people who experience these exposures is immaterial.

The averaging times have been selected to represent common exposure patterns. The one hour average standard relates to peak exposure concentrations, and also represents a frequent duration of outdoor activity for many people, for example, children playing after school, adults exercising, people doing yard work or home maintenance. In this case, the standard means that for a 1-hr exposure, the maximum ozone concentration estimated to be without adverse consequences is 0.09 ppm. Likewise, the 8-hr average standard relates to both the long, lower concentration, broad ozone concentration profile frequently observed in down wind areas, and also reflects the activity pattern of outdoor workers, and adults and children who spend multi-hour periods in outdoor activity, including work, play and recreation. In this case, the standard means that for an 8-hr exposure, the highest average concentration estimated to be without adverse effects is 0.070 ppm.

The concept of margin of safety includes the idea that a standard must be set at a level below the lowest concentration at which adverse effects have been documented, to provide protection for potentially sensitive subjects who were not included in the study group. Since state law requires that ambient air quality standards protect the most sensitive people in the population, we have looked not only at group mean responses, the basis of U.S. EPA developed ambient air quality standards, but have also evaluated individual responses. The scientific literature clearly shows that there is a very wide range of responses among individuals. This is not adequately factored into U.S. EPA ambient air quality standards. In the case of our 1-hr recommendation, multi-hour exposure studies did not find statistically significant responses with exposure to 0.10 ppm during the first one to two hours of exposure, while there were group mean and individual changes of concern with 2-hr exposure to 0.12 ppm ozone. This suggests a threshold in exercising people somewhere between 0.10 and 0.12 ppm for one to two hour exposures, the same conclusion reached in the 1987 review of the State ozone standard. We also concluded that the margin of safety applied in the existing State ozone standard was adequate, and recommended retention of the existing 1-hr standard of 0.09 ppm.

In the case of the 8-hr average recommendation, there is less guidance for determining an adequate margin of safety, since there is only one study at 0.04 ppm, and one at 0.06 ppm. The body of findings from studies of 6.6 to 8 hr exposures to 0.08 ppm ozone indicates that about 26% of people who undergo similar exposures will experience

symptoms and pulmonary function decrements of 10% or larger, with some experiencing decrements in excess of 30%. The study at 0.04 ppm found no significant pulmonary function or symptoms effects. Unfortunately, the one study at 0.06 ppm has not appeared in the peer-reviewed literature, although it has been published as a research report. The data on 6.6 to 8 hr exposures led to the conclusion that an 8 hr average concentration of 0.08 ppm was not adequately protective of public health, and that multi-hour exposure to 0.04 or 0.06 ppm ozone was unlikely to result in adverse responses. The epidemiological study by Tolbert et al. (2000), one of the few available that used an 8-hr averaging time, examined the shape of the concentration response function and found evidence for a population threshold in the ozone concentration range of 0.070 to 0.10 ppm. We selected the bottom of this range as the margin of safety. While we agree that this margin of safety is a more uncertain estimate than available for the 1-hr average standard, it incorporates all of the available data, and is substantially based on controlled human exposure data.

The primary health endpoints used to develop these recommendations are acute responses (decrements in pulmonary function, respiratory symptoms, airway hyperreactivity and airways inflammation). Reduced lung function is not a benign effect because it is due to a neural reflex. Activation of the neural reflex represents an attempt by the body to limit inhalation of a toxic substance, in this case ozone, to protect the airway lining tissues from oxidant damage, and resulting airway inflammation. Furthermore, reduced lung function and symptoms can reduce ability to work, as well as participate in healthful exercise and recreation. These seemingly minor effects, temporarily reduced lung function and symptoms, impact on ability to earn a living, and to maintain a healthy lifestyle, and clearly qualify as adverse by ATS standards, both physiologically and as aspects of quality of life. Repeated episodes of airway inflammation lead to morphological changes in the lungs, and may contribute to long-term respiratory health impacts. Animal studies clearly support this line of reasoning. There is also evidence that children who grow up in high ozone communities have lower lung function values at maturity than children who grow up in low ozone communities (Kunzli et al., 1997; Galizia and Kinney, 1999). This is a significant finding, in that low lung function is a known risk factor for chronic lung disease and premature death. Furthermore, asthmatics already have underlying chronic airway inflammation and reduced lung function. The additional ozone insult to the airway can result in exacerbation of asthma. Children are disproportionately impacted by asthma as they have higher prevalence rates and the highest hospitalization rates are for 0-4 year olds. This is likely due at least partially to physics – the airway resistance is inversely proportional to the 4th power of the radius. Thus in a small child a little airway constriction can result in serious breathing difficulty.

There are several differences in the California standard review process that may clarify for the commenter why California and the U.S. EPA review the same literature and arrive at different recommended standards. California law requires that the standards protect the most sensitive subgroup of the population. This requires that we consider the range of individual responses to different exposure protocols to understand the range of variability in the population as a whole, and then to base our recommendations

on the sensitive sub-group. In contrast, U.S. EPA primarily looks at group mean responses, with little consideration of the variability among individuals.

Second, California standards are based solely on health considerations, not on risk analysis. As noted above, our model is for selection of a concentration and averaging time combination that is unlikely to induce adverse effects in anyone who happens to undergo that exposure pattern. The exposure patterns used are based on a combination of patterns identified by ambient air quality monitoring, and on likely outdoor activity patterns. California standard setting does not consider the likelihood of exposure. As noted above, in California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects. Furthermore, the proposed standards are based on responses of subject groups most likely to have significant exposure – people who are active outdoors.

Third, when EPA last considered the ozone standard in 1996/1997, there were far fewer epidemiologic studies showing severe outcomes associated with ozone exposure.

6. Additional research on human subjects in the range of 0.04 to 0.08 ppm (multi-hour exposures) is needed.

We agree that additional research at lower exposures would be informative. However, this does not negate the evidence from studies done at 0.08 ppm that a substantial fraction of the population (26%) is likely to experience pulmonary function decrements greater than 10% as well as symptoms if they undergo 6.6 hr exposures to 0.08 ppm. It should also be noted that we are proposing an 8-hr averaging time, which is longer than that of the studies on which the standard recommendation is based. Consequently, due to the larger inhaled dose of ozone, a larger portion of the population would be expected to have decrements greater than 10% and have symptoms when exposure is extended from 6.6 hr to 8 hr.

7. Additional quantification of the uncertainties, individually and in combination, is warranted and needed.

The uncertainties in epidemiological findings are discussed in the Staff Report, and because of them, the epidemiological data were used in a <u>qualitative</u> fashion, supporting the <u>quantitative</u> findings of the controlled exposure studies. It is unclear what the commenter means by the uncertainties in controlled study results. The exposure and protocol conditions in these studies are very closely controlled, and consequently the inhaled ozone dose can be accurately estimated. On an individual level, responses to ozone are very consistent over time periods of at least one year (section 11.4.2.1.4). The range of responsiveness between individuals has also been investigated, and been shown to be very wide at all ozone concentrations investigated (section 11.4.2.1.4). For example, the range of FEV1 response with 6.6 hr exposure ranged from +10% to –40% with exposure to 0.08 ppm ozone; from +5 to –45% with exposure to 0.10 ppm ozone; and from +5% to –50% with exposure to 0.12 ppm ozone. The ranges are similar for shorter exposures with somewhat higher ozone concentrations. This information is

presented in section 11.4.2.1.4 of the Staff Report. In addition the range of individual responses is presented in the review of studies and the tables of Chapter 11 of the Staff Report.

8. The background ozone concentration is not 0.04 ppm, but is frequently much higher.

See page 50 for an in-depth discussion of the comments received on background ozone (Chapter 4).

Our analysis determined that 0.04 ppm is a reasonable average background ozone concentration. This value is in agreement with the conclusions of the 1996 U.S. EPA ozone criteria document, and also with the World Health Organization's 2000 document outlining Air Quality Criteria for Europe.

9. The exposure scenarios used in controlled studies do not reflect ambient conditions, particularly the square wave multi-hour protocol. This protocol does not consider the non-linear dose-response relationship, or that responses are related to the dose rate, not just the concentration.

The commenter is correct that the dose rate is more important than the concentration alone. This is why we have recommended two standards, one with a 1-hr averaging time, and one with an 8-hr averaging time. This will insure that during any eight hour period that meets the 0.070 ppm 8-hr standard, there will be no hour with an average ozone concentration over 0.09 ppm.

10. Controlled studies should not compare response consequent to ozone exposure with that after filtered air exposure. The baseline should be background.

The commenter asserts that the baseline for comparison of effects should be background (i.e., 0.04 ppm) rather than filtered air because responses are related to the <u>change</u> in ozone concentration, not the concentration itself. This is erroneous. The biological responses caused by ozone are not linear functions, as the commenter apparently assumes, but rather are exponential. The human exposure data clearly indicate that responses to ozone exposure are proportional to the inhaled <u>dose</u> of ozone, which is the product of ozone concentration, breathing rate, and exposure duration. Consequently, a very large number of exposure scenarios can be invented that would result in an inhaled dose that is likely to induce adverse responses. Although there are no data suggesting effects at 0.04 ppm, it is theoretically possible that a sufficiently long exposure with a high exercise level could result in an inhaled dose that is large enough to induce adverse effects, but based on available data, this is unlikely. The data also point to the existence of a threshold, particularly on the individual level, which appears to be below 0.12 ppm for 1 to 3 hour exposures, in heavily exercising subjects, and 0.08 ppm for 6.6 hour exposures, in moderately exercising subjects. Since 0.04 ppm appears to be below the threshold, use of 0.04 for the baseline for calculating responses to ozone exposure would be unlikely to change the conclusions reached.

11. It appears that staff has turned to analysis of ambient air quality to show the relationship between exposure for the 1-hr standard and alternative concentrations for an 8-hr standard.

Perhaps the commenter has misunderstood the purpose of the analysis of the relationship between 1-hr and 8-hr average ozone concentrations. The recommendations were based on the health literature. The analyses comparing the 1-hr (0.09 ppm) and recommended 8-hr (0.070 ppm) standards were presented to indicate the relationship between the two standards. The analysis indicated that either standard by itself would not be protective of public health. Specifically, an area could attain one standard but still be out of attainment relative to the other standard. Therefore, we recommended adding an 8-hour standard while retaining the 1-hour standard. We will revise the Staff Report to prevent this misunderstanding.

12. The linear rollback method is not appropriate.

The rollback method was developed using actual monitored data from California, and represents the behavior of real data. The methodology and data tables and figures that support the approach are presented in the appendix to chapter 10 of the Staff Report.

13. The 8-hr proposed standard is not attainable.

The proposed 8-hour standard will be difficult to attain. However, California law does not require that ambient air quality standards be based on ease of attainability; it requires that they be based on health effects.

Alliance of Automobile Manufacturers

1. The appropriate measurement of background ozone must be considered part of the proposed AAQS. The proposed standards are at or overlap background.

Our analysis determined that 0.04 ppm is a reasonable average background ozone concentration. This value is in agreement with the conclusions of the 1996 U.S. EPA ozone criteria document, and also with the World Health Organization's 2000 document outlining Air Quality Criteria for Europe. See page 50 for an in-depth discussion of the comments received on background ozone (Chapter 4).

2. The correlation between measured clinical health effects and impact on public health has not been established.

This issue has several parts. First, the commenter states that a new mechanism for pulmonary function decrements and respiratory symptoms has been reported - a vagal nerve reflex. Two recent papers are cited along with the claim that this is a recent finding. Actually, the vagal nerve reflex contribution to responses to ozone has been known since the 1970's. The commenter appears to believe that since a nerve reflex

mechanism is involved, there is no reason for concern. However, the reflex is a protective response to an inhaled irritant, the purpose of which is to reduce exposure of the lung tissue to the irritant. In other words, the body recognizes that inhaled ozone is potentially injurious, and attempts to reduce inhalation, and thereby exposure, by reflexively reducing lung function and tidal volume. This, in turn, can reduce work capacity. Decrements in lung function in response to ozone can be large, and can also contribute to exacerbations of lung disease including asthma. The argument that a reflex response does not represent an adverse response that is significant is incorrect.

Second, the commenter asserts that the baseline for comparison of effects should be background (i.e., 0.04 ppm) rather than filtered air because responses are related to the change in ozone concentration, not the concentration itself. This is erroneous. The biological responses caused by ozone are not linear functions, as the commenter apparently assumes, but rather are exponential. The human exposure data clearly indicate that responses to ozone exposure are proportional to the inhaled dose of ozone, which is the product of ozone concentration, breathing rate, and exposure duration. Consequently, a very large number of exposure scenarios can be invented that would result in an inhaled dose that is likely to induce adverse responses. Although there are no data suggesting effects at 0.04 ppm, it is theoretically possible that a sufficiently long exposure with a high exercise level could result in an inhaled dose that is large enough to induce adverse effects, but based on available data, this is unlikely. The data also point to the existence of a threshold, particularly on the individual level, which appears to be below 0.12 ppm for 1 to 3 hour exposures, in heavily exercising subjects, and 0.08 ppm for 6.6 hour exposures, in moderately exercising subjects.

The commenter raises issues with the Staff conclusions as to sensitive subpopulations, and asserts that the Staff Report concludes that young adults are the most sensitive population for pulmonary function decrements and symptoms, that older adults and children are less sensitive, and that COPD patients and smokers are unlikely to experience marked respiratory effects. This argument involves several misconceptions. Responses to ozone are related to the inhaled dose, not solely to the concentration. The Staff Report concluded that children, people who are active outdoors, and outdoor workers were most likely to inhale sufficient doses of ozone to induce adverse effects. While data suggest that older adults have smaller pulmonary function and symptoms responses than similarly exposed young adults, there are individual exceptions, and there are no data on airway reactivity or inflammation on older adults. However, older adults who have reduced pulmonary function with ozone exposure typically also have symptoms, as well. Consequently, a complete picture of the risks to active older adults is not available in the current literature. The available data on children suggest that they have similar pulmonary function changes as young adults who inhaled comparable doses of ozone, but they tend to report few symptoms. There are no data available from chamber studies on airway responsiveness or inflammation for children, although there is no reason to think that they would not have responses similar to those of adults. The commenter misinterprets the Staff Report statement regarding the lack of symptoms reports by children, asserting that their lack of reported symptoms indicates lower risk. The few controlled studies on children have involved children from about 8 to 12 years

of age. In reality, there are several possible explanations for this largely uninvestigated topic. It is unknown whether children really don't have symptoms, are unwilling to articulate them due to social concerns that they might disappoint the investigators, or whether they are unable to understand or articulate them. In any case, this difference in responses between adults and children is of concern from a risk management perspective, because for whatever reason, children appear to have little appreciation that their bodies have activated reflex induced pulmonary function decrements as a means to reduce toxic exposure.

Of additional importance is that asthma is an important health endpoint for children. Prevalence rates of asthma are higher in children than adults, and children 0-4 years old have the highest hospitalization rates of all age groupings. Small children have small airways and thus are more prone to breathing difficulties due to the relationship between airway caliber and resistance. Thus, children are disproportionately impacted by air pollutants that exacerbate asthma. Ozone can exacerbate asthma and may induce asthma in children who are very active outdoors (McConnell et al., 2002).

The commenter raises several issues relative to the discussion of morphological effects. While it is not clear how to extrapolate findings of animal studies to likely human responses, the fact that similar changes in morphometry have been observed in multiple animal species, albeit with differences in apparent sensitivity, makes it likely that similar responses also occur in humans. Sections 11.3.3 and 11.3.4 discuss responses of animals to long term ozone exposure and also the influence of the interexposure interval with repeated acute exposures on morphological responses. The text makes clear that the time sequence of repeated exposures affects tissue responses, and that the timing of a repeat exposure relative to the status of the injury-repair cycle influences the outcome. It is not entirely true that responses diminish over time. Animal studies clearly show that repeated acute exposures can have residual effects that accumulate over time.

It is unclear why the commenter asserts that there is no likelihood that the population most at risk (people who are active outdoors) will experience a large number of repeated peak exposures. People who are regularly active outdoors will experience a significant number of repeated high exposures if they live in areas with more than a few annual exceedances of the ozone standard (for example, the South Coast Air Basin, Sacramento, and the San Joaquin Valley). True, many people spend most of their time indoors. But the population of California includes a large number of children, many of whom spend a significant amount of time outdoors, many recreational athletes, and outdoor workers. These people will experience multiple peak exposures per year by virtue of their lifestyle patterns. The southern California Children's Study suggests that ozone may induce asthma in very active children (McConnell et al., 2002). The fact that ozone concentrations have declined considerably over the past 40+ years does not negate scientific data indicating that significant adverse effects are still possible in people who inhale a sufficient dose of ozone. The magnitude of these effects may be smaller due to the lower peak ozone concentrations currently observed, but this does

not alter the conclusion that current ozone concentrations can induce adverse responses in people who inhale a sufficient dose due to their activity patterns.

The commenter requests that the issue of an effect threshold be discussed more fully. While the Staff Report does not explicitly use the term threshold in discussion of the controlled exposure studies, it does clearly present the lowest effect levels found in the available literature for all available endpoints, and details them both in the summary of the controlled studies chapter, and in the recommendation (Chapter 8).

