State of California

Air Resources Board

Governor Arnold Schwarzenegger

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

March 11, 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 Benefits of Reducing Ozone Exposure

Quantifying the Health Benefits of Reducing Ozone Exposure

The objectives of this appendix are to quantify the adverse health effects of current ozone levels in California by estimating the health benefits that would accrue from a hypothetical control strategy that achieves the proposed ambient air quality standards for ozone. This health effects assessment is not being used to set the health standards or in any formal cost-benefit analysis. As such, the results from this appendix are provided for public information about the expected benefits of attaining the proposed standards and do not include monetary values.

There have been several recent published efforts to estimate the health benefits associated with reducing population exposures to ozone (U.S. Environmental Protection Agency 1999; Levy et al. 2000). Numerous epidemiologic studies conducted in the United States and other countries point to the 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. Below we describe the methods, data, results and uncertainties involved with estimating the health benefits of the proposed California ambient air quality standards.

Health Effects Estimation Approach

Section 812 of the federal Clean Air Act required the U.S. EPA to 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 prior efforts at the federal level, particularly in the development of concentration-response functions. We have also added studies published from around the world since the U.S. EPA report. The selection of the studies and functions to include in our analysis has undergone review by several independent experts on the subject of air pollution and health.

Estimating the health benefits associated with reductions in levels of ambient ozone involves four elements:

1. Estimates of the changes in ozone concentrations due to a hypothetical control strategy.
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 produce a beta coefficient, indicating the percent reduction in a given health outcome due to a unit change in ozone.

Ultimately, the product of these elements generates estimates of the expected number of avoided adverse health outcomes associated with a hypothetical control strategy to reduce current levels of ozone to the proposed standard. Each of these elements is discussed below. Our methods make use of U.S. EPA's development of the Environmental Benefits Mapping and Analysis Program (BenMAP) with modifications where appropriate to reflect the application to California's setting and more recent studies. All methods and results presented herein are consistent with U.S. EPA (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 benefits of reducing ozone.

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, the value that reflects the highest concentration expected to occur on any given year based on the past three years of data. The use of three years 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 done mostly 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 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

To assess the daily reductions in current ozone concentrations estimated to result at all monitoring sites when the standards are achieved, rollback factors from the 1-hour and

8-hour ozone design values to the applicable standard were calculated for each air basin. The ozone design value selected was the highest for the three-year period (2001 to 2003). 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.

This methodology assumed that under the hypothetical attainment setting, 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 statewide health benefits are projected to accrue in the SoCAB. For this region, the downward trend was consistent for both 1-hour and 8-hour concentrations from the 1980s to current levels. 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 from the 1980s. More importantly, when we examined the rate of change in the concentrations above background from the 1980s, it was similar among the percentiles. This analysis justifies our application of a constant percentage rollback to all sites within an 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 monitoring site in the State, the rollback factor necessary to move from the basin-high value to the proposed standard was calculated for both the 1- and 8-hour averages. These rollback factors were then applied on a site–by-site basis to the ozone readings for every day. The difference between the observed value and the rolled-back value was calculated for each day of the year.

Health effects 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 benefits 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 for the rollback assumption 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.

For mortality, the incidence rates were obtained from the U.S. Centers for Disease Control (CDC) derived from the U.S. death records and 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-17 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 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 10, a wide range of adverse health effects has been associated with exposure to current ambient concentrations of ozone. 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 exposure on premature mortality, hospital admissions for respiratory disease, emergency room visits for asthma, school absenteeism, and minor restrictions in activity. Although other effects have been related to ozone exposure – such as asthma exacerbations, respiratory symptoms, hospital admissions for cardiovascular disease with short-term 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 quantitative CR function 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 benefit assessment are generally 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 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 do not provide the general population response to exposure to ozone. For the latter purpose, epidemiological studies which incorporate varying subgroups, exposure scenarios, behaviors, and health outcomes will best serve to determine the overall potential human response to a particular pollutant and be the source of quantitative estimates for 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 (WHO) 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, a national average of 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 of 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 are similar. 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 effects estimates. One important issue in estimating ozone health effects 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 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 benefits 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 no threshold was in evidence and used the background level of 0.04 ppm as the no effects level. As an alternative for a sensitivity analysis, we assumed a no effects level at 0.075 ppm 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 represent 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 increase 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 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 ($= \Delta O_3$) 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 is currently funding several meta-analyses of the ozone-mortality association but this information is currently not 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.

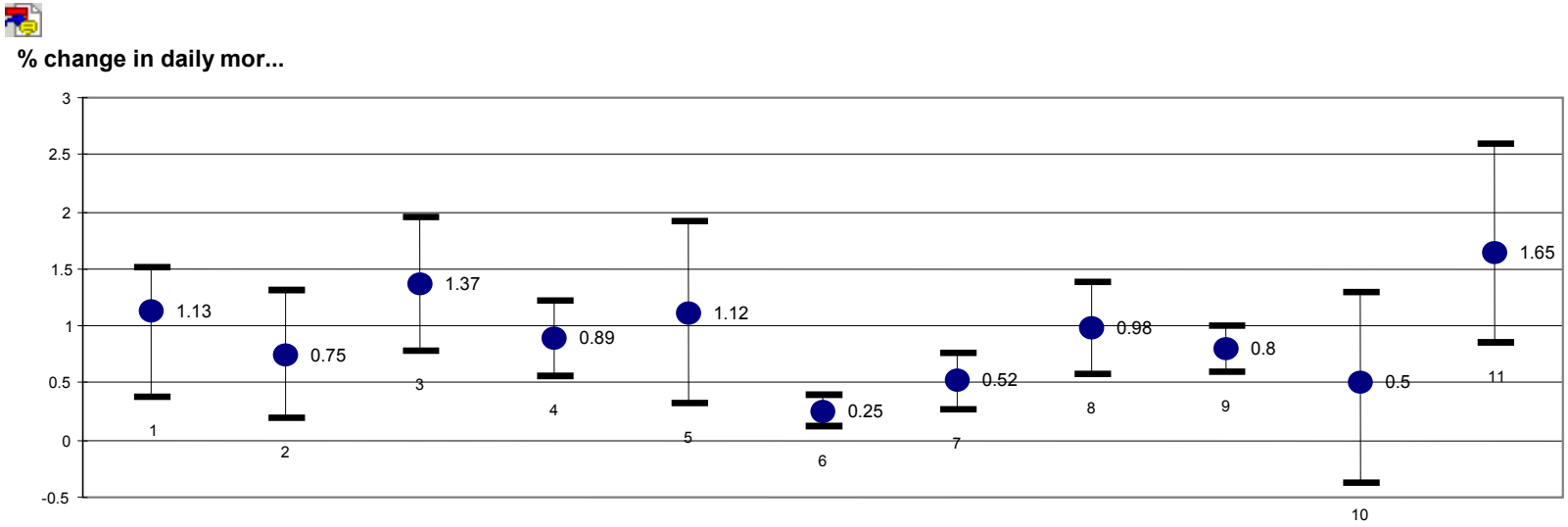
The World Health Organization (WHO) conducted a meta-analysis of the 15 cities in Europe (Anderson et al. 2004). Their meta-estimates indicate a relative risk of 1.003 (95% CI = 1.001 – 1.004) for a 10 $\mu g/m^3$ change in 8-hour ozone. For standard pressure (1 atmosphere) and temperature (25º C), 1 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 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 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 APEHA2 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 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 ozone. This summer-specific estimate might be particularly relevant for California due to its milder 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 of 0.25% (95% CI = 0.12 – 0.39) per 10-ppb change in 24-hour 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., 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 attempt to reflect the range provided in the above cited studies. Figure 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 ozone, corresponds to the best estimates from the NMMAPS (using a one-week cumulative lag) and the APEHA2 European study, but is below most of the other central estimates. A central estimates of 1% per 10 ppb is very similar to the