The commenter requests modification of a sentence in the final paragraph of the chapter summary that refers to epidemiology studies and their limitations. The subject of the limitations of epidemiology studies is discussed at length in Chapter 12, which addresses at length the concern of the commenter.

3. The inherent weaknesses in epidemiology studies need to be formally recognized.

We have attempted to outline the various weaknesses of the epidemiology studies in each of the four major sections of the epidemiology chapter, including extensive discussion of uncertainties, issues related to statistical modeling, and potential weaknesses of epidemiology studies in general, as well as specific to individual studies that impact on the conclusions that can be drawn from the literature. It is true that this chapter is not as comprehensive as the controlled studies chapter. This is because we relied primarily on the chamber studies for the development of the standard with the epidemiology studies playing a supportive role and weighing in on the margin of safety. Regarding the issue of GAM-related problems in the mortality studies, many of these studies have now been reanalyzed, although mostly for PM. The general conclusion from this reanalysis is that, for the most part, using other smoothing functions such as penalized or natural splines does appear to drastically alter the general results. In some cases the estimated effect estimate falls and in some cases it rises or stays about the same. In multi-city analyses, the general findings are often the same as the original GAM results. In general, the ozone studies have not undertaken as much examination. However, new analyses of the NMMAPS focusing on mortality confirms an association between ozone and premature mortality, with an effect estimate generally similar to that previously reported.

We agree that the statistical modeling strategy is extremely important in evaluating and interpreting these studies. This is why the chapter spends quite a few pages discussing modeling and interpretation issues as they relate to each of the four topical categories of studies evaluated. But two general findings seem apparent: (1) that the results do not appear to change when other smoothing models are used, including parametric smoothing techniques; and (2) that, in general, more careful control of weather tends to increase the size and statistical significance of the ozone effect.

Publication bias is unlikely an issue with this literature, as almost all of it was designed to investigate PM effects, and any ozone results presented were part of the sensitivity analyses and investigation of potentially confounding factors relative to the main focus,

PM. Consequently, there is little reason to suppose that negative findings have been suppressed. In fact, there is reason to suppose that any ozone related findings would be presented to show that the PM results were not influenced by ozone. In addition, the new analysis of the NMMAPS data on ozone has been recently published, and this is a study that inherently has no publication bias. Finally, the WHO has adjusted their estimates of their meta-analysis of European studies to address the possibility of publication bias. They still report an association between ozone and both all-cause and cardiovascular mortality.

4. Chapter 7 is an analysis of potential peak exposure, not actual exposure. Exposure analysis should include consideration of the probability that a person will receive an exposure of concern.

The comment suggests a misunderstanding of the purpose of Chapter 7. The chapter is an analysis of statewide air quality. It gives an indication of the number of people who live in areas where ozone concentrations reach the level of concern. It is not a risk analysis. The source of confusion may be that the Health & Safety Code calls this sort of characterization of statewide air quality "exposure", although it is not exposure in the sense of personal exposure assessment.

5. The staff recommendation is not adequately substantiated. The selected margin-of-safety interval has not been quantified or substantiated.

The commenter begins this topic by disagreeing with identification in the year 2000 of the standard for ozone as being possibly inadequate (SB25 standard prioritization process). The prioritization process involved a brief review of recent scientific literature, and a determination as to whether or not there was evidence that the various air quality standards might be inadequate, particularly in regards to infants and children. Standards deemed possibly inadequate were prioritized for full review, partially based on the frequency of exceedences of the existing standards, as well as the sorts of effects identified. Chapter 7 clearly shows that most Californians live in areas where peak ozone concentrations frequently exceed the current state standard. Personal exposure is not the issue here.

The commenter seems to misunderstand the meaning of ambient air quality standards. In California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects (H&S Code 39014). The standards represent the greatest outdoor exposure that is acceptable. The number of people who experience these exposures is immaterial. The commenter recommends inclusion of a risk analysis, such as performed by U.S. EPA. Such an analysis is not required for California ambient air quality standards. California ambient air quality standards are based solely on health effects, and as noted above, the risk of exposure has no bearing on what constitutes the maximal exposure that is unlikely to induce adverse responses. Such an analysis would not change the conclusions Staff has drawn.

The averaging times have been selected to represent common exposure patterns. The one hour average standard relates to peak exposure concentrations, and also represents a frequent duration of outdoor activity for many people, for example, children playing after school, adults exercising, people doing yard work or home maintenance. In this case, the standard means that for a 1-hr exposure, the maximum ozone concentration estimated to be without adverse consequences is 0.09 ppm. Likewise, the 8-hr average standard relates to both the long, lower concentration, broad ozone concentration profile frequently observed in down wind areas, and also reflects the activity pattern of outdoor workers, and adults and children who spend multi-hour periods in outdoor activity, including work, play and recreation. In this case, the standard means that for an 8-hr exposure, the highest average concentration estimated to be without adverse effects is 0.070 ppm.

The concept of margin of safety includes the idea that a standard must be set at a level below the lowest concentration at which adverse effects have been documented to provide protection for potentially sensitive subjects who were not included in the study group. Since state law requires that ambient air quality standards protect the most sensitive people in the population, we have looked not only at group mean responses, the basis of U.S. EPA developed ambient air quality standards, but have also evaluated individual responses. The scientific literature clearly shows that there is a very wide range of responses among individuals. This is not adequately factored into U.S. EPA ambient air quality standards. In the case of our 1-hr recommendation, multi-hour exposure studies did not find statistically significant responses with exposure to 0.10 ppm during the first one to two hours of exposure, while there were group mean and individual changes of concern with 2-hr exposure to 0.12 ppm ozone. This suggests a threshold in exercising people somewhere between 0.10 and 0.12 ppm for one to two hour exposures. We have included a margin of safety, and recommended a 1-hr standard of 0.09 ppm because the total population studied at these concentration was small, and would not have included people who represent the full range of sensitivity.

In the case of the 8-hr average recommendation, there is less guidance for determining an adequate margin of safety, since there is only one study at 0.04 ppm, and one at 0.06 ppm. The body of findings from studies of 6.6 to 8 hr exposures to 0.08 ppm ozone indicates that about 26% of people who undergo similar exposures will experience symptoms and pulmonary function decrements of 10% or larger, with some experiencing decrements in excess of 30%. The study at 0.04 ppm found no significant pulmonary function or symptoms effects. Unfortunately, the one study at 0.06 ppm has not appeared in the peer-reviewed literature, although it has been published as a research report. The data available led to the conclusion that an 8-hr average concentration of 0.08 ppm was not adequately protective of public health, and that multi-hour exposure to 0.04 or 0.06 ppm ozone was unlikely to result in adverse responses. Also, an epidemiological study by Tolbert et al. (2000) that examined the shape of the concentration response function suggested that a population threshold might be evident in the ozone concentration range of 0.070 to 0.10. We selected the bottom of this range to incorporate a margin of safety. In addition, several other epidemiologic studies

suggest the possibility of effects below the concentrations where effects are observed in the chamber studies.

Next, the commenter suggests that the effects reported in the scientific literature are isolated, transient and reversible, and therefore not of significance. We believe that we have appropriately applied the ATS guidelines for adverse health effects. Admittedly, the most common effects attributable to ozone based on the chamber studies (pulmonary function changes, respiratory symptoms, airway hyperreactivity and airways inflammation) are acute and are reversible once exposure decreases below a threshold level. We agree that these are in some sense "potential effects" in that not all people will have the exposures on which the recommendations are based. But, to reiterate, the number of people is not the issue. The standards represent the maximum single exposures unlikely to induce adverse effects in exposed people. As we discussed in the Staff Report, there is evidence that repeated responses can lead to morphological changes in the lungs.

Reduced lung function is not a benign effect because it is due to a neural reflex as the commenter asserts. Activation of the neural reflex represents an attempt by the body to limit inhalation of a toxic substance, in this case ozone, to protect the airway lining tissues from oxidant damage, and resulting airway inflammation. Furthermore, reduced lung function and symptoms can reduce ability to work, as well as participate in healthful exercise and recreation. These seemingly minor effects, temporarily reduced lung function and symptoms, impact on ability to earn a living, and to maintain a healthy lifestyle, and clearly qualify as adverse by ATS standards, both physiologically and as aspects of quality of life. Repeated episodes of airway inflammation lead to morphological changes in the lungs, and may contribute to long-term respiratory health impacts. Animal studies clearly support this line of reasoning. There is also evidence that children who grow up in high ozone communities have lower lung function values at maturity than children who grow up in low ozone communities (Kunzlie al., 1997; Galizia and Kinney, 1999). This is a significant finding, in that low lung function is a known risk factor for chronic lung disease and premature death. Furthermore, asthmatics already have underlying chronic airway inflammation and reduced lung function. The additional ozone insult to the airway can result in exacerbation of asthma. Children are disproportionately impacted by asthma as they have higher prevalence rates, and the highest hospitalization rates are for 0-4 year olds. This is likely due at least partially to physics – the airway resistance is inversely proportional to the 4th power of the radius. Thus in a small child a little airway constriction can result in serious breathing difficulty.

The next section of the comments recommends that ARB/OEHHA adopt the federal process and procedures for development of ambient air quality standards. As discussed above, federal law related to processes and procedures governing establishment of federal ambient air quality standards does not apply to California. California law dictates the process and procedures that must be followed in development and promulgation of ambient air quality standards. We have followed the process required by the California Administrative Procedure Act, and do not have jurisdiction to change it.

There are several differences in the process that may clarify for the commenter why California and the U.S. EPA review the same literature and arrive at different recommended standards. California law requires that the standards protect the most sensitive subgroup of the population. This requires that we consider the range of individual responses to different exposure protocols to understand the range of variability in the population as a whole, and then to base our recommendations on the sensitive sub-group. In contrast, U.S. EPA primarily looks at group mean responses, with little consideration of the variability among individuals. Second, California standards are based solely on health considerations, not on risk analysis. As noted above, our model is for selection of a concentration and averaging time combination that is unlikely to induce adverse effects in anyone who happens to undergo that exposure pattern. The exposure patterns used are based on a combination of patterns identified by ambient air quality monitoring, and on likely outdoor activity patterns. California standard setting does not consider the likelihood of exposure. As noted above, in California, ambient air quality standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects. Furthermore, the proposed standards are based on responses of subject groups most likely to have significant exposure – people who are active outdoors. Finally, since the U.S. EPA review in 1996/97, dozens of epidemiologic studies have been published documenting an effect of ozone on several severe health outcomes including mortality and hospitalization.

With reference to the controlled exposure studies, the commenter points out that airway hyperresponsiveness and pulmonary inflammation occur at 0.18 to 0.20 ppm, with one to three hour exposures with heavy exercise, and at 0.08 ppm with 6.6 hr exposure. Since these are the lowest concentrations at which these endpoints have been evaluated, as is noted in the Staff Report, it is unknown whether these effects occur at lower concentrations. While a single episode of airways inflammation induced by ambient concentrations of ozone is unlikely to have long-term consequences, the reality is that the most populated parts of California have multiple exceedances of the State ozone standard each year. In addition, large, heavily populated parts of the state often have concentrations at or near those reported in the literature to induce airways inflammation. As noted in the section on morphological effects of repeated ozone exposures, such a pattern of injury and repair cycles causes changes in the kind of cells lining the airways, increases collagen formation which can lead to reduced airway compliance, a feature of several chronic lung diseases, and in children exposed early in life, to changes in airway architecture and lung development. There is ample evidence that these constitute effects of concern.

The commenter has misunderstood the statement referring to the value of animal studies in elucidating human health effects. Animal studies have provided considerable information on biological mechanisms and tissue effects that can not be studied in humans. The fact that these effects have been documented in more than one mammalian species and in multiple strains of animals suggests that these effects are common to mammals. True, they do not inform as to the relative sensitivity of humans

compared to the various species and strains, but that does not negate the value of the information they provide.

The commenter complains that the Staff Report does not include discussion of the statistical form of the standard (attainment test, or expected peak exposure concentration - EPDC). The EPDC methodology is not part of the standard setting process in California. The procedure is established in section 70306 Appendix 2 of Title 17 of the California Code of Regulations. This section is unrelated to those that have been opened in the present regulatory action. The EPDC method can be changed, but a completely separate regulatory action would be required from that for standard review.

6. The EPDC method for determining attainment is too complex, not robust, and is too stringent. The federal method should be adopted. Alternate method proposed by commenter.

We thank the commenter for the suggested alternate attainment designation method. However, this is not relevant to the standard review process. The area attainment designation process is dealt with under a separate regulatory framework (Title 17, California Code of Regulations sections 70300 through 70306)

7. The federal method/process of standard review should be followed by CA.
8. A more iterative process would allow an opportunity to reconcile differences in the interpretation of the science.

These two comments are related to the process used by ARB/OEHHA to propose revision of the CA ozone standard. The Alliance recommends that ARB/OEHHA adopt the federal model in which there are several drafts of a document similar to the EPA criteria document, several rounds of public peer review, and then recommendation of a standard. They also state that the public has been excluded from participation in development of the policy recommendation.

The requirements of the federal Clean Air Act, and those governing promulgation of federal regulations do not apply to state regulations. California law dictates the process and procedures to be followed for standards review and revision. We have followed the requirements of the California law governing review of ambient air quality standards in our review and in the development of our recommendations. The public has the opportunity to participate in the process. The public is free to comment on each draft of the Staff Report and its recommendations, to comment to the Air Quality Advisory Committee, and directly to the Board at its public hearing of the item.

Engine Manufacturers Association

1. The report needs to better address whether the results of human exposure studies actually meet the criteria as adverse health effects established by the American Thoracic Society.

Adverse effects were evaluated in accordance with the American Thoracic Society guidelines outlined in the Staff Report. An effect was considered significant if it was large enough to reduce or limit work or exercise capacity, or was sufficient to impact quality of life. Obviously, some of the categories suggested in the guidelines do not pertain to effects observed with ozone exposure, however, we believe that we have properly applied the recommended criteria. Admittedly, the most common effects attributable to ozone (pulmonary function changes, respiratory symptoms, airway hyperreactivity and airways inflammation) are acute and are reversible once exposure decreases below a threshold level ends. They are not, however isolated, given that the literature shows that about 25% of people who undergo an 8 hr exposure to 0.08 ppm ozone are likely to have reductions in lung function and respiratory symptoms, along with airway inflammation.

Reduced lung function is not a benign effect because it is due to a neural reflex as some commentators assert. Activation of the neural reflex represents an attempt by the body to limit inhalation of a toxic substance, in this case ozone, to protect the airway lining tissues from oxidant damage, and resulting airway inflammation. Furthermore, reduced lung function and symptoms can reduce ability to work, as well as participate in healthful exercise and recreation. These seemingly minor effects, temporarily reduced lung function and symptoms, impact on ability to earn a living, and to maintain a healthy lifestyle, and clearly qualify as adverse by ATS standards, both physiologically and as aspects of quality of life. Repeated episodes of airway inflammation lead to morphological changes in the lungs, and may contribute to long-term respiratory health impacts. Animal studies clearly support this line of reasoning. There is also evidence that children who grow up in high ozone communities have lower lung function values at maturity than children who grow up in low ozone communities (Kunzli et al., 1997; Galizia and Kinney, 1999). This is a significant finding, in that low lung function is a known risk factor for chronic lung disease and premature death. Furthermore, asthmatics already have underlying chronic airway inflammation and reduced lung function. The additional ozone insult to the airway can result in exacerbation of asthma. Children are disproportionately impacted by asthma as they have higher prevalence rates and the highest hospitalization rates are for 0-4 year olds. This is likely due at least partially to physics – the airway resistance is inversely proportional to the 4th power of the radius. Thus in a small child a little airway constriction can result in serious breathing difficulty.

2. A better evaluation of the human exposure/chamber studies is needed.

The commenter raises questions about the design of the controlled studies with regard to undue physiological stress, measurement and form of the ozone exposure, possible subject response bias, statistical analysis methods, applicability of the results to the overall population, and differences among studies. This series of comments reflects a misunderstanding on the part of the commenter as to what an ambient air quality standard represents under California law, and the considerations that state law requires when reviewing ambient air quality standards.

The basic protocols and methodologies used for the human chamber exposure studies are standardized, and have been essentially unchanged for about 30 years. These protocols were designed to simulate several possible outdoor exposure scenarios. Typically, people who are outdoors are not continually at rest, but are at least intermittently involved in some sort of physical activity. The one-hour continuous exercise protocol simulates the sort of outdoor exposure a recreational athlete or person pursuing an exercise program, such as jogging, would experience. The two-hour intermittent exercise protocol simulates children playing, after school sports and less intense personal exercise programs, outdoor home maintenance, moderate recreational activity, and yard work. The 6.6- to 8-hour protocols simulate a full day of outdoor work. The ventilation rates used in these studies are based on research that has measured ventilation for a variety of activities. Because of these factors, we believe that the protocols adequately simulate real-world activity patterns, and disagree that the protocols cause undue physiological stress. We will add some text more fully describing the basic protocols and methodologies.

It is unclear why the commenter focuses on only four human exposure studies in their commentary, when there many studies that have similar findings. The usual statistical design for these studies is a repeated measures analysis of variance design in which each subject completes all exposures, and serves as his/her own control. A few studies from the U.S. EPA lab have assigned each subject to only one exposure group, but in these cases, the groups for each condition were considerably larger to provide sufficient statistical power to the analyses. It is typical to investigate whether the data set is normally distributed, and then to use parametric or nonparametric analysis of variance, as appropriate. Since this method focuses on the variance of the responses to the different conditions, it does reveal information as to between subject variability. The commenter raised an issue regarding the parametric t-tests used to compare intra-exposure time points in Horstman et al. (1990). The investigators compared the intraexposure time points using both t-tests and MANOVA, and point out that while the latter is more appropriate for the data set, it is also negatively biased due to the small number of degrees of freedom. The commenter also points out that many of the subjects in this study did not demonstrate clear dose-response relationships on an individual level. As Horstman et al. discuss, this may be partly due to the similarity of the inhaled effective dose for the 0.08 and 0.10 conditions, in addition to within subject variability, and the nonlinearity of the dose response relationship, which is typically exponential.

It is true that not all studies present individual level data, but we evaluated the range of responses to ozone exposure to the extent that individual data were available. The difference in mean responses between different studies also gives some information on the range in responses between individuals. Subjects are more likely to conclude that they have been exposed to ozone if they begin to develop respiratory symptoms or perceive that it is more difficult to breathe than if thy smell it because ozone quickly dulls the sense of smell. In fact, some investigators put a trace of ozone in the chamber at the time the subject enters so that initial entry conditions seem the same, no matter what the actual exposure is. The commenter suggests that subjects could be faking

their responses because they conclude, based on smell, that they are exposed to ozone. It is impossible to fake consistent lung function tests. If the subjects had been faking, their test values would be highly inconsistent. In fact, the reason it is customary to have subjects perform two to three tests per sampling period (that must agree within 5%) is to preclude the possibility of the subject failing to make maximal effort.

The commenter recommends that since we have proposed standards for one and eight hour averaging times only studies that used these exposure durations should be considered. We disagree. Analysis of the database includes consideration of the total inhaled dose of ozone, in addition to the averaging time.
While the ozone concentration is the most important determinant of effects, total dose also matters. This allows comparison of studies with different durations of exposure (i.e., 1-3 hours). It is true that most of the multi-hour exposure studies used a 6.6 hour exposure protocol, while the recommended multi-hour standard is an eight hour average. Since responses are proportional to inhaled dose, if anything, we would expect that the effects with exposure to 0.08 ppm would be greater if the 6.6 hour exposures were extended to eight hours. This in itself justifies a lower ozone concentration on the grounds of the longer averaging time, and the correspondingly increased inhaled dose of ozone.

The commenter attempts to attribute at least part of the ozone effect to temperature and humidity, and asserts that we have not adequately considered this potential confounder. The literature does not support the reviewer's contention. We reviewed all available studies addressing this subject in the Staff Report. The data indicate that temperature and humidity do not affect responses to ozone. In addition, there is no literature suggesting that heat or humidity, in the absence of ozone, alter lung function or respiratory symptoms.

The commenter questions whether the general population is capable of the sorts of exposures that were used in the published literature, and expresses the opinion that the exposure patterns studied are irrelevant for the general population. This is not the point, and the commenter appears to misunderstand the definition of ambient air quality standards in California, which is different from that used by U.S. EPA. In California, ambient air quality standards represent the highest concentration for a given averaging time that is unlikely to induce adverse responses in people who experience that exposure. It is irrelevant how many people might actually experience that exposure. Active people and outdoor workers are not less deserving of adequate protection from adverse effects caused by air pollution than less active people.

3. The report does not adequately convey the caveats or conflicting results contained in the epidemiology literature on ozone.

The commenter expresses the view that longitudinal cohort studies are more powerful than time series studies for evaluating air pollution health effects. This is not likely true for ozone, although it appears to be the case for PM. In addition, each type of epidemiologic study design has advantages and disadvantages. The prospective cohort

studies are powerful in terms of the importance of their health endpoint and the implications of the findings for both standard setting and impact assessment. On the other hand, both panel studies and time-series studies have some very powerful aspects as well such as the ability to minimize confounding, deal with seasonality, and reduce measurement error in exposure. Human and animal exposure studies indicate that ozone effects are more acute than chronic, although there is evidence for morphological effects with long-term, high concentration exposure, and some evidence for reduced lung function with long-term exposure. On this basis, we would expect time-series studies to more likely show positive associations with adverse effects.

The commenter goes on to make comments on the various types of epidemiological studies. Under longitudinal studies, the commenter discusses Gent et al. (2003) at length. This is actually a field-type study, and is discussed in section 12.1 of the Staff Report. The commenter does not raise any issues that are not pointed out in the chapter. The commenter goes on to discuss the findings of several papers from the Children's Health Study, and offers nothing that has not been considered in the chapter. The study by Frischer et al. (1999) is discussed as a long term study of lung function growth. This is not the case. The Frischer study investigated the influence of seasonal ozone exposure on lung function by comparing measurements obtained at the beginning and end of the summer ozone seasons. The study and the Staff Report indicate that it is unknown whether the somewhat lower lung function measured at the end of the summer ozone season represents a permanent change, or whether it would reverse over the low ozone season. Consequently, the study adds nothing to the commenter's argument. The commenter reaches pretty much the same conclusion as the Staff Report with reference to long-term consequences of ozone exposure.

The commenter next discusses time-series studies, largely on the issue that most have not been reanalyzed since discovery of the default convergence criteria problem in the S-Plus software for the generalized additive model. The issues raised by the commenter regarding the S-Plus software and model specifications and sensitivity analyses are all acknowledged and discussed in the chapter. We made it clear that we did not consider time series studies using the S-Plus generalized additive model, unless they had been reanalyzed. The commenter raises significant statistical modeling issues, but the report acknowledges them. Furthermore, as stated previously, we did not use epidemiological literature as the primary basis for the ozone standards recommendations. Epidemiological literature served in a supporting, qualitative capacity.

The paper by Koop and Tole (2004) asserts that there are multiple statistically acceptable models to describe time series data sets, and that there is no consensus as to which is/are the "real" one(s). This is true – the subject has been raised before. Koop and Tole suggest a Bayesian averaging methodology to address this problem. They claim that the available time series literature includes too few potentially explanatory variables. They propose an approach that is purely statistical, and includes every possible variable they can think of, and all possible interactions of these variables. Unfortunately, they also include variables and lag times that have been shown by physiological research to have no biological plausibility. There is no reason to include

variables or lag times in the models that can be excluded a priori on physiological grounds. Inclusion of such variables complicates the models, can lead to computational difficulties, and confuses interpretation of the results. Contrary to the commenter's assertion, a great deal is known about ozone that is useful in selecting a particular lag time or potential confounder. In addition, the approach included weather variables in the regression model that relate to mortality only because they impact air pollution concentrations, and that would not have an independent effect. Therefore, these variables should not be considered confounders if one is trying to assess the causal effects of air pollution. Finally, it is a problem that the authors of the comment base all of their conclusions on findings from only one city where up to 90 cities have been used in some of the meta-analyses. Single city studies have limited ability for inference in this case.

The commenter's statement that people are generally eating better, exercising more and smoking less is belied by even a cursory look at recent public health reports that obesity is epidemic, and at the high sales volume of foods of questionable nutritional value.

We believe that the caveats, limitations and various statistical modeling issues raised by the commenter with reference to the epidemiological literature have been acknowledged in the report, and taken into consideration in the conclusions drawn. As noted above, epidemiology is not the primary basis of the recommended standards.

While we agree that the relative sensitivities to ozone of rodents, monkeys and humans is unknown, the results from animal exposures provide important information as to biological mechanisms by which ozone could induce adverse effects, and that could support a conclusion as to whether chronic ozone exposure could plausibly have adverse consequences. These results are presented, not as proof of effects at ambient concentrations, but as showing that such effects are plausible. These studies also provide important mechanistic support for epidemiological findings. We have not used this literature as a basis for our recommendations, but as supportive material.

4. A more thorough discussion of the effects of ozone on susceptible populations including children and asthmatics needs to be included in the final report.

We have evaluated what literature there is on responses of children and potentially sensitive groups to ozone, and believe that we have drawn fair and reasonable conclusions. We agree that the number of studies available on these subgroups is limited. We will add additional discussion on asthma as a health endpoint that disproportionately impacts children.

We will attempt to clarify the justification for the margin of safety, and discuss our reasoning in more detail.

5. The report needs to assess the impacts on human health from historical and documented reductions in ozone levels.

We agree that this would be interesting and helpful information. Unfortunately, there is no data available that would address the issues raised. In the case of PM, there are the historical London, Meuse Valley and Donora, PA episodes of extremely high PM concentrations. There are no similar ozone events, although Friedman et al. (2001) in Atlanta reported a reduction in asthma ER visits when ozone levels decreased when city traffic was rerouted during the Atlanta Olympics. In addition, there are no studies that have investigated the magnitude of public health benefits that have accrued from the reductions in ozone concentrations over the past 40 years.

Natural Resources Defense Council

1. The proposed standards are not adequately protective, and do not include an adequate margin of safety.

We believe that the commenter has misinterpreted the epidemiological literature used to support the conclusion that the proposed standards are not adequately protective. The concentrations cited by the commenter are the annual average of daily peak concentrations. This average includes values obtained on days there was little ozone because it rained, was winter, or the meteorological conditions were not conducive to ozone formation. The only conclusion that can be made from the data cited is that effects have been reported in cities with low annual averages of the peak daily measures. This does not mean that the effects in those cities actually occurred at the annual mean of the daily peak concentrations.

Hal Levine

1. There is not enough emphasis on indoor contributions to exposure.

The ambient air quality standards are for outdoor air, and reflect the highest concentration for a given averaging period that is unlikely to induce adverse responses in anyone who undergoes outdoor exposure.

Carl Selnick from San Diego APCD

1. Several typos were pointed out.

Thank you for pointing out these errors – we will correct them in the final report.

Joint submission endorsed by: American Lung Assoc. of CA, Environment California, Environmental Defense, Kirsch Foundation, National Parks Conservation Assoc., Merced/Mariposa County Asthma Coalition, Fresno Metro Ministry, Sierra Club CA, Medical Alliance for Healthy Air, Community Medical Centers

1. Support the recommendations.

Over 200 submissions from private citizens

1. All in favor of the recommendations.

Summary of Responses to Comments on Chapter 10 (now listed as Appendix B): Quantifying the Health Benefits of Reducing Ozone Exposure

A. Key Comments

1. Question of causality from epidemiologic studies; use chamber studies instead

Usually, epidemiologic studies by themselves cannot "prove" causality. However, it is important to mention in this context that ozone has the benefit of numerous human chamber and animal studies, and extensive knowledge about biological mechanisms, so there is more than sufficient information supporting a causal relationship between ozone and cardiopulmonary health. The key question is the magnitude of the relationship and the shape of the CR function (including thresholds) for the population at large which epidemiologic studies can provide including a wide range of potential health outcomes.

There are a number of reasons for using epidemiologic studies. While human chamber studies have the merit of being controlled experiments, they usually involve small sample sizes that do not include the most sensitive subpopulations, and cannot capture severe outcomes like hospitalization or premature death. Lagged or cumulative effects are similarly omitted, and only a limited range of exposures is examined. In short, human chamber studies are helpful to support causality and to determine effects of short-term exposure on measures like lung function in generally healthy individuals, but they cannot give us the general population response to exposure to ozone in the presence of other pollutants. For the latter purpose, epidemiologic studies which incorporate varying populations, exposure scenarios and behaviors, and health outcomes would best serve to isolate the human response to a particular pollutant and be the source of quantitative estimates for health impact assessment.

2. Ozone mortality estimates

There's some misinterpretation of the long-term epidemiologic evidence. In the Harvard Six Cities, ozone levels were similar in the six cities, so the study did not have the power to detect ozone-related effects, (which is different from not finding associations). In the most recent American Cancer Society publication, summer ozone shows a positive and nearly statistically significant association with cardiopulmonary mortality, so there is consistency with the time-series literature.

The two meta-analyses of the worldwide literature by WHO and Levy et al. have yielded consistent estimates, so the real question remains as to whether NMMAPS is a better approach for estimating the effects than a literature meta-analysis. There are concerns regarding publication bias in the meta-analyses, but there are concerns that the

NMMAPS statistical approach overcontrols for weather. Given this, it is entirely appropriate to have bounding estimates that have NMMAPS as a lower bound and WHO/Levy/Steib as an upper bound – as was discussed in the Chapter. In addition, staff plans to revise the WHO estimate to consider correction for publication bias and to consider results of recently completed meta-analyses of ozone mortality studies when they are published. These meta-analyses indicate associations between ozone and mortality and do not include an effect estimate of zero within their range of estimates. Further, there is some possibility that the technique used to correct for potential publication bias is not appropriate and therefore may lead to an underestimate. Thus, results will be examined and presented as a probable range accordingly.

3. Threshold assumptions

In our next version, we will examine two different cases regarding thresholds: one in which no threshold is assumed and another with an assumed threshold. However, for the latter case to be empirically correct, the concentration-response functions need to be adjusted to correctly fit the assumption. We will utilize information on the ER visits studies to suggest the size of the slope coefficient with and without an implied threshold. These relative slope estimates will then be used to adjust all of the CR functions for sensitivity checks

4. Estimation of exposures

Staff recognizes the assumption of equal distribution of population across each county is an oversimplification of the true population distribution but is not likely to cause significant bias in either direction. Regardless, we plan to perform a sensitivity check on the exposure estimation methods by interpolating air quality measurements from nearby monitors to derive exposures for each census tract. Health benefits would then be calculated at the census tract level using census population.

5. Rollback scheme

During the period that begins today and ends at some future attainment date, many factors will affect how ozone levels will change in each California air basin. These factors, which include patterns of population growth, emergence of new technologies, and strategic decisions by air quality managers, are more or less uncertain. Into this uncertain future, we projected the benefits of attaining the proposed ozone standards based on rational but necessarily speculative ozone projections. All methods of projecting ozone are speculative, but we believe our approach is subject to fewer difficulties compared to the other approaches that we considered or that others have recommended.

The concern regarding the same proportional change (above 0.040 ppm background) in ozone applied at all locations within an air basin reflects the observation that ozone usually does not change at the same rate throughout a basin. For example, our analysis of ozone changes in the South Coast Air Basin since 1980 shows that ozone at different

locations changed in somewhat different proportions. However, while past performance is not a guarantee of future performance, it is a good indicator. The factors that produced historical changes in ozone will not necessarily follow the same path in the future. In addition, these factors may not be the same in other air basins as they are in the South Coast Air Basin. Therefore, we consider it quite appropriate to focus on the required change in the ozone design value at each design site.

For a basin to attain the standard, the design value (characterizing high ozone) at the design site (the site with the highest design value) must be reduced to the level of the standard. For each basin, the proportional change required of the design value at the design site was applied to ozone at all sites in the basin. Data from the South Coast Air Basin indicate that this approach may understate the actual benefits that would accrue when the standard is attained. That is, the proportional change (historically) at the design site was less than the proportional change found for almost all other locations in the basin.

Another suggestion is that photochemical simulation models be used to project daily ozone changes within California air basins. After all, the chief use of these models is to project future ozone as a key part of the planning process. Unfortunately, that pathway is not feasible. In the planning process, it is common for the model to calculate in great detail the response of a single set of high-ozone days, called an "episode", to alternative emission reduction scenarios. To project the benefits of attaining the standard, however, the response of all days or all types of days must be addressed. To apply a reasonable set of alternative emission reduction scenarios to a set of episodes representing all types of days in all California air basins would require many hundreds of model runs. Although simplifications could be imposed to limit the number of model runs to a feasible number, the simplifications would then lead to criticisms similar to those raised concerning the method we chose to use.

6. Conversion factors for study results based on various averaging times

As we reported in the document, an empirical examination of the California monitoring data indicates that the assumed national ratios are similar to those found in the highly populated areas of the State.

B. Comments by Commenter

Donald H. Stedman

1. Commenter suggested adding 2001-2003 data in Figure B.1, which currently stops at year 2000.

The purpose of Figure B.1 is to demonstrate the rate of change in long-term ozone trends from 1980's. It now stops at year 2001. Since ozone did not change much from 2001 to 2003, adding 2 more years of data would not change the results.

2. Commenter suggested examining the health effect changes from the past to the present as a way to validate the current approach of predicting the benefits from attaining the standards in the future.

Many changes have occurred between 1980-82 and the present, including population growth, demographic shifts, health care system changes, etc. It would be nearly impossible to simply eyeball past data to validate the current estimates. However, several studies conducted in locations including, but are not limited to, the Utah Valley, Dublin, Hong Kong, and (the former East) Germany and Los Angeles *have validated that health improvement occurs after discrete changes in air pollution levels.*

3. Commenter suggested there might be errors in the rollback formula for OzAttain (ozone under attainment scenario).

The formulae are correct. In Stedman's example, a basin maximum Bmax of 0.18, a standard of 0.09 and a background BG of 0.04 would lead to the rollback factor RF of 0.64. Thus, a current ozone value of 0.15 would be rolled back to 0.04 + (1-0.64)(0.15-0.04) = 0.08, not 0.09. The rollback methodology was not designed to bring all current ozone values into attainment; rather, it was designed as a reasonable expectation of what would occur as the high values coming into attainment.*

Suresh Moolgavkar

1. (p. 1) Thurston and Ito's 2001 paper showed that the estimated effects of ozone on mortality were sensitive to how temperature was controlled

This is true; however, their conclusion was that studies that more appropriately captured weather trends (with non-linear relationships) found higher ozone CR functions. Therefore, it is likely that some of the earlier studies underestimated the effects of ozone.