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 temp
4	Thurston+Ito (2001)	19	All studes
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

central estimates generated by WHO (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). Bates (personal communication, 2005) suggested that these concentration-response relationships may be underestimated. Our range of estimates is applied to all age groups.

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 this estimate, we rely on the meta-analysis by Thurston and Ito (1999). These authors used a random effects model using 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 µ/m change in ozone. This converts to 2.05% per 10 ppb change in 1-hour maximum ozone. Burnett et al. (2001) investigated respiratory hospitalizations in children under age 2, and reported a relative risk of 1.348 (95% CI= 1.193 – 1.523), which converts to a 6.6% increase in hospital admissions per 10 ppb change in 1-hour daily maximum ozone.

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 treated hospital admissions and ER visits separately, taking account of 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 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). 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 use some of the available information on how to adjust the slope in order 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 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.

In calculating the change in school loss days, we assumed children did not attend school during weekends and holidays, that about 20% of students attended year-round schools, and adjusted 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.

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 Analysis

Several additional analyses were run to indicate 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.

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 1-hr maximum average of 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 (= 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 (= 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 (=ln(1.68)/60) and 0.0141 (=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-2 presents the estimated statewide annual health benefits from reducing the current (2001-2003) levels of ozone to achieve the 1-hour standard of 0.09 ppm. 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. For example, the results indicate that full attainment of the proposed 1-hour standard would result in 580 fewer cases of premature mortality (probable range = 290 – 870), 3,800 fewer hospital admissions (95% CI = 2,200 – 5,400) and 3,300,000 fewer days of school loss (95% CI = 430,000 – 6,100,000) per year. Since the results for premature mortality due to short-term exposures were derived from examining the evidence from several papers, rather than combining the results into a confidence interval, we use the terminology of a “probable range”.

Similar to Table B-2, Table B-3 presents statewide results from achieving the proposed 8-hour standard of 0.070 ppm. Generally speaking, the health benefits from attaining the 1-hour standard are greater than those associated with attaining the 8-hour standard. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated benefits from Tables B-2 and B-3 together. Tables B-4 and B-5 present estimates of the annual health benefits of attaining the proposed 1-hour and 8-hour standards, respectively, by air basin. Table B-6 presents estimates of the annual health benefits of attaining either of the standards, whichever provides the greatest amount of control.

Our first sensitivity analysis examined the implications of alternative exposure assessments. In the first assessment, we interpolated concentrations for each census tract using nearby monitoring data. The results for mortality are similar to those obtained using the base-case approach. Attaining the 1-hour ozone standard statewide using census-tract interpolations lead to 570 deaths compared to 580 deaths avoided using our base-case approach. In the second assessment using county-wide average concentrations, similar results, about 630 deaths, are obtained.

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 a 10% decrease in health

outcomes. For example, mortality would decrease from 580 to 520. The breakeven point associated with a 40% increase in the slope would be about 55 ppb. 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 number of cases 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 140% to get the same number of cases as in base case, non-threshold model. If we assumed a threshold at 50 ppb with a 100% increase in the remaining slope, the estimate number of case would increase by about 70%.

Uncertainties and Limitations

There are a number of uncertainties involved in quantitatively estimating the health benefits associated with reductions in outdoor 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 benefits. 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 this 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 in the daily time series context 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 health benefits analysis.

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 mortality and morbidity, which is 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 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. 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 benefits 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 effect threshold. However, many of the 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 benefits 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 reducing

ozone. However, we also conducted a sensitivity analysis with an assumption of 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 benefits 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. In addition, 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 health benefits that could be associated with achieving the proposed ozone standards, since estimates are provided for only a subset of possible adverse outcomes. For example, estimates of the effects of ozone on asthma exacerbation 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 benefits of achieving the proposed ozone standards. To the extent that certain important health outcomes were excluded, we may have underestimated the health benefits 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 benefits of attainment of 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 benefits that may accrue from 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 selection of the most plausible effects for which there were high quality studies from which to derive CR functions. However, the results presented support the conclusion that significant public health benefits would result from statewide attainment of the proposed ambient air quality standards for ozone.

It is estimated that attainment of the proposed ozone standards throughout California would avoid a significant number of adverse health effects each year. The higher central estimate between the values calculated for 1-hour and 8-hour averaging times is given below.

- 580 (290 – 870, probable range) premature deaths for all ages.
- 3,800 (2,200 – 5,400, 95% confidence interval (CI)) hospitalizations due to respiratory diseases for all ages.
- 600 (360 – 850, 95% CI) emergency room visits for asthma for children under 18 years of age.
- 3.3 million (430,000 – 6,100,000, 95% CI) school absences for children 5 to 17 years of age.
- 2.8 million (1.2 million – 4.6 million, 95% CI) minor restricted activity days for adults above 18 years of age.

The reader is cautioned that since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated benefits from Tables B-2 and B-3 together.

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 exposure, and effects of chronic ozone exposure, are not included in the estimates, the health benefits associated with lowering ozone exposure are likely underestimated.

Table B-1: 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)	APEHA2 studies in Europe, all year
11	Gryparis et al. (2004)	23	1.65 (0.85 – 2.60)	APEHA2 studies in Europe, summer only

Table B-2: California Annual Health Benefits from Attaining a 1-hour Ozone Standard of 0.09 ppm*

Health Endpoint	Population	Estimated Beta (% per 10 ppb) (95% Confidence Interval)	Avoided Incidence (cases/year)
			Mean 95% Confidence Interval
Premature Mortality due to Short-term Exposures	All ages	0.0040 (0.0020 - 0.0060)**	580 (290 – 870) **
Hospital Admissions for Respiratory Diseases	All ages	0.0164 (0.0095 - 0.0228)	3,800 (2,200 – 5,400)
Emergency Room Visits for Asthma	Age < 18	0.0237 (0.01446 – 0.0329)	600 (360 – 850)
School Loss Days	Age 5-17	0.2123 (0.0334 – 0.3295)	3,300,000 (430,000 – 6,100,000)
Minor Restricted Activity Days	Age > 18	0.0222 (0.0092 - 0.0350)	2,800,000 (1,200,000 – 4,600,000)

*Base period 2001-2003. Since 1-hour and 8-hour concentrations are highly correlated, it is not appropriate to add the estimated benefits from Tables B-2 and B-3 together. **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.

Table B-3: California Annual Health Benefits from Attaining an 8-hour Ozone Standard of 0.070 ppm*

Health Endpoint	Population	Estimated Beta (% per 10 ppb) (95% Confidence Interval)	Avoided Incidence (cases/year)
			Mean (95% Confidence Interval)
Premature Mortality due to Short-term Exposures	All ages	0.0053 (0.0027 – 0.0079)**	540 (270 – 810) **
Hospital Admissions for Respiratory Diseases	All ages	0.0218 (0.0126 - 0.0302)	3,600 (2,000 –5000)
Emergency Room Visits for Asthma	Age < 18	0.0314 (0.0192 - 0.0434)	570 (340 – 800)
School Loss Days	Age 5-17	0.2440 (0.0844 – 0.4034)	2,600,000 (760,000 – 5,200,000)
Minor Restricted Activity Days	Age > 18	0.0294 (0.0121 - 0.0462)	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 benefits from Tables B-2 and B-3 together. **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.

Table B-4: Annual Health Benefits from Attaining 1-hour Ozone Standard of Air Basin of 0.09 ppm by Air Basin.

Air Basin	Mortality	HospitalAdmissions	EmergencyRoom Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	<1	<1	340	370
Lake County	0	0	0	0	0
Lake Tahoe	<1	3	<1	2,500	2,400
Mountain Counties	10	52	7	40,000	41,000
Mojave Desert	43	300	50	280,000	220,000
North Coast	<1	<1	<1	370	330
North Central Coast	1	10	2	9,000	7,700
Northeast Plateau	0	0	0	0	0
South Coast	300	2,100	330	1,700,000	1,600,000
South Central Coast	16	110	16	97,000	83,000
San Diego	24	160	22	120,000	120,000
San Francisco Bay	23	150	21	100,000	120,000
San Joaquin Valley	95	610	110	650,000	440,000
Salton Sea	20	120	20	120,000	88,000
Sacramento Valley	39	220	32	170,000	170,000
Statewide	580	3,800	600	3,300,000	2,800,000

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 benefits from Tables B-4 and B-5 together. Table B-6 should be used to estimate the maximum health benefit per air basin. The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

Table B-5: Annual Health Benefits from Attaining 8-hour Ozone Standard of 0.070 ppm by Air Basin

Air Basin	Mortality	HospitalAdmissions	EmergencyRoom Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	1	<1	650	820
Lake County	<1	<1	<1	35	53
Lake Tahoe	1	9	1	6,100	6,600
Mountain Counties	12	62	8	41,000	50,000
Mojave Desert	51	350	59	280,000	260,000
North Coast	<1	1	<1	720	750
North Central Coast	2	12	2	9,100	8,800
Northeast Plateau	<1	<1	<1	88	140
South Coast	260	1,700	270	1,100,000	1,300,000
South Central Coast	17	120	18	92,000	91,000
San Diego	25	170	23	110,000	130,000
San Francisco Bay	14	92	13	51,000	72,000
San Joaquin Valley	100	670	120	600,000	470,000
Salton Sea	21	130	21	110,000	91,000
Sacramento Valley	39	220	32	140,000	160,000
Statewide	540	3,600	570	2,600,000	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 benefits from Tables B-4 and B-5 together. Table B-6 should be used to estimate the maximum health benefit per air basin. The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

Table B-6: Annual Health Benefits from Attaining Both 1-hour and 8-hour Ozone Standards by Air Basin

Air Basin	Mortality	HospitalAdmissions	EmergencyRoom Visits	School Absences	Minor Restricted Activity Days
Great Basin Valley	<1	1	<1	650	820
Lake County	<1	<1	<1	35	53
Lake Tahoe	1	9	1	6,100	6,600
Mountain Counties	12	62	8	41,000	50,000
Mojave Desert	51	350	59	280,000	260,000
North Coast	<1	1	<1	720	750
North Central Coast	2	12	2	9,100	8,800
Northeast Plateau	<1	<1	<1	88	140
South Coast	300	2,100	330	1,700,000	1,600,000
South Central Coast	17	120	18	97,000	91,000
San Diego	25	170	23	120,000	130,000
San Francisco Bay	23	150	21	100,000	120,000
San Joaquin Valley	100	670	120	650,000	470,000
Salton Sea	21	130	21	120,000	91,000
Sacramento Valley	39	220	32	170,000	170,000

Note: The higher central estimate for the benefit values (either 1-hour or 8-hour averaging times) is given above for each endpoint by air basin. The uncertainty behind the mortality estimates is on the order of +/- 50% and varies for other endpoints.

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(http://www.epa.gov/sab/pdf/council_adv_04002.pdf)

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 = proposed standard (0.09 ppm for 1-hour and 0.070 ppm for 8-hour), 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-2 through B-11 and Tables B-7 through B-16 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-15.