2. (p. 1) At the end of the first paragraph, and elsewhere in this critique and others, it is stated that the associations in epidemiologic studies cannot lead to inferences of causality.

Usually, epidemiologic studies by themselves cannot "prove" causality. However, it is important to mention in this context that ozone has the benefit of numerous human chamber and animal studies, and extensive knowledge about biological mechanisms, so there is more than sufficient information supporting a causal relationship between ozone and cardiopulmonary health. The key question is the magnitude of the relationship and the shape of the CR function (including thresholds), which epi studies, and only epi studies, can provide.

3. (p. 1-2) Disagreed with the assumption of a threshold for emergency room visits but not for other health outcomes.

We are re-examining the studies to address this inconsistency. In our next version, we will examine two different cases regarding thresholds: one in which no threshold is assumed and another with an assumed threshold. However, for the latter case to be empirically correct, the concentration-response functions need to be adjusted to correctly fit the assumption. We will utilize information on the ER visits studies as to the slope coefficient with and without an implied threshold. These relative slope estimates will then be used to adjust all of the CR functions for sensitivity checks.

4. (p. 2) Commenter states that the long-term exposure studies do not report associations between ozone and mortality.

There's some misinterpretation of the long-term epidemiologic evidence here. In the Harvard Six Cities, ozone levels were similar in the six cities, so the study did not have the power to detect ozone-related effects, (which is different from not finding associations). In the most recent American Cancer Society publication, summer ozone shows a positive and nearly statistically significant association with cardiopulmonary mortality, so there is consistency with the time-series literature.

5. (p. 2) Regarding the conversion factors applied to epidemiologic study results for various averaging times of ozone measurements, commenter suggests that the national ratios may not be precise.

Commenter may overstate the potential level of imprecision and the implications. As we reported in the document, an empirical examination of the California monitoring data indicates that the assumed national ratios are similar to those found in the highly populated areas of the State. In any case, it is likely that the conversions contribute only a small amount of uncertainty, under the assumption that there is not significant dose-rate dependence.

6. (p. 3-4) Commenter questioned ARB's use of mortality estimate by WHO and that if we do use these estimates, suggests that we should use estimate that corrects for publication bias.

The two meta-analyses of the worldwide literature by WHO and Levy et al have yielded consistent estimates, so the real question is whether NMMAPS is a better approach for estimating the effects than a literature meta-analysis. There are concerns regarding publication bias in the meta-analyses, but there are concerns that the NMMAPS statistical approach overcontrols for weather. Given this, it is entirely appropriate to have bounding estimates that have NMMAPS as a lower bound and WHO/Levy/Steib as an upper bound – as was discussed in the Chapter. In addition, staff plans to revise the WHO estimate to consider correction for publication bias and to consider results of recently completed meta-analyses of ozone mortality studies when they are published. These meta-analyses indicate associations between ozone and mortality and do not include an effect estimate of zero within their range of estimates. Further, there is some possibility that the technique used to correct for potential publication bias is not

appropriate and therefore may lead to an underestimate. Thus, results will be examined and presented as a probable range accordingly.

7. (p. 4) Regarding ozone and hospital admissions, commenter suggests that results from the WHO report be used and questions whether results in Thurston & Ito are peer-reviewed.

Staff will take a closer look at the WHO report and investigate whether a bounding distribution parallel to that for mortality might make sense. However, it is reasonable to use the WHO estimates for mortality but not hospitalization. While death is death everyplace, the health care systems vary significantly between the US and Europe, so what one is hospitalized for may also vary. The argument that the book chapter is not peer-reviewed does not hold since the three studies underlying the estimate are peer-reviewed, and the pooling approach was simple inverse-variance weighting, a method commonly used for meta-analyses.

Stan Hayes

1. (p. 2) At a number of points, commenter (and others) raises the argument that human chamber studies should be used instead of epidemiological studies for benefits assessment.

There are a number of reasons for using epidemiologic studies. While human chamber studies have the merit of being controlled experiments, they usually involve small sample sizes that do not include the most sensitive subpopulations, and cannot capture severe outcomes like hospitalization or premature death. Lagged or cumulative effects are similarly omitted, and only a limited range of exposures is examined. In short, human chamber studies are helpful to support causality and to determine effects of short-term exposure on measures like lung function in generally healthy individuals, but they cannot give us the general population response to exposure to ozone in the presence of other pollutants. For the latter purpose, epidemiologic studies which incorporate varying populations, exposure scenarios and behaviors, and health outcomes would best serve to isolate the human response to a particular pollutant and be the source of quantitative estimates for health impact assessment.

2. (p. 3) Commenter questions the assumption of log-linearity or linearity of the CR functions for values below the levels of the standards.

As mentioned previously, the concern about linearity can be partially addressed with an explicit sensitivity analysis that captures the "hockey stick" CR function in an appropriate way. As a result, Staff will perform some sensitivity analysis which assumes a threshold model and which adjusts the estimated slope.

3. (p. 4) Commenter suggests examining the log-linearity issue by using human clinical data from controlled chamber studies. He mentions explicitly in the first paragraph that healthy young adults were the target population in the controlled chamber studies by Avol, Kulle, and McDonnell.

Using the shape of the dose-response curve for healthy young adults to draw inferences about the shape of the population dose-response curve is highly suspect. One would certainly expect that there may be susceptible individuals that would have a greater response to ozone and that are not included in the chamber studies. If individuals have heterogeneity in the levels at which they respond, there may be a tendency toward linearity (or at the very least, toward lower thresholds than were observed in chamber studies).

4. (p. 5) Commenter suggests including chronic effects such as those studied in Gauderman et al.

Since the Chapter did not include any chronic exposure effects, the discussion about Gauderman is not relevant. However, there are multiple other epidemiologic studies that have documented effects of ozone on lung development, and these have been supported by animal studies. Regardless, Staff thought the evidence of an effect associated with long term exposure, while plausible, was not sufficient at this time.

5. (p. 6) Commenter states that quantitative estimation of mortality and morbidity benefits should be deferred until substantial additional research is conducted.

While caution is warranted in using the current literature to estimate benefits, deferring this work altogether effectively assumes zero benefit and implies that one can never proceed with risk assessment in the presence of uncertainty (since uncertainty is always present). It is a better approach to acknowledge the uncertainties and come up with reasonable bounding estimates, rather than to ignore the effect altogether. With some additional sensitivity analyses and discussion of key uncertainties, the output will be superior to not having any quantitative analysis. There are a compelling number of studies linking both morbidity and mortality to exposure to ozone at current ambient concentrations. In addition, several meta-analyses on ozone mortality are being submitted for publication, representing a reasonable basis for quantification.

Allen Lefohn

27. (p. 3) Commenter questions the background level assumption of 0.04 ppm and asserts that the benefits assessment is <u>sensitive</u> to the selection of a level for background ozone.

Various citations included in the comment imply that our use of 0.04 ppm as the background level for ozone was based on analyses for which the commenter asserts a detailed array of flaws. We do not consider it necessary to defend the questioned analyses in this venue, because our choice of 0.04 ppm for background was not based on the analyses cited. Instead, it was based on the simple empirical observation that as ozone has improved in California, the distributions of 1-hour and 8-hour daily maximum concentrations have "piled up" around the 0.04 ppm level. That is, ozone concentrations higher than 0.04 ppm decrease, but they tend to stop improving in the neighborhood of

0.04 ppm. Please see our more detailed response to the comment regarding the background assumption in the set of comments on that chapter of our report.

Further, the commenter states that the benefits assessment would be sensitive to the choice of background level. A table supporting this contention was included in the commenter's submittal. Based on the example presented in the table, it seems more appropriate to say that the calculated benefits are affected by the choice of background rather than sensitive to the choice of background. We agree that the calculated benefits are affected by the choice of background. However, the magnitude of the effect is relatively small and would not alter the general picture.

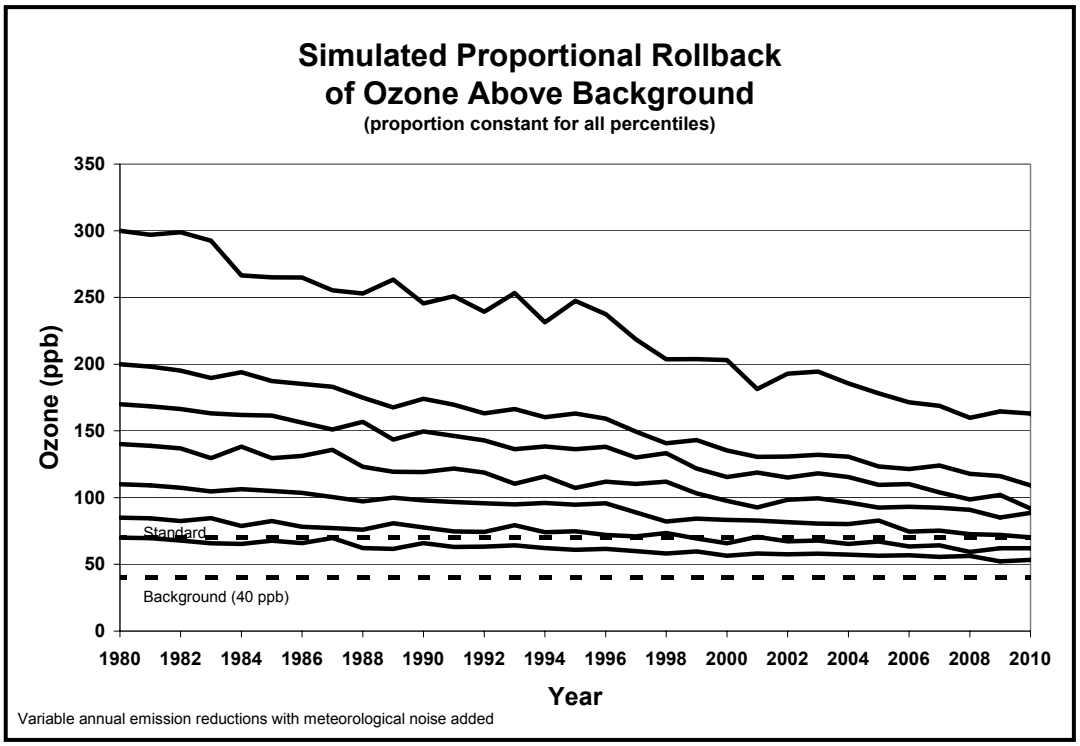
The table includes alternative choices for background from 0.040 to 0.070 ppm. Since the vast bulk of the benefits attributed to ozone reductions represent the highly populated coastal and valley regions of the state, any background level significantly above 0.04 ppm is highly doubtful. In these areas, a very few days under extremely unusual circumstances might have a "policy-relevant" background greater than 0.04 ppm ozone, but the incidental frequency of such days means they would have a negligible effect on the overall assessment of benefits. When one compares the results in the table provided for 0.04 ppm to the results for 0.05 ppm, the differences are minor. Accordingly, we take the writer's analysis as more supportive than critical of our choice of 0.04 ppm for background ozone.

28. (p.3-4) Commenter questions the proportional linear rollback method, making the observation that the percentile trends in the South Coast analysis do not convince that a constant rate of reduction occurs across the range of ozone concentrations.

The Appendix shows a similar downward trend in percentiles of ozone maximum observations at each site in the South Coast Air Basin. This suggests that the rollback method used is a reasonable approximation. In fact, it is the most defensible approach among alternative methods, for the alternative methods would likely lead to results that are well within the uncertainty bounds presented in our report

Our interpretation of the South Coast analysis is that the rates of reduction in the portion of each concentration that is above background are more similar than they are different. The rates do not need to be identical to support our rollback methodology. They need to be similar enough to support the general application of one proportion in an uncertain future.

The figures we provided support our rollback approach because the lines are roughly parallel. The lines need not be "straight" to support our "linear" rollback method. They need only be "proportionally" parallel and converge as they near 0.04 ppm or some lower concentration. The following picture is a simulated example of the ideal pattern that would support our method. The figure shows striking similarity to those based on measured data from the South Coast Air Basin from 1981 - 2001.



29. (p. 4-5) Commenter is concerned with the use of epidemiologic data for this work and whether causality can be implied.

As indicated above, the epidemiological studies are not meant to establish causality by themselves, but in the presence of many other studies, are meant to quantify the relationship between ozone concentrations and population health effects. It is not inconsistent to document large uncertainties in the epidemiological literature and to quantify health benefits, as long as the uncertainties are acknowledged and quantified to some extent – as was done in our Chapter.

30. (p. 6) Commenter objects to accruing health benefits below the proposed standards.

As mentioned above, the fact that human chamber studies do not show statistically significant effects below 0.08 ppm does not imply that there are no health risks for susceptible individuals at those levels. All of the epidemiological studies used document effects below the 0.08 concentration.

31. (p. 13) Commenter states that the benefits would have been reduced from the estimated values to 14-24% if one only considers benefits between the current levels and the proposed standards only.

This estimate may not hold since an analysis incorporating a threshold at the proposed standard would have needed to have a significantly greater slope above that point to appropriately capture the information from the epidemiological studies. Exactly what the difference would be requires careful analysis, and staff plans to address this issue via a sensitivity analysis for at least one health endpoint.

32. (p. 44) Commenter notes that there are seasonal differences in ozone CR relationships.

The fact that there are seasonal differences in ozone CR relationships does make interpretation of annual average estimates somewhat problematic, but the argument is overstated. It is unclear what is driving the seasonal differences, but activity patterns related to ambient temperature likely play a role. Given the mild climate in CA, the seasonality is likely more muted, which would imply that using a US-wide estimate would tend to underestimate the effects in CA (by including too much of the wintertime relationship). Regardless, we are not able to re-estimate the original studies published by other researchers.

33. (p. 46) Commenter argues that the sharp disagreement between summer and winter does not argue that weather has not been adequately addressed and that higher ozone appears to be beneficial in the winter.

This is not true. Rather, the sharp disagreement may point out the importance of different activity patterns (time outdoors) during the seasons, the possibility of a population threshold, or the possibility of poor modeling of potential confounders such as weather. Also, the statement that higher ozone appears to be beneficial in the winter is overstated – most of the literature seems to show highly statistically insignificant relationships in the winter and there is no biological mechanism to support this assertion. Finally, the most recent reanalysis of NMMAPS shows very similar effect estimates using the full year of data versus the warm season.

34. (p. 47, 49) Commenter is concerned with the inter-city differences among ozone effect estimates.

Inter-city differences are accounted for in random effects modeling, so the uncertainty behind these differences has been addressed in the epidemiologic studies. There are many possible explanations for the heterogeneity in the effect estimates. For example, the fact that NMMAPS imposed very similar weather and time smoothers for all cities may have resulted in model mis-specifications. In addition, factors such as monitor placement, spatial variability, socioeconomic factors, background health status, use of air conditioners, and housing characteristics all could contribute to heterogeneity in

response. So long as it is addressed properly, variation should not be the reason for ignoring the positive associations reported in many of the existing studies.

35. (p. 52) Commenter argues that a relation between exposure and response may be non-linear, hence opting for a linear model can result in regulatory decisions that will not produce the desired mitigation of health effects.

Just because a linear CR function might have significant regulatory implications does not mean that one should not use a linear CR function if the evidence shows that it is appropriate to do so. As stated above, staff plans to address the assumption of linearity via a sensitivity analysis.

36. (p. 52-53) Commenter questions the compatibility of linear CR functions in the context of individual variations.

The argument regarding the compatibility of linear CR functions in the context of individual variations is not correct. If there are individual-specific response thresholds to ozone, and those thresholds are distributed normally across the population (which the central limit theorem would support), then the population CR curve would resemble a cumulative normal distribution, which is linear at low doses. See Schwartz et al., The Concentration-Response Relation Between PM2.5 and Daily Deaths, Environ Health Perspect 110: 1025-1029 (2002) for a detailed discussion of this point.

37. (p. 53) Commenter argues that the re-analyzed results of the NMMAPS study show a negative effect of ozone mortality in the winter, hence acute mortality studies do not show sufficient evidence for calculating mortality effects from ozone exposure.

The most recent reanalysis of the NMMAPS shows no appreciable difference between ozone and mortality relationships for the whole year versus the warm season. Our analysis relies on WHO results and discusses NMMAPS as a lower bound. Additional meta-analyses of U.S. mortality may be published soon and if so, these estimates will be incorporated into our analysis. Although uncertainties in the estimates clearly remain, it would be inappropriate to ignore the vast scientific literature suggestive of a mortality effect.

38. (p. 55) Commenter states that Pope et al (2002) did not discern an ozone effect on total mortality even when restricted to summer months and to specific causes of death.