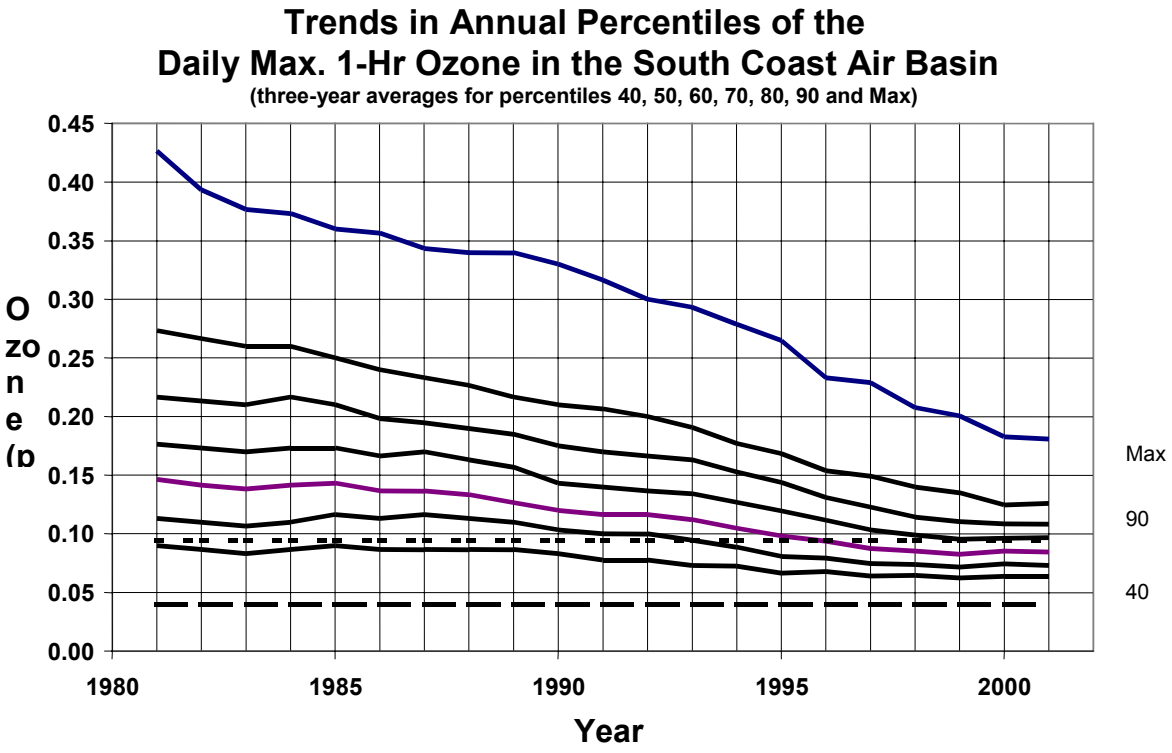

Figure B-2: Trends in Annual Percentiles of Daily Max 1-hour Ozone in the South Coast Air Basin

Table B-7: 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.

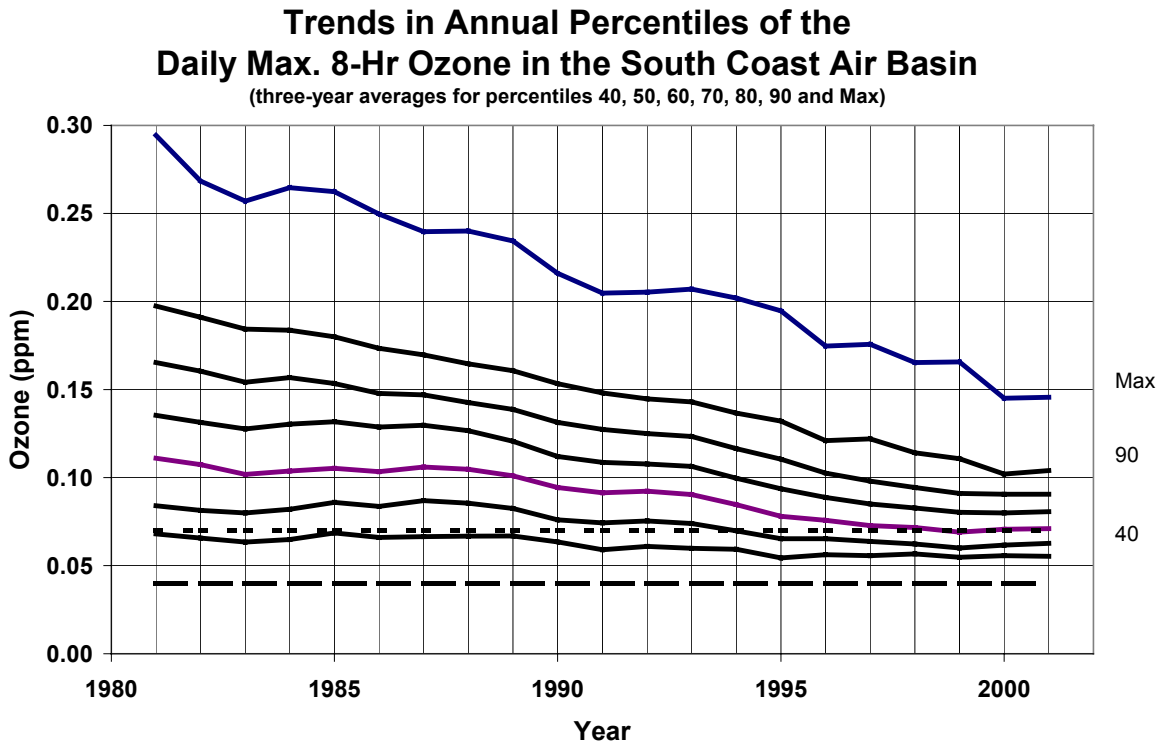

Figure B-3: Trends in Annual Percentiles of Daily Max 8-hour Ozone in the South Coast Air Basin

Table B-8: 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.