The estimate in the ACS cohort using summertime ozone is of borderline significance (p~ 0.07). Most epidemiologists would agree that using a p-value of 0.05 as the only indicator of association is inappropriate. Rather, one has to consider the potential biases that may exist in the study (i.e., factors that, in this case, may lower the likelihood of finding an association), the biologic mechanism involved (i.e., in this case, the evidence for inflammation and other effects), and, the related evidence (i.e.,

epidemiologic evidence of effects on hospital admission and emergency room visits, and toxicological evidence of inflammation and lung restructuring)

Cover letter from WSPA

On page 4, the author argues that benefit estimates predicated on mortality be removed given artifacts of the analysis methodology.

The points about human chamber studies and the appropriateness of conducting analyses in the presence of uncertainty have already been made. One additional point can be made here: what is the logic in arguing for the omission of mortality but the inclusion of morbidity effects? Similar issues regarding seasonality and the use of observational epidemiology would hold for other health endpoints.

John Heuss

1. (p. 2) Commenter questions whether the health effects estimation approach has drawn well from methods used at the federal level.

The Section 812 analysis from U.S. EPA is not specifically focused on ozone, but it includes ozone with explicit determination of ozone exposures and CR functions. Numerous other regulatory impact analyses by EPA have included ozone and followed an identical approach. Since the intent of the CA analysis is parallel with that of EPA's analysis, it makes sense to use similar methods. It would be worthwhile to mention the ozone health risk assessment conducted by EPA as part of their recommendation for the ozone standard in 1997. However, staff would like to point out that it does not have direct relevance for quantifying the full scope of benefits to the population from attaining the ozone standard since the 1997 analysis only used human chamber studies.

2. (p. 3) Commenter asserts that a linear rollback method is not appropriate because the proportional linear rollback was applied to concentrations above a 0.04 ppm background level and that ozone formation is highly non-linear

Our rollback calculation was calibrated and applied based on the "portion above 0.04 ppm" for each measured concentration. This means that 0.04 ppm rather than 0.00 ppm was the effective rollback target. We believe this target is well established from the empirical data, which indicate a range around 0.04 ppm is suitable for background ozone. The overall evaluation of benefits is not especially sensitive to alternative background levels in the neighborhood of 0.04 ppm.

The well-known non-linear nature of ozone chemistry relates chiefly to the quantitative response of ozone to quantitative reductions in the ozone precursors, VOC (a.k.a., ROG) and NOx. In this case, we are not postulating any particular reductions in VOC and/or NOx. Rather, we only assume that the standard has been attained by whatever emission reductions were needed. The linear aspect to our rollback calculations is the

use of a basin-specific proportion (a linear factor) applied to the portion above background for each measured value.

3. (p. 3) Commenter suggests that ozone trends in other California locations (than the South Coast Air Basin) be evaluated to test the assumption of the linear rollback method.

We do not believe that additional study of other California air basins is needed. South Coast covers a vast population, and it is where we have seen a dramatic downward trend in ozone concentrations. Many people found the results of our analysis in the South Coast Air Basin quite "surprising". It seems that experience and general scientific understanding did not correctly align expectations in this case. We believe that the South Coast work sufficiently demonstrates that our roll-back procedures "make sense".

4. (p. 3) Commenter suggests using GIS methods and population by census tract to assign exposures to each monitor.

Staff recognizes the assumption of equal distribution of population across each county is an oversimplification of the true population distribution but is not likely to cause significant bias in either direction. Regardless, we plan to perform a sensitivity check on the exposure estimation methods by interpolating air quality measurements from nearby monitors to derive exposures for each census tract. Health benefits would then be calculated at the census tract level using census population.

5. (p. 3) Commenter argues that due to time spent indoors, the population surrounding a monitor is not actually continuously exposed to the concentrations at the monitor.

It is factually correct that actual exposure will tend to be less than the reported ambient concentration, given time spent indoors. However, the epidemiological studies are based on the central site monitors, making exposure estimates at these monitors the most appropriate values to use in the health impact assessment.

6. (p. 4) Commenter states that the finding of a cardiovascular but not respiratory mortality signal from ozone in single-pollutant models is hard to explain as a causal relation.

The fact that effects were seen with cardiovascular but not respiratory mortality could be explained by the relatively low baseline rate for the latter, resulting in low statistical power to detect an effect. Also, many deaths from respiratory disease are likely to be coded as related to cardiovascular death. Finally, there are biological mechanisms, which would render cardiovascular deaths to be a plausible outcome.

7. (p.4) Commenter mentions that Anderson et al. evaluated the potential for publication bias.

Staff recognizes the publication bias correction used in the Anderson et al. study for the WHO. However, it is also possible that the trim and fill method used to correct for potential bias is not correct since that method was initially proposed for estimates that all came from the same population. Nevertheless, our analysis will include a new estimate that corrects for publication bias. In addition, if the new meta-analyses funded by EPA are published prior to completion of our standard development, we will incorporate those estimates into our quantification as well.

8. (p. 5) Here and elsewhere, commenter raises the argument that the staff should include a lower effect estimate of zero.

There is some non-zero probability that the effects are not causal. However, staff proposes to use the existing meta-analytic studies currently available. These studies do not include zero within the confidence interval. Over the last several years, many studies have reported associations between short-term exposure to ozone and resultant mortality with intervals that do not include zero. This is particularly the case when temperature and time trend are carefully modeled with non-linear smooth terms. However, unlike the examination of particulate matter, researchers have not conducted the full range of sensitivity analyses using ozone. Therefore, we will add some discussion in our text regarding the uncertainties in the estimates that are not incorporated into the confidence intervals..

9. (p. 5) Commenter mentions that Thurston & Ito's work on hospital admissions relied on the meta-analysis based exclusively on studies in cold climates.

Staff notes that the cold climate/warm climate argument made in Levy et al (2001) and Thurston & Ito (1999) in regard to hospital admissions had to do with air conditioning, which is far more prevalent in Alabama than in California. The lack of air conditioning in many parts of California may serve to increase the penetration of ozone into the homes and increase the estimated effect. However, we do not have enough California-specific studies at this time and we cannot ignore the existing literature on this issue.

Ozone Standard Review Staff Report
Summary of Comments (by commentor)

Chapter 4 – Background Ozone

Note to Reader: There is considerable overlap among the comments on Chapter 4. In order to avoid repetition, comments/responses are numbered so that redundant comments can be referenced to a single response.

General:

Extensive comments on the discussion of background ozone were provided by two commentors, the Alliance of Automobile Manufacturers and joint comments submitted by the American Petroleum Institute and Western States Petroleum Association. Both contend that the 8-hour standard may be exceeded due to “background” ozone. These commentors also provided extensive lists of citations of scientific papers, and criticized the Draft Staff Report for failing to review all the literature.

The issue of attainment status is addressed in responses to comments presented elsewhere in this document. In summary, ARB Staff’s position on this issue is that California law requires the standard to be based on health effects alone. California standards represent the highest concentrations for selected averaging times that are unlikely to induce adverse effects. Furthermore, the proposed standards are based on responses of subject groups most likely to have significant exposure – people who are active outdoors. Problems that may be encountered in attaining the standard through emission control programs are relegated to the air quality control planning process, and are not properly part of the standard setting process. The presence (of absence) of a “background” concentration of any particular pollutant is not specifically addressed in State law regarding setting air quality standards.

The question of the extent of the review of scientific literature derives directly from the previous point regarding the relevance of “background” ozone to the standard-setting process. The discussion of "background" ozone in the Staff Report is provided as part of a general review of the characteristics of ozone as an atmospheric pollutant. In this context the information is supporting material designed to acquaint the non-specialist reader with the nature of ozone pollution. The review focused primarily on recent literature to avoid discussion of the evolution of understanding about ozone, and to avoid revisiting past controversies about the causes of "background" ozone. Much of the literature cited by the commentors has been superceded by more recent work, and the ARB Staff disagrees with the central assumption of some of the cited papers, that observed elevated ozone in non-urban, non-industrial sites can be presumed to be due to natural causes. ARB Staff believe that such observations need to be supported by

chemical and meteorological data that preclude anthropogenic influences if the measurements are to be accepted as "background."

The following discussion addresses comments bearing on "background" ozone concentrations.

Alliance of Automobile Manufacturers, General Comments by Casimer J. Andary, Director, Regulatory Programs; Technical Comments prepared by Jon M. Heuss and Dennis F. Kahlbaum, Air Improvement Resources, Inc.

The appropriate measurement of background ozone must be considered as part of the proposed Ambient Air Quality Standard. This issue will impact whether the proposed standards overlap with natural (or transported from outside of California) levels of pollutants in the air. The staff review uses one model (Fiore *et al*., 2002) to evaluate background ozone concentrations. We identify specific concerns with that modeling approach and present analyses and data from a variety of sources that conflict with the assessment. The scientific literature on background ozone indicates that the proposed standards overlap with background concentrations. We also provide an analysis demonstrating that the elimination of essentially all human activity in California will still leave portions of California unable to attain the proposed standards. We recommend a broader discussion of background level ozone in the document, including natural fluctuations and measurements at clean sites to allow comparison of concentrations with the proposed standards. We also note that, from a policy perspective, the overlap of background concentrations with the proposed standards is in conflict with implementation requirements for California air districts to develop plans to meet the standards.

As stated above, the discussion of "background" ozone is provided as informative supporting information. Under California law, the level of the standard is to be based on health effects data, and in this context "background" levels of a pollutant are not relevant to standard setting. The commentor is incorrect in suggesting that the discussion relies solely on the work of Dr. Fiore; the text summarizes the work of several investigators and does not rely solely on models. We do not agree with the commentor's contention that the proposed standard "overlaps background." While there is considerable uncertainty regarding background ozone in California, the lack of such information, does not preclude California from acting on this standard. Data presented in the section on health benefits indicate that the frequency of concentrations at or near the proposed standard is decreasing in multiple locations in California, which would not be the case if background concentrations were commonly near 70 ppbv. Finally, ARB's Area Designation Criteria (17 CCR Appendix 2 sections 70300 through 70306) already allow for the exclusion of exceptional events that are beyond reasonable regulatory control. This includes stratospheric ozone intrusion, and wildfires to the extent that it can be demonstrated that they impact ozone value. However, under the current regulations, neither ozone background nor transport is a cause for an exclusion.

There are several concerns with the analysis. First, it relies on one modeling study and does not account for known criticisms and limitations of the model. Second, we have found a large body of ozone observations that show annual maximum ozone concentrations in remote monitoring sites in the western United States that equal or exceed the proposed 8-hour standard. Third, the conclusions of several other researchers and the United States Environmental Protection Agency (USEPA) concerning maximum background levels should be considered in the review. Fourth, there are studies of stratospheric ozone, which demonstrate that its impact is larger, more widespread, and more difficult to identify than assumed in the review. Fifth, the review uses the standard as the typical case when various background studies shows it is an extreme value. Sixth, the analysis of background is not consistent with the background assumed by ARB in its assessment of the impact of transported pollutants on ozone in California.

Each of these criticisms is addressed in turn below.

The review relies on the Fiore et al. (2002) modeling study to estimate the various components of background ozone. There are a number of problems with this approach. First, it is a model calculation with a global transport model that was not designed to address the components of background specifically in California. The model was run for the summer of 1995, so it was not aimed at evaluating the various sources of ozone over the entire year. As documented in the following, it is not a reliable tool to estimate the mean value or the range of background in California that might influence the attainability of the proposed standards.

Since there is no measurement record for "background" ozone in California, ARB Staff believes that the Fiore et al. (2002) study provides a reasonable first estimate. We also cited global and regional modeling and analyses from other authors (e.g. Lelieveld and Dentener, 2000; Galani et al., 2003). The reviewer correctly states that the model is uncertain, but does not suggest an alternative model or systematic estimation procedure.

The GEOS-CHEM model Fiore et al. used employs a coarse 2° latitude by 2.5° longitude horizontal grid that the authors acknowledge cannot resolve the steep gradients in surface heating near coastal sites that determine the depth of the mixed layer. The authors indicate that this compromises the simulation over coastal urban environments. In addition, the authors list the inability to resolve topography in California as another problem that manifests itself in the Central Valley of California. The limitations of the model in simulating coastal urban environments and the Central Valley are important in that these are the areas of California with the greatest population and hence man-made emissions.

To begin with, characterizing the Staff Report analysis as solely based on the Fiore et al. (2002) modeling is incorrect. The comment's assertion that the model may understate down-mixing over California is speculative and contradicts the bulk of information available. For global-scale processes, the coarse resolution is reasonable –

even localized stratospheric intrusions in mid-latitudes are the result of synoptic scale "Tropopause Folding Events" (TFEs) in which stratospheric air is incorporated into the upper troposphere due to vertical motion induced by cold fronts. These tend to have geographic scales on the order of several to tens of degrees and occur at altitudes generally above 10 km (30,000 ft). As noted in section 1.1.2.1 of the Staff Report, most TFEs produce layers of enhanced ozone at elevations of 5 – 6 km (15,000 – 18,000 ft) with weaker ozone signals down to about 3 km (10,000 ft) – altitudes well removed from the populated coastal lowlands or the low-altitude San Joaquin Valley. Terrain interactions with these layers will be generally restricted to higher elevations of the Sierra Nevada, not the lower Coast Ranges or the floor of the Central Valley. Detailed study of TFEs over Europe (referenced in the Staff Report) showed only about 2% of TFEs deliver stratospheric ozone to elevations below 1 km (3000 ft).

Regarding the problem of not resolving surface ozone in populated coastal zones, Fiore et al. (2002) observe that the model tends to ***overpredict*** *surface ozone concentrations in grid cells that include coastal ocean and highly populated land areas due to extending the coastal shallow mixing layer too far inland (and contradicting the commentor's assertion that background is underestimated). This error would tend to overpredict grid-cell-wide natural ozone concentrations, so that applying the Fiore et al. (2002) results in the coastal areas of California incorrectly extends elevated coastal plain ozone concentrations into the coastal mountains. In determining the ozone contribution due to long range transport or stratospheric downmixing, this error is irrelevant outside the coastal zone.*

Dynamical considerations support this interpretation. The cold Pacific Ocean causes strong, persistent inversions to overlie California's coastal plains at elevations from 300 m to 500 m (1000 – 1500 ft), and similar shallow nocturnal inversions are also common in the Central Valley – thus down-mixing of mid-troposphere ozone (whether from in-situ formation or TFEs) below about 1 km (3000 ft) is even more unlikely in California than elsewhere in North America. For free troposphere ozone to descend to near sea level would require an extraordinary degree of vertical mixing in the atmosphere – a situation antithetical to accumulation of high concentrations of pollutants near the surface, thus these events are not expected to be additive with local accumulation of anthropogenic ozone. Moreover, TFEs generally occur in late winter or early spring, well outside the California ozone season (summer and fall). Observational data presented by Newchurch et al. (2003) further support the case that the impact of this error is overprediction. Ozonesonde data from coastal California show that the local inversion drives surface "background" ozone down in summer – the reverse of the pattern at other ozonesonde sites in the U.S. The Newchurch data will be added to the Staff Report to clarify the effect of shallow inversions on surface ozone in undeveloped areas.

A source of non-anthropogenic ozone that is important in California is photochemical production from reactions of NO_x that comes from microbial action in the soil and lightning with biogenic hydrocarbons from vegetation. Another complicating factor in California is increased NO_x emissions from soil related to fertilizer use. The model was not designed to accurately simulate these sources and processes in California.

The estimates of lightning caused ozone cited in the Staff Report are independent of the Fiore et al. (2002) model. The literature cited indicates that lightning is not a significant source of ozone at low altitudes. Furthermore, lightning is a relatively rare phenomenon in Mediterranean climates (compared to most mid-latitude land masses).

Pedogenic (soil-produced) NOx is highly uncertain, but it is thought to be dependent on temperature and the activity state of vegetation and soil organisms. The protracted dry season in most of California forces natural vegetation over much of the State into semi-dormancy during the dry months, and areas with substantial summer precipitation (mountains, northwest coast) do not experience high temperatures. While ARB Staff cannot precisely estimate the ozone production due to natural soil NO_x emissions in California, it is unlikely to be atypically large. Surface ozone production in areas remote from anthropogenic precursor sources has been observed to be limited to concentrations well below the level of the proposed standard. Data on 19th century ozone concentrations measured in Europe and the U.S. (Bojkov, 1986) show that spring peak ozone partial pressures were about 4 $\pm$ 1 mPa (30-50 ppbv) in the Midwestern U.S. and ranged from 2 – 3 mPa (20-30 ppbv) in Europe. This point will be clarified by adding a discussion of the Bojkov (1986) data to the Staff Report.

Fertilizer emissions are considered in ARB's own modeling for ozone management in the Central Valley, and are not reasonably included in "natural" sources of ozone precursors. Biogenic hydrocarbons have been observed to react with anthropogenic NO_x to enhance ozone downwind of urban areas, as discussed in the Staff Report (Sect. 1.1.3.2) but this, because it is dependent on a local anthropogenic precursor whose sources are already within ARB's regulatory purview, is not properly considered "background" ozone.

Any global model contains many assumptions and simplifications that simply cannot be fully evaluated. The GEOS-CHEM model is but one of a number of such models. Fusco and Logan (2003) evaluated the GEOS-CHEM model and report that the model estimates somewhat higher production and loss rates of ozone than other chemical transport models, as much as 15 to 30%. Since the net photochemical production of ozone is determined by the difference between these two large numbers (a large chemical source term and a large chemical sink term), the net production cannot be precisely determined. They note that differences in modeled photochemical production and loss rates affect the relative importance of the stratospheric source giving examples of other models that indicate a much larger role for the stratospheric source in summer and in winter. Adding to the complexity is that assumptions have to be made about the cross-tropopause flux of ozone and ozone deposition at the surface, quantities that each have significant uncertainty, too. There are other aspects of the chemical transport models that are also highly uncertain. For example, there is disagreement over how many ozone molecules are produced, on average, from each NO molecule emitted. The recent NARSTO Synthesis Report indicates that more recent studies have reduced the estimated ozone production efficiency from 7 to 10 molecules ozone per molecule NO_x emitted down to 1 to 3. In addition, the NARSTO report acknowledges there is

substantial disagreement over key factors such as the magnitude of United States biogenic VOC emissions (uncertain by a factor of 2 or 3) and natural NO_x emissions from soil and lightning.

The Staff Report discusses the work of Fiore et al. (2002) because it is the only modeling study to date that explicitly treats background ozone in California. Other modeling studies addressing hemispheric to global-scale ozone distributions are available; to the degree they can be compared with the Fiore et al. (2002) results, they do not contradict ARB Staff's interpretation. This particular comment is based on a selective reading of the Fusco and Logan (2003) paper. Their critique of GOES-CHEM is presented in the context of using that model to estimate global ozone trends. Fusco and Logan (2003) present comparisons with ozonesonde data that show GEOS-CHEM to perform well in this application, with errors compared to low altitude measurements on the order of 10 ppbv or less – generally within the standard deviation range of the measurements.

Fusco and Logan (2003) also express concern that with the accuracy of the method imposed to simulate the annual flux of ozone across the tropopause, noting that an incorrectly modeled seasonal cycle, as appears likely in the case of the GEOS-CHEM model, could adversely affect the response of the modeled ozone to the stratospheric flux. In summary, there are a large number of questions concerning the conclusions derived from the model, in general, and more specifically in California. Thus, it is not a reliable tool to estimate mean background in California much less the range of background that might influence the attainability of the proposed standards.

Again, this is a selective reading of Fusco and Logan (2003). A full reading of their report shows that GEOS-CHEM tends to overpredict stratospheric downmixing, while being somewhat uncertain on in-situ tropospheric ozone dynamics, with the result that it has a small positive bias in springtime. This would tend to make GEOS-CHEM overestimate background ozone in California.

Since Staff recommends that the proposed standards be defined as concentrations not to be exceeded, the Chapter should evaluate the extreme values or yearly maxima of policy relevant background. There is now a substantial body of ozone observations that shows annual maximum 8-hour ozone concentrations in remote monitoring sites in the western United States that equal or exceed the staff's proposed 8-hour standard. This data is relevant to the issue of a regional, policy relevant background that will hinder the attainability of the proposed standard. Therefore, it should be included and discussed in the Chapter. In California, the ARB has provided the peak 8-hour indicators for all the air basins in the 2004 Almanac as well as in Chapter 7. The yearly maximum 8-hour concentrations in Lake County have averaged 0.069 ppm for the past 20 years and equaled or exceeded 0.07 ppm in 11 of the past 20 years. In the North Coast, the yearly maxima have averaged 0.072 ppm over the past 20 years and equaled or exceeded 0.070 ppm in 13 of the past 20 years. Inspection of the figures in Chapter 7 shows that the proposed 0.070 ppm standard would put the entire state out of attainment.

This comment again refers to attainment issues, not the health effects that drive the standard setting process (see response to comment 1). Note that the terminology "not to be exceeded" is superceded by State law and ARB's Area Designation Criteria. In addition, ARB Staff notes that any attainment designations under this standard will be made no sooner than 2006, and will be based on data collected between 2003 and 2005, so that the problematic values cited by the commentor are unlikely to represent air quality during the designation period.

[Alliance commentors present data on ozone concentrations in rural areas across the U.S., them conclude:] The idea that peak background is 0.04 ppm is inconsistent with the data from the cleanest of the California air basins where the population and emissions density is only a minute fraction of that in California. While transport from other more populated California air basins may play a role from time to time in the ozone values measured in the most remote air basins, the large fraction of daily 1-hour maximum and daily 8-hour maximum concentrations that are reported as 0.04 ppm and greater in the tables and figures in section 7.3.6 demonstrate a much higher policy relevant background than indicated in Chapter 4.

Data for a much less industrialized period (Bojkov, 1986) suggest that the 40 $\pm$ 10 ppbv average presented in the Staff Report is consistent with surface ozone concentrations observed in the absence of modern transportation, utility, and industrial emissions. Recitation of rural concentrations, absent dynamical analyses to support interpretation as "background" are not, of themselves, persuasive that <u>mean</u> background is much higher. The commentors themselves cite modeling they performed for the South Coast Air Basin based on the episode of August 3-7, 1997 that showed that, with "all anthropogenic emissions in the modeling domain turned off (both U. S. and Mexican)… the peak 8-hour ozone during the episode was 37 to 46 ppb."

ARB Staff agrees that there are occasional events of "background" ozone that show higher concentrations, but, as argued in the Staff Report, such events are unlikely to coincide with local ozone production sufficient to exceed the proposed air quality standard.

[Alliance commentors present an extended discussion of stratospheric downmixing and putative observations of surface ozone impacts of stratospheric downmixing. They conclude:] The known patterns of tropospheric folds together with the ground-level ozone- ^{7}Be analyses by Wolff et al. suggest that stratospheric ozone also contributes significantly to ground-level ozone during times when man-made ozone is present.

The processes controlling the concentrations and survival of stratospheric ozone after it moves into the lower troposphere are very complex. Although ^{7}Be is radiogenically produced in the stratosphere, it's concentrations are not linearly related to stratospheric ozone, especially after movement into the troposphere. The referenced paper suggests recurring downward stratospheric ozone transport over the eastern U.S. More recent analyses of ^{7}Be and ^{10}Be data from Europe (Zanis et al., 2003) indicates that most stratospheric transport events (STEs) are short lived, and that ^{7}Be observed during

periods of anticyclonic circulation (associated with regional ozone events in the eastern U.S.) is potentially a biased estimator for stratospheric ozone transport due to reduced Be removal rates in dry air and the accompanying high insolation that accelerates in-situ ozone formation by tropospheric photochemistry.

In addition to the examples in the references noted above, there are several cases in the references presently included in the chapter of elevated ozone transported long distances that contain a mixture of anthropogenic and stratospheric air. In these situations, routine monitoring data will not be able to distinguish the anthropogenic contribution from the stratospheric contribution. Although the ARB and the USEPA have "exceptional event" policies, it is likely that only a small portion of the stratospheric intrusions that affect ground-level ozone concentrations will be uniquely identified and thereby qualify for the exceptional event policy.

An air mass carrying ozone from a "classic" STE can be distinguished from one carrying anthropogenic ozone by its chemistry. An anthropogenic ozone plume would contain elevated concentrations of long-lived combustion-related gases, such as CO and CO_2; the CO/CO_2 ratio would be elevated, and there would be accompanying combustion-related aerosols, including sulfates and carbonaceous species. In contrast, stratospheric ozone would not be accompanied by other gaseous or aerosol pollutants and would be marked by very low relative humidity (RH) since there is little water in the stratosphere to begin with, and compressional heating during descent to the surface would drive RH very low. Determination of an "exceptional event" would rely primarily on meteorological analyses, and chemical evidence where available, that support a showing that synoptic conditions were compatible with stratospheric ozone intrusion..

We would like to see this available literature included in the review of the role of stratospheric ozone on ground level background. It appears from these other studies cited that the Galani et al. (2003) study is not typical of Europe or of the U. S.-relevant studies.

The characterization of ARB Staff's review as being based solely on Galani et al. (2003) is incorrect. ARB Staff believes that the observational record presented by Galani et al. (2003) is representative. Moreover, we find broad consistency in the balancing of stratospheric intrusion and in-situ formation as explanation for tropospheric ozone across the many papers coming from the large, integrated STACATTO program, the general discussion of Lelieveld and Dentener (2000), ozonesonde data, GEOS-CHEM modeling, and other sources. ARB Staff will update the Staff Report to reflect discussions in these responses, but we do not intend wholesale inclusion of all the material presented by the commentors.

Problems with Comparing Average Behavior with an Extreme Value Standard: Chapter 4 focuses on background as it may apply to the stable, stagnant conditions that produce the highest ozone concentrations from man-made emissions. For example, it is argued that some background sources generally peak in other seasons than man-made ozone and that they are generally not major contributors to observed peak ozone. However,

the review proposed an extreme value standard that applies everywhere in California all the time. So the range of background during worst case urban episodes is not the only concern. The evidence from observations around the globe and modeling is that the factors and processes that affect ozone levels in the atmosphere are very complex. There are complex chemical and dynamic processes involved that interact in a variety of ways. Stratospheric intrusions create elevated ozone plumes that may persist or mix with neighboring air. Under certain conditions, long-range transport of man-made ozone or its precursors from continent to continent is observed. Large-scale plumes originating in the stratosphere and plumes from long-range transport and plumes from nearby urban areas can all cause elevated ozone levels exceeding the proposed state standards. Sometimes ozone from these sources is mixed together so that one cannot identify a specific source. It just takes one combination of the many different combinations of these sources to violate the state standard.

Determination of attainment or violation of State standards does not solely rely on identifying the highest measured concentration at a monitoring site. Statistical filtering is used to avoid arbitrary determination of attainment status due to very rare or unique situations (Guidance for Using Air Quality-Related Indicators in Reporting Progress in Attaining the State Ambient Air Quality Standards, ARB Research Report 93-49, 1993, pp. 21-26). In addition, measured values above the standard that can be shown to be very rare or associated with unusual weather or sources beyond regulatory control can be removed from consideration as exceedances of the standard through the State's Attainment Designation process. Designations and the procedures for designation are subject to public review and comment since the California Health and Safety Code (H&SC) requires the Board to periodically review the criteria it uses for making State area designations and both the H&SC (section 39608) and the regulations covering designation criteria (17 CCR, section 70306) require the Board to review the area designations annually and to redesignate areas as new information becomes available.

The Background Used in ARB Transport Assessments: The March 2001 ARB Report, in reference to background level ozone, states the following: "For instance, clean air, such as the air mass over the Pacific Ocean has a normal background of 4 pphm. Areas in the mountains may have background concentrations of 5 or 6 pphm…" (March 2001 Staff Report at page D-2) Since 4 pphm is the same as 0.04 ppm or 40 ppb, the ARB, in assessing transport, considers the normal or average clean air background coming off the Pacific to be 0.04 ppm. This contradicts the statement on page 4-11 that the maximum clean air background is 0.04 ppm. The "clean air" boundary conditions used in photochemical modeling also specify 0.04 ppm ozone because it is widely accepted as an average clean air background. The normal background at elevation noted in the March 2001 Staff Report of 0.05 or 0.06 ppm is very close to the proposed 8-hour standard of 0.070 ppm, so that fluctuations around the normal background will likely cause violations of the proposed standard.

The reviewer has identified an error in the text of the 2004 Staff Report – the referenced statement in the Summary should read "The models reviewed here indicate that average "natural background" ozone near sea level is in the range of 15 – 35 ppbv, with

a maximum monthly mean of about 40; at altitudes above 2 km stratospheric intrusions can push peak "natural background" concentrations to 45 – 50 ppbv." This will be corrected in future versions of the Staff Report.

Policy Relevant Background Levels Given that the extreme values of background can approach or exceed the proposed standards, the proposal allows little or no room for ozone from mankind's activities: With a policy relevant background that varies substantially, there will be times and places where the background approaches the 70 ppb level of the proposed 8-hour standard. The Chapter limits the discussion of policy relevant background to the meteorological conditions conducive to peak urban ozone formation. While this is currently the limiting case for development of control plans, it may not be under a 70 ppb standard. If the policy relevant background is 40 ppb and the standard is 70 ppb, the amount of ozone that can be formed from man-made emissions is only 30 ppb. So even with a 40 ppb background, the proposed standard allows little room for man's activities. On a day when the background is 60 ppb, the margin for man's activities will be only 10 ppb. On a day when the background is 70 ppb, there is no margin for man's activities. While this illustration over-simplifies the complex chemical and meteorological processes involved in ozone formation and transport, it demonstrates that transport of ozone from upwind natural and non-California man-made sources can make the proposed standard unattainable.

The case presented has an internally modeled "background" that is in the range of 37 – 46 ppb, well within the range discussed in the Staff Report. Peak ozone events in Southern California depends on strong local temperature inversions and overlying synoptic high pressure. These conditions preclude rapid down-mixing of stratospheric air to the surface. Slow downmixing from the stratosphere at this time of year is relatively weak, and any stratospheric air present near the surface will be many days old and strongly diluted, thus high natural " background" ozone is not expected during such an episode. No case is made for why much higher "background" ozone can be assumed in discussing these findings. Note that ARB Staff cannot comment in detail on the commentor's modeling exercise since the details of the model specifications have not been included in the comments.

Even with a 40 ppb background advected into the South Coast Air Basin, the degree of emission control required to attain a 70 ppb standard is unreasonable. The Alliance of Automobile Manufacturers asked ENVIRON International to carry out photochemical modeling of Southern California to investigate whether an 8-hour ozone standard of 0.070 ppm could be achieved… even 95% additional control of the man-made VOC and NO_x from current 1995 baseline is not enough to attain the proposed 8-hour standard… The difficulty in finding additional emission reductions to enable the South Coast to attain the federal 1-hour standard is well known. It has led to the use of long-term or "black box" emission reductions within the Basin in order to demonstrate attainment of the federal 1- hour standard… The 90% control of man-made emissions beyond the 2003 AQMP did bring [other] air basins below the 70 ppb proposal, but when Mexican emissions were added back in, the proposed 8-hour standard was exceeded in the San Diego and Salton Sea air basins… For other situations in which there is an additional

contribution from natural sources or transport of non-California man-made ozone or ozone precursors, the margin for manmade ozone associated with the 70 ppb standard will be reduced. In much of California, the reactions of biogenic NO_x emissions (that maybe increased due to fertilizer use) and biogenic VOC will contribute additional uncontrollable ozone that will add to the regional background coming off the ocean.

See response to comment 1.

Summary of Chapter 4: Background Ozone in California: In summary, the scientific literature on background ozone indicates that it is highly variable and can reach levels close to the current California 1-hour standard. There is ample evidence that the proposed 8-hour standard will be exceeded, as a result of the regional background from natural and non-California sources, in all California air basins and throughout much of the Western U. S. including many national parks. The ARB discussion of background relies on an unverified model calculation and discounts the large body of observations and analyses around the world that indicate higher maximum background concentrations than ARB assumes.

ARB Staff does not concur in the commentor's assertion that ozone in California, absent a contribution from in-State anthropogenic precursor sources, can approach the current 1-hour standard. ARB Staff accepts that there may be some (as yet unquantified) potential for exceedances of the 8-hour standard due to a combination of natural and anthropogenic ozone production, however we believe that these will be infrequent in time and space, generally restricted to high altitude locations. Nonetheless, the putative existence of such events is not relevant to the standard setting process (see response to Comment 1).

In addition, the scientific literature and the USEPA ozone scientific review support a higher maximum background than ARB assumes. The review states that the influence of tropopause folding events that insert high concentrations of ozone from the stratosphere into the troposphere will be easily recognized and dealt with by the exceptional events policy. However, as documented in the references noted above, there is evidence that these events may not be easily identified. The policy relevant background varies spatially and temporally. It varies substantially on both seasonal and short-term time scales, and policy relevant background levels leave little room for man's activities.

ARB Staff recognizes that there may be occasions when "background " ozone contributes to exceedances of the proposed standard, however, as discussed above, health effects levels, not attainability of the standard, are the primary determinants of the standard.

Allen S. Lefohn, Ph.D., A.S.L. & Associates, Helena, Montana: Comments prepared for American Petroleum Institute, Washington, D.C., and Western States Petroleum Association, Sacramento, CA, Dated August 27, 2004.

Note: Dr. Lefohn submitted nearly 50 pages of comments. Much of his submission is devoted to a literature review and presentation of extensive monitoring data. Point–by-point citation and reply would reiterate discussions in the Staff Report and revisit issues dealt with above. ARB staff have reviewed Dr. Lefohn's materials, and we present here responses to his major points, conclusions, and criticisms of the Staff Report. The responses here are organized according to Dr. Lefohn's summary.

In the comments, specific focus is provided on the following issues:

Estimates of *policy-relevant background* concentrations need to consider the important contribution from stratospheric O_3, as well as other natural sources;

Stratospheric ozone intrusion is explicitly treated in the Staff Report. Discussion of significant historical ozone data cited by Dr. Lefohn (Bojkov, 1986) will be added to the Staff Report. The measurements cited by Dr. Lefohn are within the range discussed in the Staff Report.

There is large variability among global models on the attribution of the contribution of natural O_3 to the background;

ARB Staff agrees that there is wide variation among models, however much of the disagreement is due to the difficulty of comparing results across models with different vertical and horizontal resolutions. In preparing the Staff Report ARB Staff selected recent modeling studies that are constrained by recent observations and theoretical understanding of ozone formation and transport.