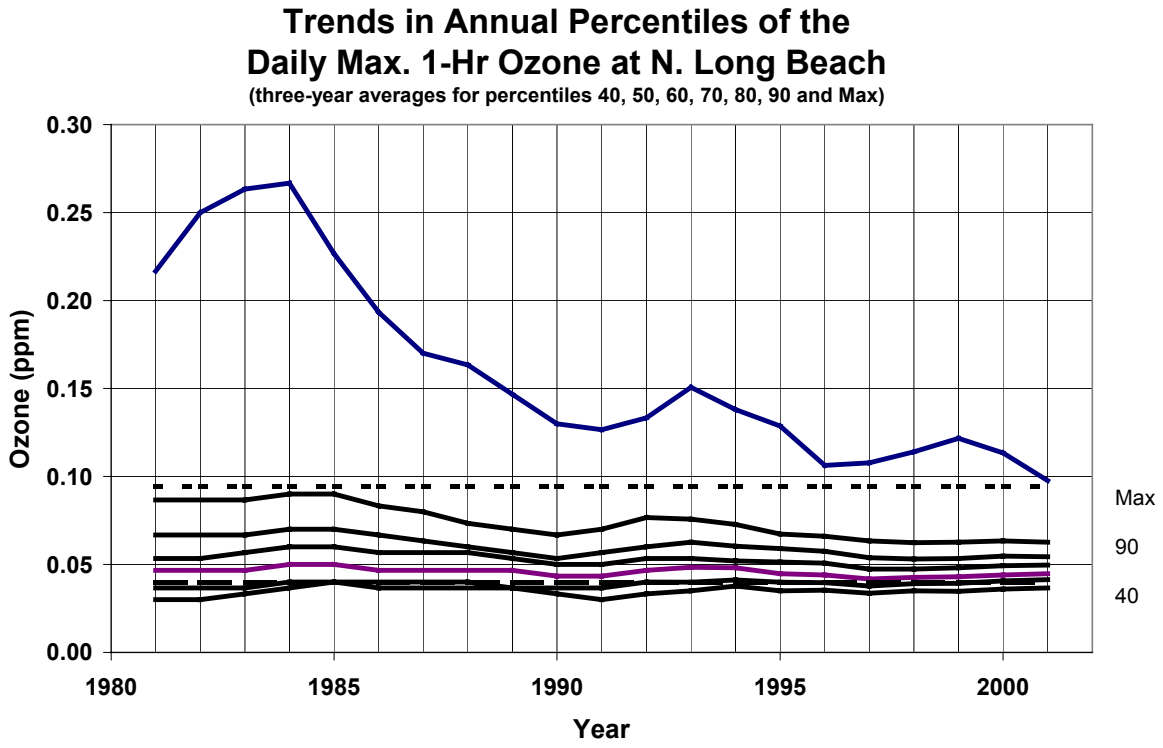

Figure B-4: Trends in Annual Percentiles of Daily Max 1-hour Ozone at N. Long Beach

Table B-9: 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.

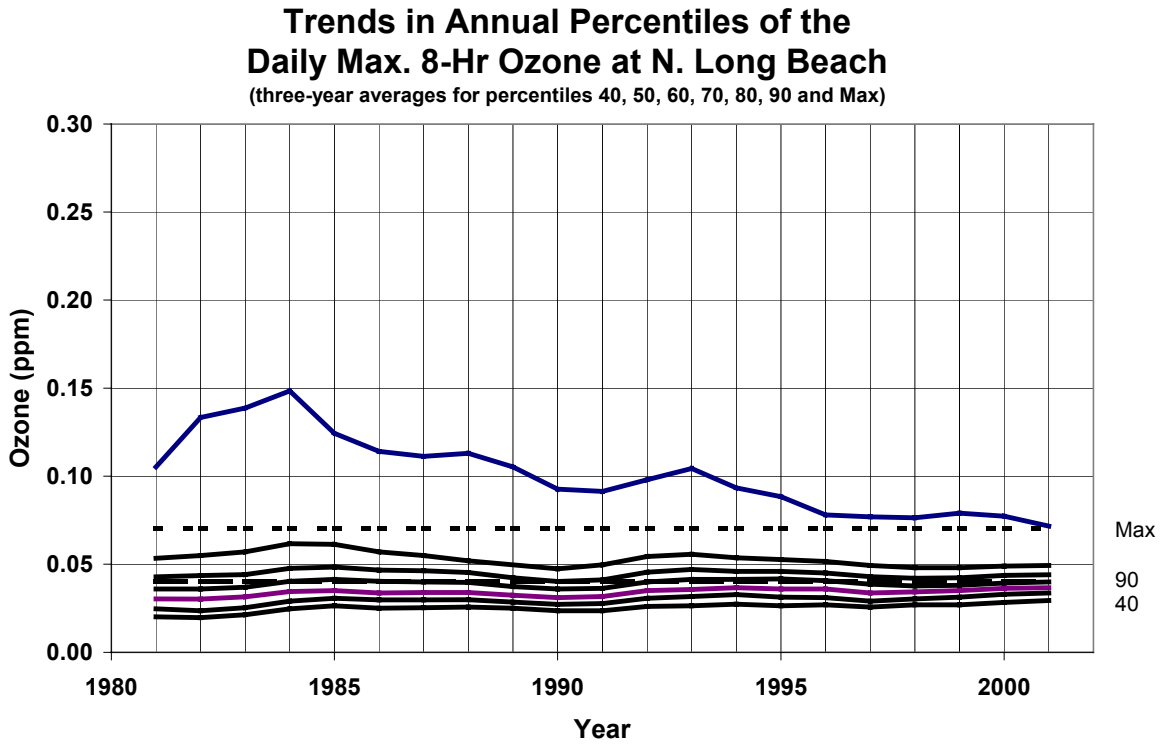

Figure B-5: Trends in annual percentiles of daily max 8-hour ozone at N. Long Beach

Table B-10: 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.

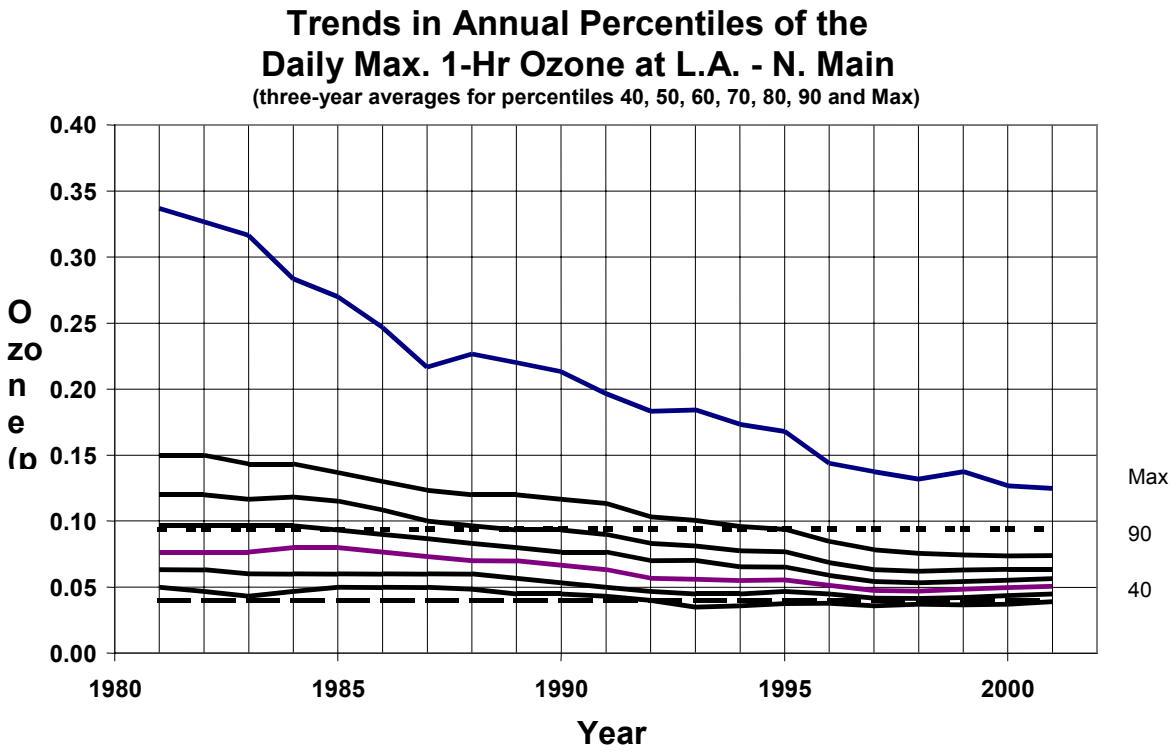

Figure B-6: Trends in annual percentiles of daily max 1-hour ozone L.A. – N. Main

Table B-11: 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.

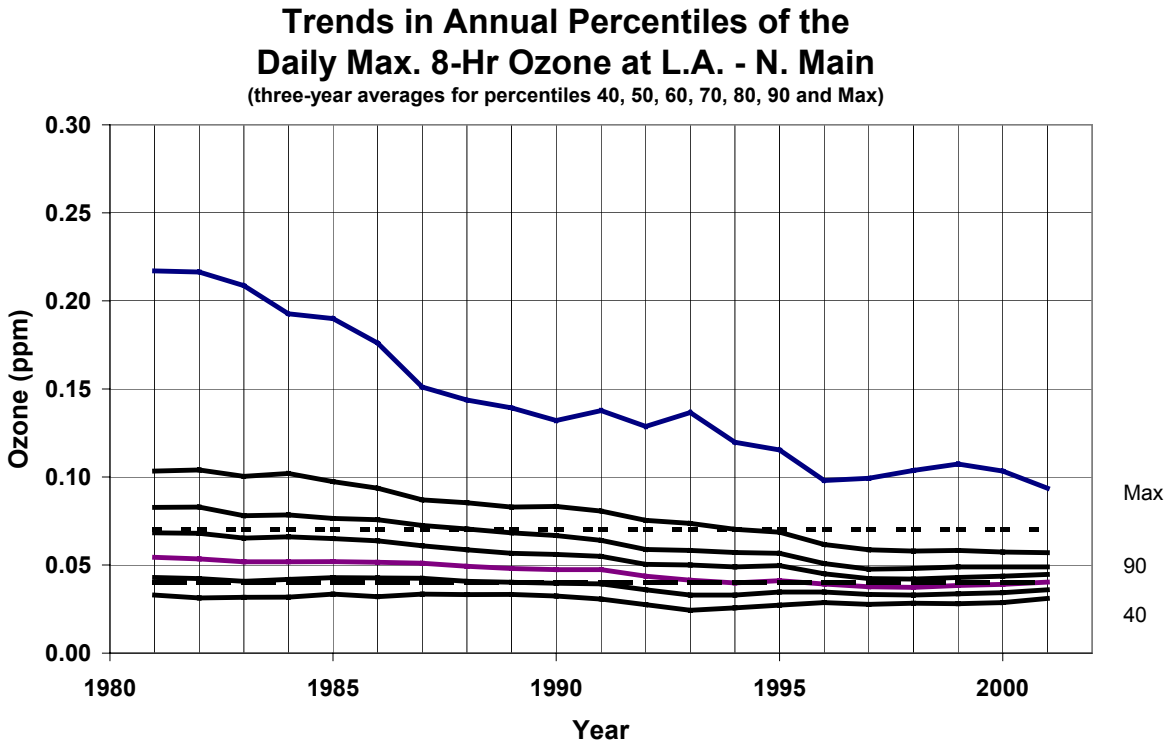

Figure B-7: Trends in annual percentiles of daily max 8-hour ozone at L.A.-N. Main

Table B-12: 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.

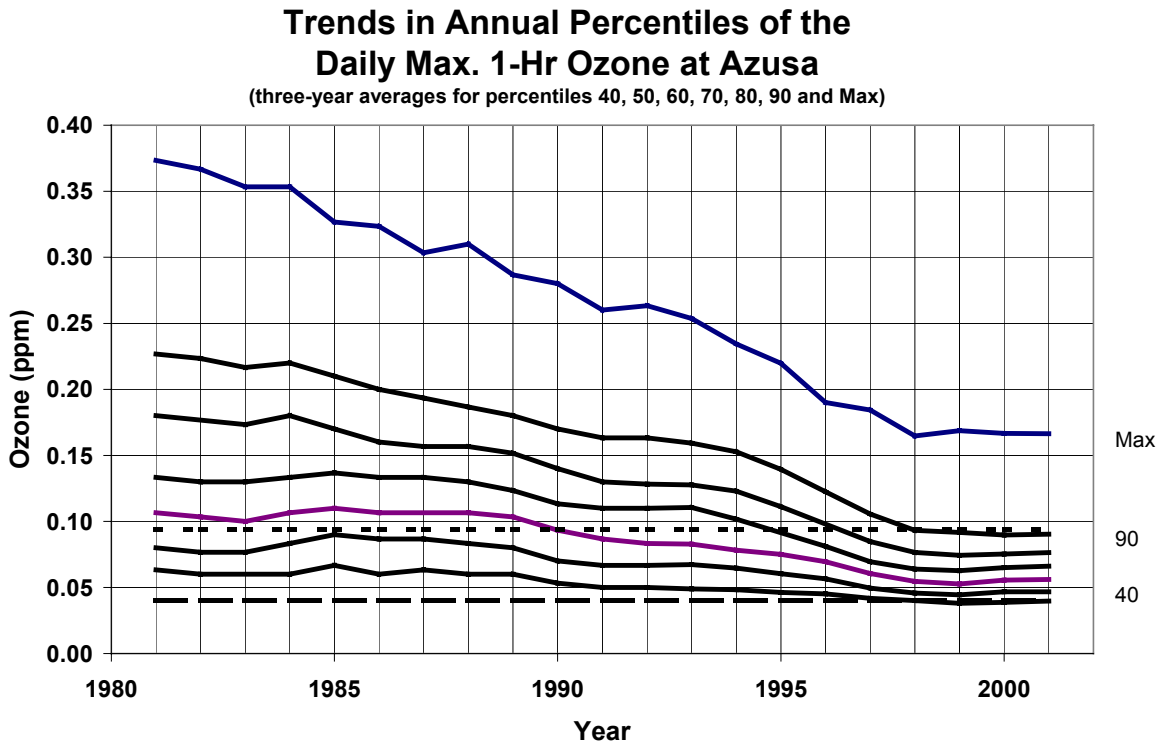

Figure B-8: Trends in annual percentiles of daily max 1-hour ozone at Azusa

Table B-13: 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.