The California Ambient Air Quality Standard Document (CAAQSOD) states that ground level impacts from fires are typically in the range of 15-25 ppb. Such is not necessarily the case;

ARB Staff agrees with Dr. Lefohn that there are documented cases of very large fires, such as the Yellowstone fires of 1988, that have produced elevated ground level ozone measurements. We disagree with Dr. Lefohn's contention that such an event may be missed by California's exceptional event policy. The Yellowstone fires were, by their nature, a very rare ("once a century") event, with smoke impacts across several states – such an event would be impossible to overlook as an "exceptional event." The lower ozone impact numbers presented in the Staff Report were based on California's fire experience and relate to recurring large fires in the State, not extreme events.

Given the limitations discussed in this report with the Lin et al. (2000) and Jaffe et al. (2003b) trending analyses, the scientific evidence for an Asian influence on surface O_3 concentrations on the United States is weak and further research efforts are required;

ARB Staff agrees that Asian ozone is not, at present, a significant source of enhanced ozone in California. The Staff Report included the modeling and anecdotal evidence in order to provide the reader with a complete picture of the exogenous ozone sources

that may enhance locally formed ozone in California. We agree that further research on this problem is needed. We disagree with Dr. Lefohn's interpretation of the data presented by Jaffe et al. (2003b) as suggestive that elevated springtime ozone at Mt. Lassen is more likely to be due to stratospheric intrusion rather than long range transport. The persistent vertical stratification characteristic of the meteorology in the region makes both sources more likely to be detected at the Lassen site. Regardless of the source, the Mt. Lassen springtime ozone peaks are the type of event that ARB Staff expects would be subject to review as "exceptional events."

The CAAQSOD emphasizes that the violations associated with the proposed 0.07 ppm 8-hour average standard would occur during the summertime, when stratospheric O3 contributions are thought to be minimal. However, when one characterizes the hourly average concentrations collected in 2003 for 184 monitoring sites in California, one finds that violations of the proposed 8-hour average standard occur during spring, summer, and fall;

ARB Staff disagrees with Dr. Lefohn's interpretation of the monitoring data. The Staff Report acknowledges the potential for elevated background ozone concentrations at high altitude sites, but we are unconvinced of the "natural" origin of many of the ozone peaks listed, and the commentor does not provide dynamical analyses to support such interpretation. ARB Staff does admit to the potential for infrequent standard exceedances due in part to influx of exogenous ozone, however any exceedance caused by stratospheric ozone intrusion or wildfire would be subject to "exceptional events" review under State law.

Because violations of the proposed 8-hour average standard occur during spring summer, and fall, policy-relevant background concentrations that occur during seasons other than summer will have to be characterized so that emission control actions result in optimum reductions in hourly average O_3 concentrations;

ARB Staff agrees that "off season" ozone peaks above the proposed standard will be encountered at California monitoring sites. The sources of these peaks will need to be investigated in the course of designating "design days" for control plans and to insure that exceedances due to natural or transport processes outside ARB's regulatory control are not misinterpreted.

At some monitoring sites in California, when stratospheric O_3 predominates in comparison to anthropogenic sources during the spring, it may not be possible for regulators to control hourly average concentrations in the 0.05 – 0.06 ppm range using emission reduction strategies;

ARB Staff agrees that current control strategies may not be effective for ozone concentrations in this range, however, as discussed in the response to comment 1 above, the standard is to be based on health effects, and, moreover, the proposed standard would not require any regulatory action for ozone concentrations in this range.

The empirical data provide a solid indication to CAAQSOD that policy-relevant background O_3 hourly average concentrations, as defined on page 4-1, are more than likely higher than the 15-35 ppb discussed in the document. Using models that provide highly uncertain concentration estimates provides an overly optimistic message to those who are responsible for implementing control strategies.

We agree that reading the long term mean values presented in the Staff Report as absolute maxima would be misleading. The text in the Staff Report will be reviewed to insure such a misreading in precluded.

In some of the modeling efforts to estimate natural background O_3 concentrations within North America, investigators removed all anthropogenic emissions of NOx, CO, and nonmethane hydrocarbons (including NO_x emitted from aircraft and fertilizer, but not biomass burning). Because the State of California does not plan to eliminate all anthropogenic emissions of NO_x, CO, and nonmethane hydrocarbons (including NO_x emitted from aircraft and fertilizer), the estimates for the range of hourly average policy-relevant background concentrations will be greater than the 4-hour afternoon average background (i.e., natural background, in North America and anthropogenic and natural background outside of North America) values estimated by these models.

ARB Staff does not propose or project that all anthropogenic sources of ozone precursors in California could be eliminated. The background discussion is intended to give readers a sense of the scale of the in-State anthropogenic contribution to observed ozone concentrations. Comparing model results with and without in-State anthropogenic sources provides a cross-check on estimates based on interpreting the literature on global ozone formation processes and reporting the scant data available from preindustrial sampling.

The proposed 8-hour standard of 0.07 ppm is violated in pristine places, such as Yellowstone National Park in Wyoming. The ambient concentrations experienced at Yellowstone National Park in the springtime represent policy-relevant background as defined in Chapter 4 of the CAAQSOD. This implies that the proposed 8-hour standard will be difficult to attain in some areas that are affected by stratospheric O_3 during the spring and that perhaps the methodology used by Staff to propose the form and level of the 8-hour standard provides highly uncertain results.

As stated above, ARB Staff accepts the possibility that some rural sites, especially those at high elevation, may experience occasional ozone concentrations in excess of the proposed standard that are not obviously linked to local or upwind in-State emissions of ozone precursors, and we plan to address these though an "exceptional events" policy

Appendix F

March 4, 2005 Letter from Joan Denton, Director of the Office of Environmental Health Hazard Assessment to Catherine Witherspoon, Executive Officer, Air Resource Board

Submission of OEHHA Recommendations to the ARB for an Ambient Air Quality Standard for Ozone

Office of Environmental Health Hazard Assessment

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Alan C. Lloyd, Ph.D.
Agency Secretary

Arnold Schwarzenegger
Governor

MEMORANDUM

TO: Catherine Witherspoon
Executive Officer
Air Resources Board

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

DATE: March 4, 2005

SUBJECT: RECOMMENDATION FOR AMBIENT AIR QUALITY STANDARDS FOR OZONE

I am transmitting to you the Office of Environmental Health Hazard Assessment (OEHHA) recommendations for Ambient Air Quality Standards for ozone. Our draft recommendations and their underlying scientific rationale have undergone public comment and a full review by the Air Quality Advisory Committee (AQAC), the external scientific peer review committee appointed by the President of the University of California, and appropriate revisions to the rationale have been made. The OEHHA recommendations and underlying rationale have been incorporated into the "recommendations" chapter of the ARB staff report, developed for the review of the ozone standard under SB25.

California ambient air quality standards have four elements (California Health and Safety Code Section 39014, and Title 17, California Code of Regulations, Article 2, Section 70101): (1) definition of the air pollutant, (2) an averaging time, (3) a pollutant concentration, and (4) a monitoring method to determine attainment of the standard. OEHHA recommends the following revision be made to the California ambient air quality standard for ozone:

1. Ozone will continue to be the pollutant addressed by the standard.
2. Ozone 1-hour-average standard – Retain the current 1-hour-average standard for ozone at 0.09 ppm, not to be exceeded.
3. Ozone 8-hour-average standard – Establish a new 8-hour-average standard for ozone at 0.070 ppm, not to be exceeded.
4. These recommendations are based on the following findings:

   a. Reduced lung function, and increased respiratory symptoms following 1-hour exposure to 0.12 ppm ozone with moderate to heavy exercise.

b. Increased airway hyperreactivity following 2-hour exposure to 0.18 ppm in exercising subjects.

c. Airways inflammation following 2-hour exposure to 0.20 ppm ozone in exercising subjects.

d. Reduced lung function, increased respiratory and ventilatory symptoms, increased airway hyperreactivity, and increased airways inflammation following 6.6- to 8-hour exposure to 0.08 ppm ozone.

e. Evidence from epidemiological studies of effects of ozone on lung function, respiratory symptoms, asthma exacerbations, emergency room visits, hospitalization and premature mortality.

f. Evidence from epidemiological studies of emergency room visits for asthma suggesting a possible threshold concentration between 0.075 and 0.11 ppm from analyses based on a 1-hour averaging time, and a possible threshold concentration between 0.070 and 0.10 ppm from analyses based on an 8 hour averaging time.

g. There is no evidence that children and infants respond to lower ozone concentrations than adults. Their risk is primarily related to their greater ventilation rate and greater exposure duration.

h. The dose-rate of ozone inhalation influences the magnitude of observed effects.

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

Should you have any questions or concerns, please call me at (916) 324-7572.

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Appendix G

Review of Animal Toxicological Studies on the Health Effects of Ozone

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-hyroxypropyl)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_2	nitrogen dioxide
PHA	phytohemagglutinin
PM2.5	particulate matter with an aerodynamic size cutoff of 2.5 microns
PM10	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_2	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^3	milligrams per cubic meter

Review of Animal Toxicological Studies on the Health Effects of Ozone

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).

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 ^{18}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 ^{18}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 ^{18}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 ^{18}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}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}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}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}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}O of 32.2 µg/g dry weight) and the parenchyma the lowest concentration (excess ^{18}O of 7.8 µg/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}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}O content by airway level can predict airways that will exhibit oxidant injury.

In rats and guinea pigs exposed to 1 ppm ^{18}O-labeled ozone for 2 hours, the content of ^{18}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.

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, 1989a; 1989b; 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. (1989a) 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.

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_2) 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.

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^3/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 ^{18}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.

Respiratory Tract Effects

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 34ºC (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 phophatase activity at 22ºC. 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., 2003b). 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, 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.

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.

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. Maintainence of the new clearance rate night 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^3), 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^3) 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.

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 E2, 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 polystyene 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) demostrated 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, peroxynitrite) 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).

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 in to 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 interstitum 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 (Bleavins 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.

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 phagocytzing *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 aureas (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. miribilis 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 (backgroud 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 produced superoxide anion.

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* hydbridization 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 hydroxypyridinium, 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, hydroxypyridinium 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 hydroxypyridinium 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 (μmol/min/g supernatent 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 phophatase and glucose-6-phosphate dehydrogenase) in lung homogenates.

Levels of antioxidant substances (i.e., ascobate, 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 occured 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 (34ºC) while uric acid levels in BAL fluid increased or were similar to controls in a cold ambient temperature (22ºC). 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.

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 methological 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™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 $B6C3F_1$ 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 the1.0 ppm level (Herbert et al., 1996). In male $B6C3F_1$ 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 (Hassett et al., 1985b). In studies investigating the tumor promotion potential of ozone, male Wistar rats administered a single dose of N-bis(2-hyroxypropyl)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 (Hassett 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 carcingenicity 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 carcingenicity, and by extrapolation, human carcinogenicity, at ambient air levels is presently uncertain.

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.

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.

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 microbiocidal 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 (Burleson 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.

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.

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 seratonin 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 seratonin 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.

Hematology and Serum Chemistry

In the previous Ozone Review (ARB, 1987), ozone was reported to a have 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 measuered 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).

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*CO2 (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 (22ºC) 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.

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 (22ºC) 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 extrapumonary 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 bradyarrythmic 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.

Pre- and Post-Natal Effects of Ozone

Particular attention has recently been focused on assessing the adverse effects of ozone exposure in infants and children, particularly because the young may inhale a greater relative dose of ozone as a result of their increased ventilation rate per unit body

weight compared to adults. Physiologically based pharmacokinetic (PBPK) modeling estimates show that the regional extraction of ozone is relatively insensitive with age, but the extraction per unit surface area is two- to eightfold higher in infants (≤1 yr of age) compared to adults (Sarangapani et al., 2003). Extraction per unit surface area differences between adults and infants were greatest for the pulmonary region, suggesting that up to eight times the amount of ozone reaches and reacts with target regions of the deep lung in infants compared to adults. Additionally, lung development occurs over the entire perinatal period. Thus, exposure effects can have significant consequences whether they occur during the pre- or postnatal period and can result in long-term effects persisting into adult life.

Effects of Prenatal Exposure to Ozone

Maternal exposure of rats to 1.0 ppm ozone continuously during mid- or late gestation (Days 9-12 or 17-20) resulted in reduced neonatal growth rates, with late gestational exposure resulting in retardation of early reflex development and open field behavior (Kavlock et al., 1980). However, maternal toxicity was not discussed. Lower maternal exposures of mice or rats to ozone during gestation had little or no impact on developmental measures of the offspring. Intermittent and continuous maternal exposure to ozone concentrations between 0.2 and 0.8 ppm during gestation produced occasional transient decreases in maternal body weight and food and water consumption, but physical developmental effects and major neurobehavioral developmental effects of the pups were not apparent (Bignami et al., 1994; Petruzzi et al., 1995; Kavlock et al., 1979; Sorace et al., 2001). Recently, a wide-ranging battery of neurobehavioral tests was conducted in mice exposed continuously to 0.3 or 0.6 ppm ozone prenatally up to day 17 of gestation (Sorace et al., 2001). Results from the low concentration prenatal exposure condition (0.3 ppm) suggested long-term neurobehavioral impairment when the animals were tested at adulthood, but the data failed to show a concentration-dependent effect. Impaired reversal learning in the Morris water maze test, longer latency to step-through on the passive avoidance test, and a decrease in wall rearing in the hot-plate test were recorded at 0.3 ppm but not at 0.6 ppm. Petruzzi et al. (1995) also reported possible neurobehavioral findings only at low ozone concentrations, suggesting that low exposure levels do not yield to adequate compensatory mechanisms for protection from oxidant injury compared to high concentrations of ozone.

In other studies using high ozone concentrations, exposure of pregnant female rats to 1.0 ppm for 12 hr/day during gestation resulted in morphological anomalies of the cerebellum of the offspring, including damaged Purkinje cells, a decrease in total area and number of Purkinje cells, abnormal fibrillar structures in the molecular layer, and a diminished folding pattern over the surface of the anterior lobe (Rivas-Manzano and Paz, 1999; Romero-Velazquez et al., 2002). 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 sleep cycle. It was theorized that ozone reaction products permeated the circulatory system and reached the brain to produce these effects. However, it was also acknowledged that ozone-induced maternal effects and subsequent decreased body weights of pups might also be responsible for the CNS changes.

Effects of Both Pre- and Postnatal Exposure of Ozone

A few studies examined the effects of pre- and postnatal ozone exposure in mice. Continuous exposure to 0.6 ppm ozone from several days before start of pregnancy up to 26 days after birth produced transient depressed dam body weight and long-lasting depressed body weight in pups. There was also attenuation of sex differences in some activities that suggest persistent neural and endocrine changes similar to early stress effects (Bignami, 1996; Dell'Omo et al., 1995a; Dell'Omo et al., 1995b). Exposed offspring subjected to swimming navigation tests did not show consistent developmental effects with the exception of left-turning preference. Swimming navigation tests are reported to be a sensitive indicator for hippocampal damage. Subsequent tests for handedness of mice 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 did not produce clear differences in paw preferences for delivery of food pellets (Petruzzi et al., 1999). Offspring tested for morphine reactivity to a hot plate also did not produce consistent results across exposure concentrations, though there was general tendency towards reduced drug sensitivity at the highest concentration (0.9 ppm).

Effects of Postnatal Exposure of Ozone

Shore et al. (2002) investigated age-related pulmonary function responses to acute ozone exposure (0.3, 0.5, 1.0 ppm or greater for 3 hr) in immature and adult mice. Pulmonary function tests showed that ozone concentrations >0.3 ppm caused a concentration-related decrease in minute ventilation in mice of all ages, but the response was significantly less in 2-week-old mice than in mice 4 to 12 weeks of age. This change resulted in a two- to threefold increase in the inhaled dose of ozone normalized for body weight in the immature mice. Subsequent tests noted greater protein content in BAL fluid in exposed immature mice compared to exposed adult mice, but exposures were conducted only at ozone concentrations greater than 1 ppm.

The earliest studies investigating age-related differences in ozone susceptibility reported conflicting findings. A qualitative morphological examination by Stephens et al. (1978) reported that postnatal rats are resistant to ozone-induced pulmonary injury until weaning. In groups of 1 to 40-day-old rats exposed to 0.85 ppm ozone for 1 to 3 days, the appearance of “tissue nodules” (denser toluidine blue-staining areas), hypertrophy of epithelial lining, loss of cilia from ciliated cells, and type I cell injury in the centriacinar regions did not occur until rats were 20 days of age or older. The tissue nodules were reported to be due to proliferation of nonciliated and type II cells following ozone injury. In another study, 5 to 15 day-old neonatal rats exposed to 0.9 ppm ozone continuously for 3 days had reduced body and lung weights, while exposed weanling rats 21 to 41 days of age displayed reduced body weights and increased lung or lung/body weights (Tyson et al., 1982). Nursing mothers also had increased lung/body weight (suggestive of reduced body weights) and protein/DNA ratios, indicating slight ozone-induced pulmonary edema compared to neonatal rats. GSH-shuttle enzymes in lungs were unchanged or decreased in neonatal rats, but increased in lungs of weanlings 21 days old or older. Elsayed et al. (1982) conducted a similar exposure study (0.8 ppm ozone for 3 days), but reported that the effects seen were indicative of increased susceptibility in neonatal rats. Effects included increased mortality (7- and 12-day old), decreased

body weight, lung weight, and enzyme activity in lung homogenates of neonatal rats (7-day old) compared to rats 18 days old or older.