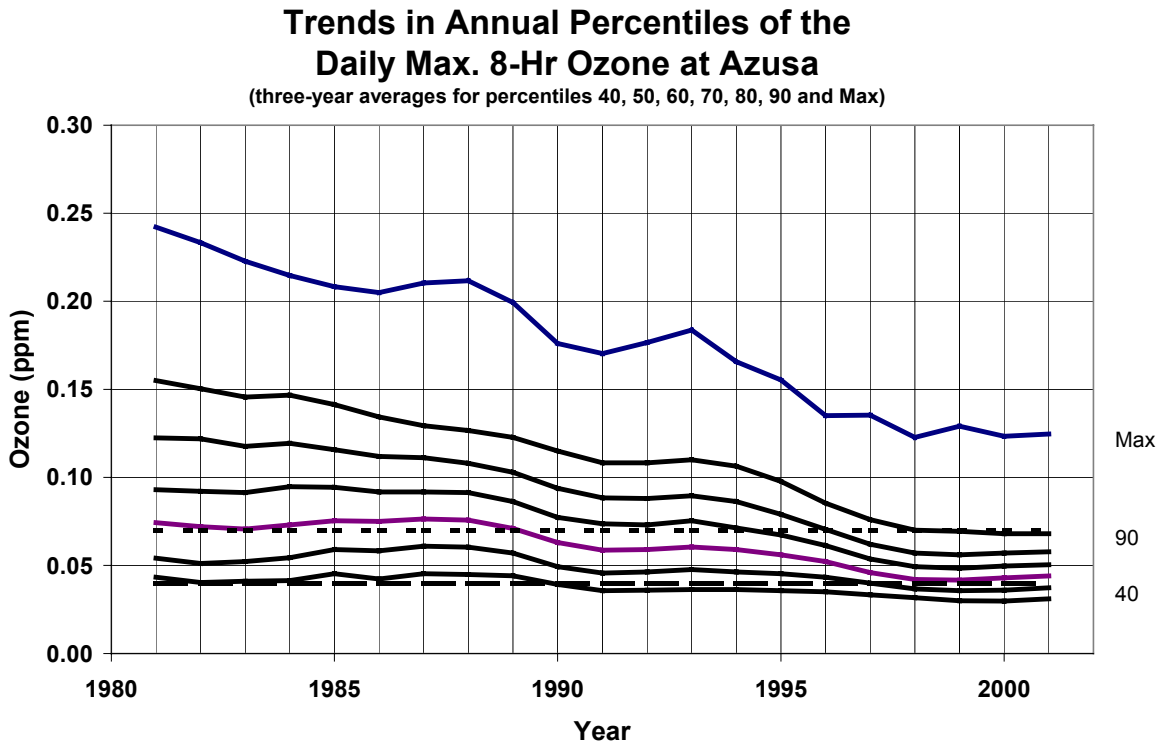

Figure B-9: Trends in annual percentiles of daily max 8-hour ozone at Azusa

Table B-14: 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.

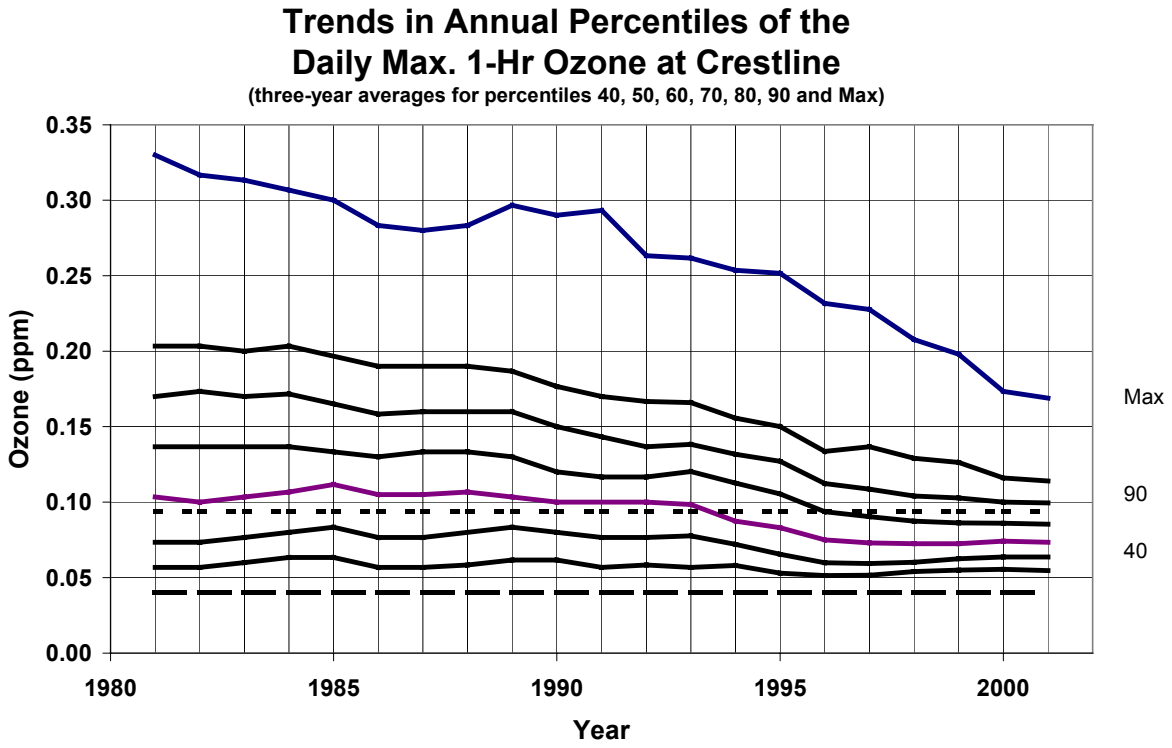

Figure B-10: Trends in annual percentiles of daily max 1-hour ozone at Crestline

Table B-15: 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.

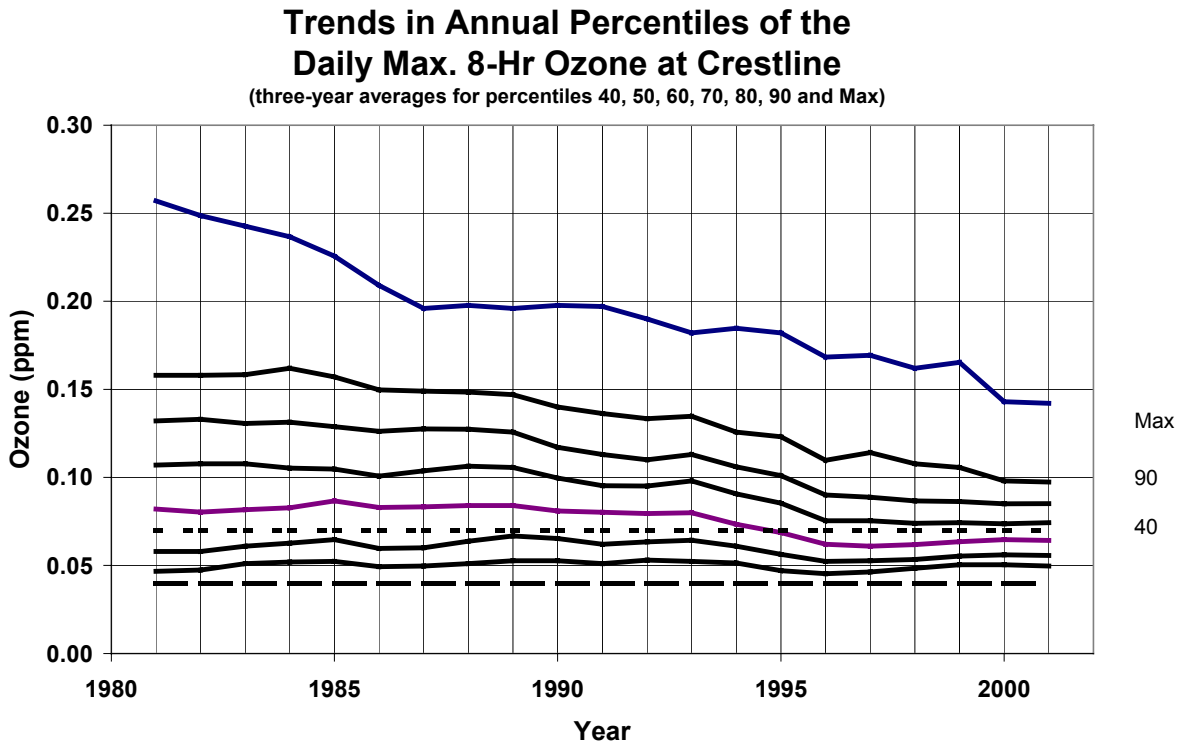

Figure B-11: Trends in annual percentiles of daily max 8-hour ozone at Crestline

Table B-16: 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-17: 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
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California Air Resources Board
1001 I Street
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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 90^{th} 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 4^{th} 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 4^{th} 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 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. 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 4^{th} 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 4^{th} 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 sensitive 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.

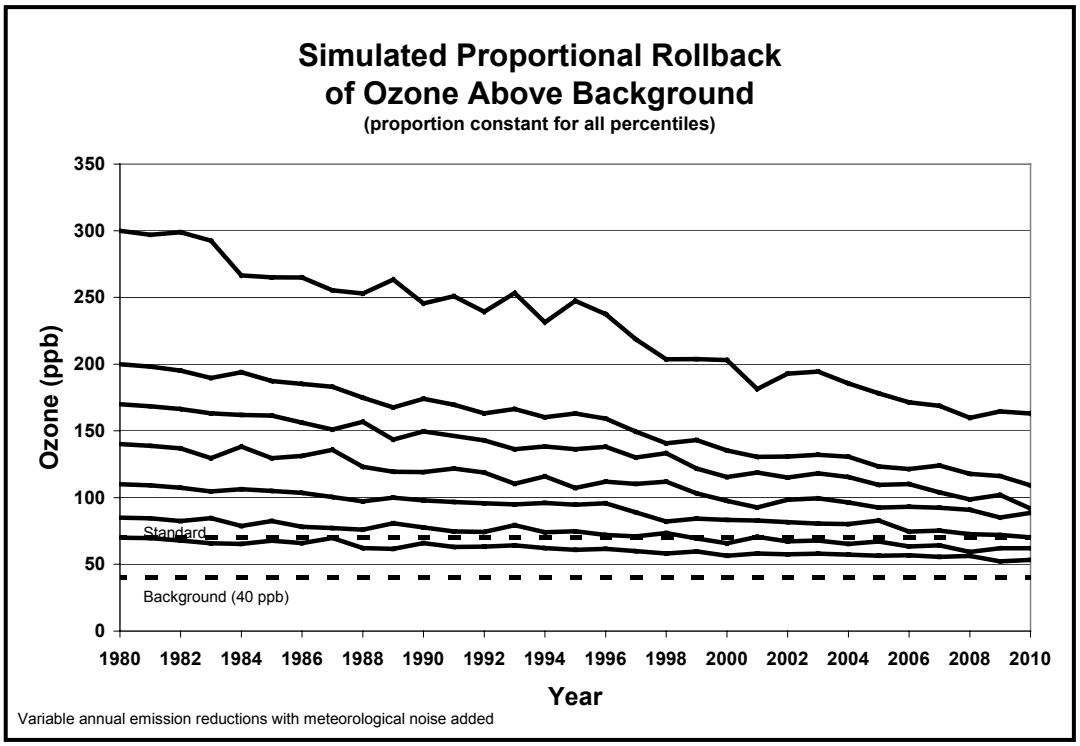
The table includes alternative choices for background from 0.040 to 0.070 ppm. Since the vast bulk of the benefits attributed to ozone reductions represent the highly populated coastal and valley regions of the state, any background level significantly above 0.04 ppm is highly doubtful. In these areas, 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.

cc: Val. F. Siebal
Chief Deputy Director

George V. Alexeeff, Ph.D.
Deputy Director for Scientific Affairs

Melanie A. Marty, Ph.D., Chief
Air Toxicology & Epidemiology Section

Bart E. Croes, Chief
Research Division

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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^{TM}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 São 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-Garcidueñas 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-Garcidueñas 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