Further support for the notion that immature rats are more sensitive to the acute inflammatory effects of ozone comes from a study of neonatal rats (13-days of age) exposed to 1 ppm ozone for 2 to 6 hr. The results showed a considerably greater peak concentration of lavaged prostaglandin E_2, a greater percentage of lavaged leukocytes that were non-viable, and larger numbers of lavaged dead epithelial cells compared to rats that were 18 or more days old when exposed (Gunnison et al., 1992). The concentration of protein in lavage fluid following exposure did not show age-dependence, possibly as a result of lavaging before protein increases can be measured in lung airways. Juvenile rodents have also shown increased sensitivity to the acute inflammatory effects of ozone compared to older rodents. Dormans et al. (1996) observed that exposure to 0.8 ppm ozone for 12 hours resulted in highest levels of protein and albumin in BAL fluid in one month old rats, with lesser increases occurring in 3, 9, and 18-month-old rats. A decrease in the net percentage of PMN influx in BAL fluid was also observed in older rats. Semiquantitative morphological evaluation following acute or 7-day exposure showed that the extent of centriacinar lesions was significantly lower as age increased.

Many of the enzymes that play a critical role in lung metabolism are not fully developed at birth (Pinkerton and Joad, 2000). A number of these enzymes, including antioxidant enzymes, are responsible for both activation and detoxification of xenobiotic compounds. The effect of age on changes in antioxidant enzyme activities in homogenized rat lungs was assessed following 72-hr continuous exposure to 0.9 ppm ozone (Tyson et al., 1982). GSH-shuttle enzyme activity was elevated in young adult and, to a lesser degree, in weanlings. Enzyme levels in exposed neonates 5 to 15 days old remained unchanged or were lower than weanling rats. Elsayed et al. (1982) noted similar age-related differences in enzyme activities resulting from acute ozone exposure (0.8 ppm for 3 days). Dormans et al. (1996) observed increased GSH-shuttle enzyme activities in both juvenile and adult rats (1 to 18 mo of age) following 12 hr or 7 day exposure to 0.8 ppm ozone. However, no overall age-related change in enzyme activities was apparent, suggesting that ozone-related pulmonary induction of GSH antioxidant activities are near, or at, adult capacities by 1 month of age. Other age-related effects of ozone on biochemical indicators of inflammation investigated changes in chemokine and cytokine expression. Adult mice showed early increases in mRNAs encoding antioxidants, chemokines, and cytokines after acute ozone exposure (1 ppm) compared to newborn mice, indicating more extensive epithelial cell injury in adult mice (Johnston et al., 2000). In contrast to the ozone findings, the researchers observed similar responses of newborn and adult mice in response to an agent not causing epithelial injury (endotoxin), suggesting the altered inflammatory control observed between newborn and adult mice following ozone exposure is secondary to epithelial cell injury. The relationship of age to rat lung collagen synthesis has also been investigated. Three-day exposure of 24-365 day-old rats to 0.8 ppm ozone resulted in increased collagen synthesis, indicated by greater incorporation of ^{14}C into ^{14}C-hydroxyproline in all age groups (Hacker et al., 1986). However, there was a relatively greater increase in older rats roughly starting at 60 days of age.

Studies investigating age-related susceptibility to infection following ozone exposure are also conflicting. 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., 1993). Ingestion and intrapulmonary killing of the bacteria by alveolar macrophages (AM) were reduced in all ozone-exposed mice, but the apparent reduction of AM phagocytosis in younger mice was more marked. In contrast, Dormans et al. (1996) observed no effect of age on the reduced pulmonary clearance of *Listeria* bacteria among 1, 3, 9, and 18 month-old rats exposed to 0.8 ppm ozone for 12 hr, or 12 hr/day for 7 days.

A few studies investigated the effects of 6-week ozone exposures in young or neonatal rodents. Pulmonary function tests on rats exposed to ozone (0.08-0.25 ppm, 12 hr/day, 7 days/week) beginning at birth for 6 weeks and compared to adult rats exposed to the same ozone regimen indicated greater sensitivity in the neonates (Raub et al., 1983). Exposed neonates, but not exposed adults, showed evidence of increased lung distensibility (i.e., increased inspiratory reserve volume, reduced peak inspiratory flow, and increased inspiratory reserve volume, inspiratory capacity, vital capacity at high distending pressures). Image-analysis quantitation of lungs of mice exposed to 0.3 ppm ozone 7 hr/day for the first 6 weeks of life showed small but statistically non-significant increases in alveolar wall area and mean type II cell area immediately after end of exposure (Sherwin and Richters, 1985). Similar trends were noted in an earlier exposure study of adult mice, though the newborn mouse findings suggested a greater propensity for type II cell aggregation than in adults. Morphometric studies by Barry et al. (1985;1988) observed altered centriacinar epithelium in both one-day-old and 6-week-old rats exposed to 0.25 ppm ozone (12 hr/day) for 6 weeks, but did not find age-related differences in lung structure or lung maturation in this lung region. It was speculated that the 3 weeks of exposure following weaning might have resulted in the overall changes, which were not substantially different from those occurring in adult rats. In another morphometric study, exposure of rats to 0.64 or 0.96 ppm ozone for 6 weeks (8 hr/night) beginning at 28 days of age resulted in larger lungs and greater volumes of parenchyma, alveoli and respiratory bronchioles (Tyler et al., 1987). Exposed rats also had reduced body weights and lengths compared to rats fed *ad libitum*, but not compared to pair-fed rats. At the end of a 6-week post-exposure period, body weights of both ozone groups were reduced compared to both control groups, and lung volume and centriacinar changes had not fully recovered in high exposure rats.

Age-related differences in ozone susceptibility have also been carried out in higher mammals. Phalen et al. (1986) exposed 6-week old beagle dogs to 1 ppm ozone, 4 hr/day for 5 days to investigate the effects of ozone on postnatal lung maturation. Beagle dogs were selected because postnatal lung development is similar to humans and alveolar development occurs over a period of a few months. Six weeks after exposure, morphometric analysis of mean linear intercept changes showed a small but statistically significant decrease in lung surface area of about 4%, indicating anatomical retardation in formation of new alveoli. However, no other gross or histologically observable defects in lung morphology were observed. 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 tracheal mucociliary system by decreasing epithelial cell density, retarding the normal developmental decrease in the number of mucus cells, altering the lectin detectable carbohydrate composition of mucus in these cells, reducing tracheal ciliated and basal cell populations, increasing total mucus load, and reducing mucus velocity (Mariassy et al., 1990; Mariassy et al., 1989). 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. In comparison, 4-hour exposure of adult sheep to 1 ppm ozone did not alter lung clearance of a radiolabeled tracer instilled in the lungs (Hornof et al., 1989).

Monkeys provide an ideal model for developmental effects of ozone exposure in children because of similarities in postnatal lung and immune system development. Juvenile, 7-month old male cynomolgus monkeys exposed to 0.25 ppm ozone 8 hr/day either daily or daily only during alternate months for 18 months showed abnormal lung growth, with increased volume fraction of respiratory bronchioles and their lumens (Tyler et al., 1988). Both groups exhibited respiratory bronchiolitis but the seasonal model of exposure had significantly increased total lung collagen content, chest wall compliance, and inspiratory capacity indicating alterations in growth of pulmonary functions and delay in maturation. A follow-up study in male juvenile cynomolgus monkeys exposed to 0.64 ppm ozone 8 hr/day for 12 months investigated distal lung remodeling changes at end of exposure and after a 6-month post-exposure period (Tyler et al., 1991). As with the previous study, ozone exposure resulted in greater volume fractions and volumes of respiratory bronchioles, but had progressively worsened after a 6-month clean air post-exposure period. No changes in volume fractions of the alveolar compartment were observed.

Structural remodeling and airway immune changes in the developing Rhesus monkey lung was investigated in a series of studies using a cyclic regimen of ozone exposure and allergen inhalation in sensitized animals (Schelegle et al., 2003a; Larson et al., 2004; Evans et al., 2003). Groups of 30-day old monkeys were exposed to ozone (0.5 ppm, 8 hr/day) and house dust mite allergen (HDMA), or ozone and HDMA alone for 11 cycles (5 days exposure followed by 9 days of clean air). Cyclic exposure of nonsensitized monkeys to ozone alone had only mild, non-significant effects on most immune, structural, and functional end points examined. However, combined inhalation exposure to ozone and HDMA in sensitized monkeys acted synergistically to produce an allergic-reactive airway phenotype characterized by increased serum histamine, increased airways eosinophilia, altered structural development resulting in longer, narrower tracheobronchial airways, elevated airway resistance, and non-specific airway hyperresponsiveness to histamine challenge. In addition, highest levels of serum IgE and mucous cell content in terminal bronchioles were observed in the ozone + HDMA group. Altered development of neural innervation within the epithelium was also observed, including fewer airway generations with abundant nerve plexuses in allergen + ozone groups, and the appearance of a new population of undefined neuroendocrine or neuroendocrine-like cells in both ozone-exposed groups (Larson et al., 2004). Abnormal development of the basement membrane zone of lung airways was also observed in both groups of ozone-exposed monkeys, with irregular and thin areas of

collagen in the zone and altered levels of cytokines and molecules important for responses to lung injury (Evans et al., 2003).

Prenatal or combined pre- and postnatal exposure to episodic, low ambient ozone concentrations has not been performed in animals. At higher concentrations, prenatal or combined pre- and postnatal exposure to ozone in rodents produced suggestive evidence of subtle neurobehavioral effects, cerebellum changes and long-term body weight reductions. However, considering the high ozone concentrations (0.8 to 1.0 ppm) and extended exposure durations needed to produce these affects, maternal toxicity likely had a significant impact on development of the offspring. The earliest studies investigating age-dependent susceptibility to ozone were not detailed enough to provide definitive evidence for increased sensitivity of newborn rodents. More recent studies in rodents and higher mammals have shown that indicators for pulmonary inflammation (e.g., increased protein and cell count in BAL fluid and morphological evidence of changes in tracheal and centriacinar epithelium) increase in neonates and juveniles relative to adults upon exposure to ozone. However, these effects were noted after relatively high ozone concentrations. Other postnatal toxicity data suggest that differences in age-related susceptibility to ozone depend on the outcome indicator examined. For example, anti-oxidants and proinflammatory cytokines and chemokines respond quicker to acute ozone exposure in adult rodents than in newborns, suggesting increased lung injury in the adults. Six-week ozone exposures in young versus adult rodents have suggested decreased pulmonary function in the young, but morphological comparisons have not shown age-related differences at the level of the centriacinar unit. The best evidence for ozone injury in the young at low, episodic ozone exposures is in monkeys, particularly when combined with house dust mite allergen (HDMA). While strict comparisons with similar adult exposures were not performed, ozone clearly enhanced the effects of allergen sensitization and altered the development of airway structural and immune system components.

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 interections 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.

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^3 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_2) 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_2 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^3), 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 bronchoalvaolar 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^3 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^3 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^3 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^3 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^3), 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^3 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^3 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^3 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^3 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^3), 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^3 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.

Ozone and Nitrogen-Containing Pollutants

Ozone and nitrogen dioxide (NO_2) 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_2. Due to ozone's greater oxidant potency relative to NO_2, ozone is often the driver of pulmonary effects. Estimates of the relative effects of ozone and NO_2 have shown that ozone can cause 15- to 20-fold greater lung injury than NO_2 at the same concentration. Thus, the relative contribution of ozone and NO_2 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_2 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_2 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_2 (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_2) 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_2 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_2), 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_2 and generates a highly reactive species that is at least partly responsible for the synergistic effects. In addition, when the concentration of NO_2 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_2 mixture for the three higher dose rates in the large airways (0.4 ppm ozone + 7.2 ppm NO_2 for 12 hr/night; 0.6 ppm ozone + 10.8 ppm NO_2 for 8 hr/night; 0.8 ppm ozone + 14.4 ppm NO_2 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_2 (2.5 ppm), or NO_2 (6 and 12 ppm). Exposure to ozone + NO_2 at rest increased bronchoalveolar permeability, but was not different from exposure to ozone alone. However, exposure to ozone + NO_2 during exercise led to significantly greater permeability than did exposure to ozone alone during exercise. Only exposure to 12 ppm NO_2 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_2 (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_2 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^3 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_2 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_2 with peaks of 1.0 ppm NO_2 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_2 with peaks of 0.1 ppm NO_2 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_2 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_2 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_2 mixture that was half the concentration (0.4 ppm ozone and 7 ppm NO_2) 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_2 exposure was examined by Fujimaki (1989). Continuous exposure of mice to 0.8 ppm ozone, 4.0 ppm NO_2, 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_2 alone had little or no

effect on these organ weights. Continuous exposure of mice for two weeks to the ozone/NO_2 mixture suppressed plaque-forming antibody production (mostly IgM) in spleens when subsequently immunized with sheep erythrocytes (Fujimaki, 1989). Exposure to NO_2 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_2 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_2 mixtures. These immune function studies suggest that NO_2 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_2, 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_2 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_2, 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_2 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_2, 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 sulhydryl 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_2, and ozone + 0.4 ppm NO_2 for up to 22 months and examined for pulmonary biochemical effects (Sagai and Ichinose, 1991). Ozone exposure duration

was10 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_2 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_2 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^3 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_2 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_2 (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_2 was characterized as additive at best, though exposure to NO_2 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_2 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_2 (0.35 ppm) enhanced lung cancer cell colonization (Richters, 1988). However, NO_2 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_2, male rats were administered a single dose of N-bis(2-hyroxypropyl)nitrosamine (BHPN) followed by exposure to a mean ozone concentration of 0.05 ppm plus 0.4 ppm NO_2 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_2 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.

Ozone and Particulate Matter including Complex Mixtures

Recent epidemiological evidence has found an association between high levels of small airborne particules and increased morbidity and mortality, particularly among individuals with preexisting lung and heart disease. Respirable particulate matter is generally referred to as PM10 (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 PM2.5 (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^3 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_2 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_2. The findings from histopathology and macrophage phagocytosis after 20 days of exposure suggested that effects of the ozone + NO_2 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^3) plus ammonium bisulfate (0.070 mg/m^3) plus ozone (0.2 ppm), a high level of carbon (0.100 mg/m^3) plus ammonium bisulfate (0.140 mg/m^3) 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^3 or 50 mg/m^3), 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^3) 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^3), 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^3 and 0.250 mg/m^3, respectively) elicited much greater suppression than did a low mixture concentration (0.2 ppm, 0.100 mg/m^3 and 0.050 mg/m^3, 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 PM2.5/ozone atmospheres on aged rats, animals were exposed 4 hr/day, 3 consecutive days/wk for 4 weeks to 0.05 mg/m^3 carbon particles alone, 0.07 mg/m^3 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 PM2.5. 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 acivity 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^3 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^3 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 preceeding 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^3 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^3; 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^3 particulate matter from Ottawa Canada (EHC-93). Groups of rats were exposed to

ozone alone or 5.0 mg/m^3 EHC-93 alone. Parameters in BAL fluid used to measure the inflammatory effect of ozone alone (total protein, alkaline phosphatase and lactate dehydrogenase acitivity, 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^3), sulfuric acid (0.6 mg/m^3), and hydroxymethanesulfonate (HMSA) (0.6 mg/m^3) 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^3). 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_3, 0.05 mg/m^3 nitric acid, 0.1 mg/m^3 NH_4NSO_4, and 0.06 mg/m^3 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^3) in the presence of 5 ppm SO_2 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_2 (2.5 ppm), SO_2 (5 ppm), ferric oxide (0.241 mg/m^3), ammonium sulfate (0.308-0.364 mg/m^3), ferric sulfate (0.411-0.571 mg/m^3), and manganese sulfate (0.007-0.009 mg/m^3). 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_2 (2.5 ppm), or NO_2 alone (6 or 12 ppm). Exposure to ozone, ozone + NO_2, 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_2, 2.5 ppm SO_2, 0.27 mg/m^3 ammonium sulfate, 0.22 mg/m^3 iron sulfate, 0.004 mg/m^3 manganese sulfate and an insoluble aerosol of 0.15 mg/m^3 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 to 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_2, 0.2 mg/m^3 ammonium bisulfite, 0.12 mg/m^3 carbon particles, 0.1 mg/m^3 nitric acid) exacerbated irritant-induced rapid shallow breathing responses while exposure to the medium concentration (0.3 ppm ozone, 0.2 ppm NO_2, 0.1 mg/m^3 ammonium bisulfite, 0.06 mg/m^3 carbon particles, 0.05 mg/m^3 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 Sǎo 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^3 particulates, and 0.029 mg/m^3 SO_2. 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-Garcidueňas 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 severly 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_2 likely contribute to the particle uptake and translocation into the interstitium by increasing epithelial permeability. In an associated study, Calderón-Garcidueňas et al. (2001b) also observed cardiac abnormalities in the dogs exposed to severly 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 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_2 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_2 interactions utilized a bacterial infectivity model in which 15-day exposure to a simulated urban pollutant atmosphere (baseline of 0.5 ppm NO_2 with peaks of 1.0 ppm, and a baseline of 0.05 ppm 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.

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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